

Cleveland State University
EngagedScholarship@CSU



ETD Archive

2009

Overcoming Barriers to Natural Orifice Translumenal Endoscopic Surgery (Notes)

Steve J. Schomisch
Cleveland State University

Follow this and additional works at: <https://engagedscholarship.csuohio.edu/etdarchive>

 Part of the [Biology Commons](#)

How does access to this work benefit you? Let us know!

Recommended Citation

Schomisch, Steve J., "Overcoming Barriers to Natural Orifice Translumenal Endoscopic Surgery (Notes)" (2009). *ETD Archive*. 264.
<https://engagedscholarship.csuohio.edu/etdarchive/264>

This Dissertation is brought to you for free and open access by EngagedScholarship@CSU. It has been accepted for inclusion in ETD Archive by an authorized administrator of EngagedScholarship@CSU. For more information, please contact library.es@csuohio.edu.

**OVERCOMING BARRIERS TO NATURAL
ORIFICE TRANSLUMENAL ENDOSCOPIC
SURGERY (NOTES)**

STEVE J. SCHOMISCH

Bachelor of Science in Biology

John Carroll University

June, 1986

Submitted in partial fulfillment of requirements for the degree

DOCTOR OF PHILOSOPHY IN REGULATORY BIOLOGY

At the

CLEVELAND STATE UNIVERSITY

MAY, 2009

This dissertation has been approved
for the Department of

BIOLOGICAL, GEOLOGICAL AND ENVIRONMENTAL SCIENCES

and the College of Graduate Studies by

Dissertation Chairperson, Crystal M. Weyman, Ph.D. Date

Biological, Geological and Environmental Sciences

Dennis Stuehr, Ph.D. Date

Biological, Geological and Environmental Sciences

Roman Kondratov, Ph.D. Date

Biological, Geological and Environmental Sciences

Girish Shukla, Ph.D. Date

Biological, Geological and Environmental Sciences

ACKNOWLEDGEMENTS

I would like to thank Jeffrey Ponsky MD, Chair, Department of Surgery, University Hospitals of Cleveland, for providing me with the resources and support to conduct this project in the Animate Skills Laboratory at CASE Medical Center. Additional thanks to the staff surgeons and gastroenterologists in the Department of Surgery including Dr. Jeffrey Marks, Dr. Conor Delaney, Dr. Michael Rosen and Dr. Amitabh Chak for educating me in the areas of laparoscopic, endoscopic and general surgery.

I extend great appreciation to Department of Surgery residents and fellows Dr. Joseph Trunzo, Dr. Michael McGee, Dr. Joseph Elmunzer and Dr. Leandro Cavazzolo for their expertise and valued contributions. Thanks to Mr. Jamie Andrews, Ms. Jessica Bailey, Mr. Joseph Furlan and Mr. Tripurari Mishra for technical assistance throughout the many years.

I would also like to recognize Dr. Donald Jacobsen, Department of Cell Biology, Cleveland Clinic Foundation, for serving as my advisor for several years and to the members of his lab, especially Dr. Patricia DiBello, for working with me and teaching me numerous methodologies.

Thanks to members of my committee, past and present, Dr. Dennis Steuhr, Dr. Martha Cathcart, Dr. Joseph Fontes, Dr. Jeffrey Dean, Dr. Girish Shukla and Dr. Roman Kondratov for their service.

I would especially like to thank Dr. Christine Schomisch Moravec and Dr. Crystal Weyman for guidance, support and refining this project to make it possible and presentable.

OVERCOMING BARRIERS TO NATURAL ORIFICE TRANSLUMENAL ENDOSCOPIC SURGERY (NOTES)

STEVE J. SCHOMISCH

ABSTRACT

Natural Orifice Translumenal Endoscopic Surgery (NOTES) avoids skin incisions by accessing the abdominal cavity through natural orifices. Benefits include less pain, fewer complications and no scars. The aims of this study were to evaluate safety and efficacy of access techniques for natural orifice surgery, to investigate safety and efficacy of closure methodologies and to compare the inflammatory response induced by NOTES with that following conventional surgery.

Access techniques were evaluated for safety and efficacy by measuring resultant injury and time required to access the peritoneal cavity in an acute porcine model. Four different gastrotomy closure modalities were evaluated for safety and efficacy by measuring clinical data, evidence of infection and closure integrity in a chronic porcine study. Markers of inflammation were measured in a chronic study comparing NOTES to conventional surgery.

70 anterior transgastric access procedures were performed without any serious injury to adjacent organs. NOTES access required significantly longer than laparoscopic access. Of the seven methods evaluated none was significantly superior to others. In distinguishing between safe and unsafe alternate access sites, endoscopic ultrasound (EUS) provided a statistically significant difference for antral and posterior stomach access points but not for rectal access. Gastrotomy closure time varied widely for the

four techniques evaluated. No leak was detected with any method and the strength of closure for each technique was equivalent to control. Three of the four methods resulted in injury and evidence of infection was found in all groups. Postoperative elevation of inflammatory markers was not significantly different between NOTES and laparoscopy except in the case of cortisol, which was greater in the laparoscopic group.

This study supports the safety of anterior transgastric access while demonstrating the potential use of EUS in minimizing risks of injury at alternate sites. Likewise, evidence for the reliability of numerous closure techniques was demonstrated with concerns for the safety. Additionally, inefficient access and closure data exposed the technical challenges facing NOTES practitioners. Most importantly, NOTES produced an inflammatory response which was not significantly greater than that produced by conventional laparoscopic surgery, validating its potential and emphasizing the need to overcome the aforementioned technical challenges.

TABLE OF CONTENTS

	Page
ABSTRACT.....	iv
LIST OF TABLES.....	ix
LIST OF FIGURES	x
CHAPTER	
I. INTRODUCTION TO NATURAL ORIFICE SURGERY	
1.1 Surgery: A Historical Perspective.....	1
1.2 Minimally Invasive Surgery: Minimizing the injury caused by the treatment	4
1.3 Natural Orifice Surgery.....	5
1.4 The Origins of NOTES	6
1.5 The Benefits of NOTES.....	10
1.6 The Feasibility of NOTES	13
1.7 Barriers to NOTES.....	15
1.8 The Current Status of NOTES	15
1.9 Specific Aims and Hypotheses	24
II. ACCESS TO THE PERITONEAL CAVITY VIA NOTES	
2.1 Background.....	25
2.1.1 Endoscopic Instruments	26
2.1.2 Choosing an Access Site.....	30
2.2 A Comparison of Transgastric Access Techniques	31
2.2.1 Introduction.....	31

2.2.2 Methods.....	34
2.2.3 Results.....	40
2.2.4 Discussion.....	49
2.3 Endoscopic Ultrasound for Identification of Safe Alternate Access Sites.....	57
2.3.1 Introduction.....	57
2.3.2 Methods.....	59
2.3.3 Results.....	64
2.3.4 Discussion.....	71
III. CLOSURE: THE SINGLE GREATEST BARRIER TO NOTES	
3.1 Introduction.....	74
3.2 Methods.....	75
3.3 Results.....	89
3.4 Discussion.....	97
IV. INFLAMMATORY RESPONSE	
4.1 Introduction.....	104
4.2 Methods.....	107
4.3 Results.....	112
4.4 Discussion.....	122
V. CLOSING REMARKS	
5.1 Summary.....	127
5.2 Technological developments.....	129
5.3 Cost of NOTES.....	130

5.4 Demonstration of a true benefit	131
5.5 Training of NOTES practitioners.....	132
5.6 Patient perception.....	132
5.7 NOTES By-products.....	133
BIBLIOGRAPHY.....	135

LIST OF TABLES

Table		Page
I.	NOSCAR potential barriers to clinical practice for NOTES	16
II.	Injury assessment following transgastric NOTES access.....	41
III.	Efficiency indicators for NOTES access	43
IV.	Complications of unsafe access performed through the gastric antrum ..	65
V.	Complications of unsafe access through the posterior stomach wall	66
VI.	Complications of unsafe access through the rectum.....	69
VII.	Complications of safe access through the rectum.....	70
VIII.	Total closure time	91
IX.	Evidence of gastrotomy leak by contrast fluoroscopy	92
X.	Failure to thrive following gastrotomy closure.....	94
XI.	Evidence of infection and injury following gastrotomy closure.....	96
XII.	Gastric burst pressure following gastrotomy closure.....	98

LIST OF FIGURES

Figure	Page
1. Natural orifice transluminal endoscopic surgery	7
2. Laparoscopic view of NOTES via transgastric access in the human.....	9
3. Modern Endoscope	28
4. Anterior transgastric access techniques	35
5. Total time required for peritoneal access.....	45
6. Effect of wire-guided or dimple.....	46
7. Effect of single device or overtube	47
8. Effect of PEG-guided or no cautery.....	48
9. EUS image of a safe access site.....	62
10. EUS image of an unsafe access site.....	63
11. Bladder puncture following an unsafe transrectal access site.....	67
12. TPD, tissue plicating device	77
13. TAS, tissue apposition system	78
14. FESD, flexible endoscopic suturing device.....	80
15. Clip/loop closure.....	81
16. TNF- α levels in peritoneal fluid	115
17. IL-1 β levels in peritoneal fluid	117
18. IL-6 levels in peritoneal fluid	118
19. CRP levels in serum.....	120
20. Cortisol levels in serum	121

I

INTRODUCTION TO NATURAL ORIFICE SURGERY

NOTES, or Natural Orifice Translumenal Endoscopic Surgery, promises an incisionless abdominal surgery, leaving the patient with no visible scars. Intentionally creating a hole in the stomach and performing an abdominal surgery using instruments passed through the mouth, is becoming a reality. NOTES is, to healthcare professionals, a revolutionary concept in minimally invasive surgery, propelling a wave of excitement, research and technological developments. To the lay person, the idea of no visible scars, fewer complications, less pain and a shorter hospital stay is welcome news, once they accept the apparent paradox that their appendix will be removed through their mouth. To properly understand and appreciate the concept, benefits and necessity of NOTES, it is important to begin with a brief explanation of its evolution.

1.1 Surgery: A Historical Perspective

According to The History of Surgery by Harold Ellis, the first surgical procedures are believed to have been performed as early as about 10,000 to 6,000 BC. Egyptian carvings dating back to 2500 BC depict surgical procedures including circumcision,

castration and amputation. Ancient Egyptian medical texts provide instructions for surgical procedures including repair of a broken bone and treatment of a serious wound (Ellis 2002).

The Middle Ages (5th century to 14th century AD) saw a decline in surgical practices as they were regarded as inferior to medical approaches. Surgical procedures were primarily conducted by barbers who traveled from town to town cutting hair, removing tumors, pulling teeth and stitching wounds (Ellis 2002). In 1316 the French surgeon Guy de Chauliac published *Chirurgia magna* (Great Surgery). This massive text describes how to remove growths, repair hernias (protrusion of an organ through surrounding structures) and treat fractures using slings and weights (Ellis 2002). During the 16th, 17th and 18th centuries, a better understanding of the human body and the study of anatomy allowed surgery to become a more accurate science. For example, the English physician and anatomist William Harvey discovered the process of blood circulation and Italian anatomist Marcello Malpighi identified the existence of tiny blood vessels called capillaries that carry blood from the major blood vessels to the cells of the body. John Hunter, a British anatomist and surgeon, stressed the close relationship between medicine and surgery and performed many experimental operations that advanced the practice of surgery (Ellis 2002).

Most surgery, however, continued to be restricted to less critical areas of the body or to operations that did not penetrate the skin too deeply. Surgeons rarely opened the abdomen, chest, or skull because of the pain it caused the patient and the risk of infection. This changed in 1846 when ether was used as a way to mask pain during surgery by American dentist William Morton and the use of anesthesia was born (Ellis 2002).

Post-surgical infections remained a serious complication of surgery until the mid-19th century when the French chemist Louis Pasteur discovered that fermentation or putrefaction, the decay and death of body tissue, is caused by bacteria in the air. In 1865 the British surgeon Joseph Lister applied Pasteur's work to surgery, developing antiseptic techniques to kill germs in the operating room before surgery, greatly reducing postoperative infection. Other physicians, including Austrian Ignaz Semmelweiss and American Oliver Wendell Holmes, determined that bacteria are also carried on the hands and clothing and transferred from patient to patient. These physicians pioneered techniques such as hand washing and changing into clean clothing before surgery to prevent wounds from being contaminated during surgery (Ellis 2002).

In the late 1800s, having reduced the problems of pain and infection, surgeons began performing new types of surgery including procedures on the abdomen, brain, and spinal cord. When the German physicist Wilhelm Conrad Roentgen discovered X rays in 1895 to "photograph" the inside of the body, he changed the way surgery was performed. At the turn of the 20th century, improved diagnostic abilities and methods of treatment helped surgery become even more effective. The introduction of antibiotics in the 1940s further minimized the risk of postoperative infection. More recent technological advances permitted surgeons to perform increasingly complex and difficult operations (Ellis 2002).

1.2 Minimally Invasive Surgery: Minimizing the injury caused by the treatment

By the late 20th century, anesthesia, antiseptics and antibiotics had brought great improvements to surgical outcomes, allowing a shift from the origins of surgery as a desperate attempt to save lives, to a specialty in which non life-threatening procedures were performed to improve a patient's quality of life. Nuclear medicine continued to expand on its non-invasive imaging capabilities with the advent of magnetic resonance imaging (MRI) and computed tomography (CT) scans making possible the diagnosis of soft tissue injury (Mervis et al. 1989).

Likewise, around this time, surgeons adopted a new goal; to reduce the injury caused by the treatment. The use of small cameras and light sources enabled surgeons to reduce the size of incisions and repair damaged tissue or organs using instruments inserted through numerous small ports rather than inserting their hands through a single large incision (Himal 2002). Even complex cardiac procedures are now frequently performed through minimally invasive techniques. While years ago the only option to restore blood flow through a blocked artery of the heart was to open the patient's chest and reroute the vessels, today stents or balloons can be inserted through a peripheral vessel and guided to the blocked artery, restoring flow to the heart while leaving just a small incision in a peripheral limb (Park 1999). Arthroscopy allows orthopedists to employ narrow instruments and a small camera to repair damaged bones and joints, leaving behind only a small scar compared to the scar left just decades ago. Laparoscopy is the similar use of narrow instruments and a video camera to perform abdominal surgery through smaller incisions. Likewise, endoscopy uses a long flexible scope to explore the gastrointestinal tract, entering through the mouth or anus. These less-invasive

surgical approaches reduce the incision size and have proved to be beneficial to the patient but are not without complications.

1.3 Natural Orifice Surgery

Access to the abdominal cavity is required for diagnostic and therapeutic endeavors for an array of medical and surgical diseases. Historically, abdominal access has required a formal laparotomy to provide adequate exposure of the target tissue and to provide working space for the surgeon's hands and instruments. Laparoscopy introduced the concept of video-assisted surgery, allowing the surgeon's hands to remain outside the body cavity while manipulating instruments passed through much smaller incisions. With the advent of minimally invasive techniques, coupled with recent technological and engineering advances, morbidity associated with access-related incisions has significantly decreased (Duepre et al. 2003).

While minimally invasive techniques are beneficial to most patients, laparoscopy is still susceptible to complications related to skin incisions, such as wound infections, hernias, and pain. Moreover, several incisions are often required to accommodate additional working ports to assist with laparoscopic manipulation of abdominal tissues. Recently, several groups have begun evaluating the feasibility of performing abdominal surgeries without external incisions. These pioneering investigators gain access to the abdominal cavity through various natural orifices such as the mouth, anus, urethra and vagina.

Natural orifice transluminal endoscopic surgery (NOTES) is an emerging experimental alternative to conventional surgery that eliminates abdominal incisions and

incision-related complications by combining endoscopic and laparoscopic techniques to diagnose and treat abdominal pathology (Rattner and Kalloo 2006). During NOTES, commercially available flexible video endoscopes are used to create a controlled transvisceral incision via natural orifice access to enter the peritoneal cavity (Figure 1).

Once the endoscope is passed into the peritoneal cavity, endoscopic devices are advanced through the endoscope's working channels, allowing manipulation of abdominal tissues. Theoretically, any abdominal procedure currently conducted laparoscopically could be performed via NOTES. At the completion of the procedure, the point of peritoneal access is closed using endoscopic devices and the scope is withdrawn from the natural orifice, obviating the need for abdominal wall incisions.

1.4 The Origin of NOTES

NOTES has evolved from more than two centuries of technological innovations and continued growth in the field of endoscopy. As technological improvements enabled innovative endoscopists to experiment with improved endoscopic equipment, the hybridized field of *surgical endoscopy* arose at the intersection of more invasive endoscopy and lesser invasive surgery.

During the past 50 years, pioneering surgical endoscopists have slowly developed means to surpass the constraints of the gastrointestinal lumen by using a flexible endoscope. For instance, endoscopic ultrasound and biopsy (Strohm et al. 1980) now enables skilled endoscopists to perform splenic (Fritscher-Ravens et al. 2003), adrenal (Eloubeidi et al. 2004), hepatic (Hollerbach et al. 2003) and pancreatic biopsies (Wegener et al. 1995) through the stomach and duodenal walls, enabling pinpoint access and

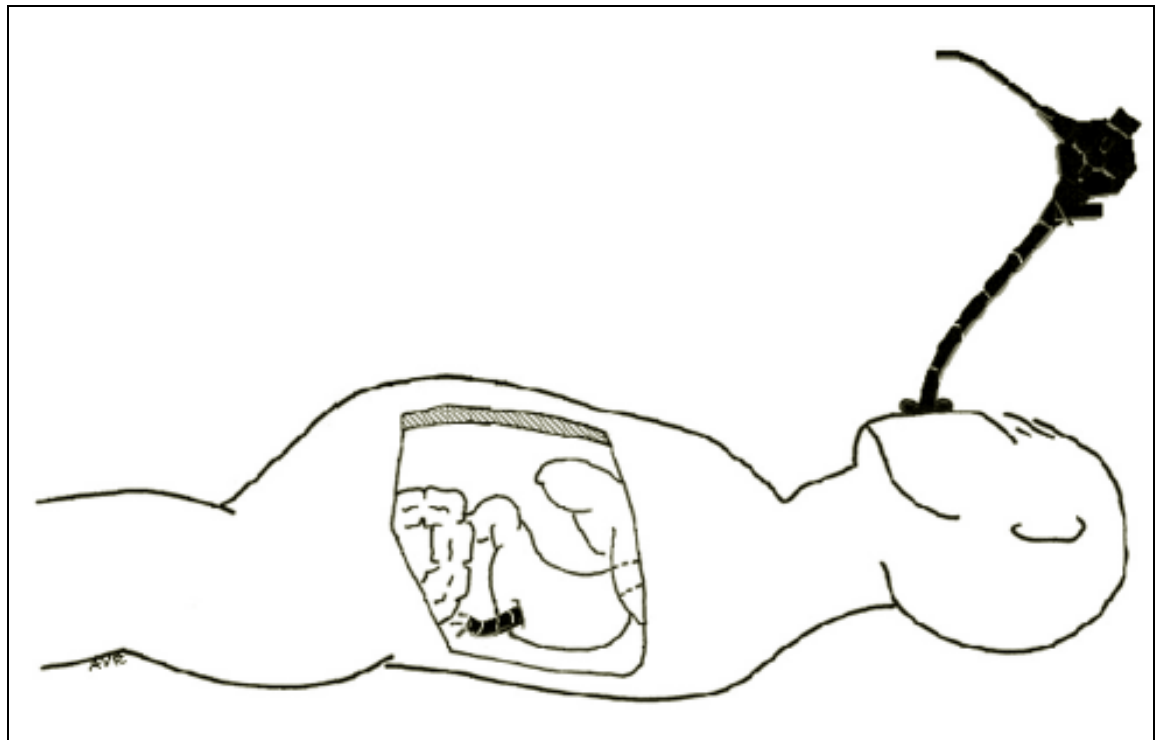


Figure 1. Natural orifice transluminal endoscopic surgery through a controlled gastrotomy.

breaching the domain of traditional intraluminal endoscopy. Endoscopic ultrasound-guided fine-needle aspiration of the pancreas has become a popular means of diagnosing pancreatic cancer, while transgastric pancreatic pseudocyst drainage has evolved into the first-line standard of care for uncomplicated pancreatic pseudocyst management (Fazel 2005, Kozarek et al. 1985). Although each of these procedures requires merely a pinpoint-sized hole, occasionally, endoscopic polypectomies have afforded endoscopists an opportunity to view the peritoneal cavity in NOTES fashion, through unintentional full-thickness resections.

Other examples of the use of flexible endoscopy for surgical procedures include cases of transcolonic treatment of acute appendicitis (Said et al. 1995) as well as colonoscopic resection of appendiceal remnants have been reported (Enander and Gustavsson 1979). Surgical endoscopists have also employed sterile endoscopes passed through abdominal wall incisions as laparoscopes (Sanowski et al. 1981) and through posterior lumbar incisions to perform pancreatic debridements (Castellanos et al. 2005). Endoscopic mucosal resection, which is currently used for mucosal lesions of the esophagus, stomach, and colon, further extends the domain of endoscopy, even if by only one layer of the gastrointestinal tract (Conio et al. 2006). The percutaneous endoscopic gastrostomy (PEG) tube (Gauderer et al. 1980, Ponsky and Gauderer 1981) represents the first report of breaching the lumen of the hollow abdominal viscera and extending through the serosa with the assistance of endoscopy.

Based on the work of these pioneering surgeons who have expanded the current applications of surgical endoscopy, several researchers have now devised incisionless



Figure 2. Laparoscopic view of natural orifice transluminal endoscopic surgery via transgastric access in the human.

access points to the abdominal cavity using novel flexible endoscopic techniques (Figure 2) opening the door to the potentially limitless possibilities of surgical procedures which can be performed in lieu of skin incisions.

1.5 The Benefits of NOTES

NOTES may facilitate surgery in the abdomen without incisions, thereby potentially avoiding incision-related complications. Although complications may develop at any point during the surgical procedure, significant morbidity is encountered while providing exposure to the target organ (Oshinsky and Smith 1992, McKernan and Champion 1995). Common post-operative incision-related complications such as wound infections, incisional hernias, and adhesions could be minimized or eliminated by NOTES.

Surgical site infections (SSIs) are the most common surgical complications. Wound infections develop in 2% to 25% of patients, depending on the type of surgery performed (Bratzler and Houck 2005, DiPiro et al. 1998). In the United States, wound infections increase costs of hospitalization by more than \$3000 per patient (Kirkland et al. 1999). A litany of risk factors is associated with developing an SSI (Buggy 2000), with bacterial load being the single most important factor (Cruse and Foord 1980). Since the most common bacterial source responsible for SSI is the skin (Nichols 2001), the absence of skin incisions in NOTES may eliminate SSI from abdominal surgeries.

Hernia formation is the second most common complication of abdominal surgery, developing in 4% to 18% of open incisions (Mingoli et al. 1999, O'Dwyer and Courtney 2003, Carlson et al. 1995) and 0.02% to 3% of laparoscopic incisions (Montz et al. 1994, Nezhat et al. 1997, Bowrey et al. 2001). Hernias can progress to catastrophic

strangulation of the entrapped organs, followed by death. In the United States, more than 100,000 patients annually will develop incisional hernias from laparotomy and require subsequent surgery (Millikan 2003). When compared to laparotomy, laparoscopic procedures have decreased rates of incisional hernia; however, up to 5% of patients undergoing laparoscopic bariatric procedures may develop trocar site hernias (Puzziferri et al. 2006). By avoiding external incisions that create a defect in the abdominal wall, the internal access afforded by NOTES may completely eradicate the incisional hernia as a postoperative complication.

Adhesions are another common complication that develops in greater than 90% of postoperative patients undergoing abdominal surgery (Menzies and Ellis 1990). Adhesions can cause small bowel obstruction (SBO), with open abdominal surgery conveying a subsequent 1% lifetime risk of developing SBO. The extent of adhesive disease is thought to be correlated with the size of the surgical incision. Studies have shown that adhesive disease most commonly involves the abdominal wall incision (Menzies and Ellis 1990). In 2003, Duepre and colleagues reported lower rates of SBO following laparoscopy when compared to laparotomy (1.9% vs. 6.1%) (Duepre et al. 2003). The authors implied that smaller incisions, when compared to larger incisions, are a mitigating factor responsible for the decreased incidence of SBO. Intestinal obstruction from adhesive disease, affecting as many as 1% of surgical patients, is a common indication for re-operation. In 1996, the United States Centers for Disease Control reported 474,000 operations for lysis of adhesions (National Center for Health Statistics 1996). Older studies reveal that \$1.1 billion was spent on lysis of adhesions, with an average hospital stay exceeding 11 days (Ray et al. 1988). NOTES may further reduce

the extent of intestinal adhesive disease following surgery, by virtue of a smaller, solitary, directed point of abdominal access coupled with elimination of the anterior abdominal wall incision, the most common nidus for adhesion formation.

Since most pain associated with abdominal surgery is due to the cutting of muscle, NOTES may also reduce the pain accompanying a procedure. Pain related to external incisions would be eliminated, which may benefit postoperative recovery times, reduce hospitalization rates, and subsequently decrease inpatient complications such as nosocomial infection, deep venous thrombosis, and pulmonary embolism.

In addition to potentially limiting the complications of traditional surgery, NOTES may expand the library of surgical indications and enlarge the role of surgery to incorporate disease processes that are otherwise technically not feasible with available surgical techniques. A specific population that may benefit from a NOTES inside-out approach is the morbidly obese, where special instrumentation is required to span the breadth of the abdominal wall. NOTES, coupled with EUS technology, may also introduce a unique approach for abdominal surgery in pregnant women, avoiding the potential injuries related to visceral displacement caused by the gravid uterus. One particularly unique population that may be well served by NOTES is children. Since surgeries in children convey nearly a lifetime risk of incision-related complications such as adhesive disease, abdominal scarring, and aesthetic disdain, NOTES may offer a novel approach for common pediatric surgical diseases while concomitantly reducing the long-term sequelae of abdominal surgery.

Additional prospective benefits derived from an incisionless technique are minimization of anesthesia and analgesia and decreased patient apprehension during

elective surgeries. Procedures which now require hospital admission and overnight stays because of concerns related to general anesthesia may be converted to outpatient procedures conducted under conscious sedation, as is done routinely for endoscopic procedures including upper gastrointestinal procedures.

1.6 The Feasibility of NOTES

The first reports of natural orifice endoscopic surgery hail from outside the United States. In Germany, Seifert described the first documented cases of NOTES in 2000, in which a posterior gastrotomy was created with an endoscopic needle-knife, enabling retroperitoneal passage of the endoscope to perform a pancreatic necrosectomy in three patients too ill to tolerate formal debridement (Seifert et al. 2000). Within a few years of Seifert's report, Reddy and Rao from India further validated the feasibility of NOTES by performing an appendectomy in a human (Hochberger and Lamade 2005). These seminal case reports of NOTES prompted several American groups to cautiously evaluate the revolutionary concept of natural orifice surgery in various animal models as described in the following paragraph.

Kaloo and colleagues demonstrated feasibility of NOTES access to the peritoneal cavity (Kaloo et al. 2001). In a series of six swine, transgastric access was achieved, peritinoscopy and liver biopsies were performed and the gastrotomy was closed using endoscopic clips. One year later, Kaloo and his group evaluated the feasibility of NOTES during pelvic and gynecologic procedures. Six swine successfully underwent NOTES tubal ligation and survived 2 or 3 weeks (Jagannath et al. 2005a). Wagh and colleagues have verified these findings, after successfully demonstrating NOTES

hysterectomy in surviving pig models (Wagh et al. 2005). The feasibility of NOTES splenectomy was established in a series of six acute pigs in early 2006 (Kantsevov et al. 2006). These early reports demonstrating the feasibility of potential NOTES applications also exposed the limitations of the existing technology.

In collaboration with engineers and surgical consultants, many investigators have focused on specialized device development to assist with NOTES. Kalloo et al. in collaboration with Olympus, have reported on a prototypical endoscopic suturing device that enables curved-needle control and intracorporeal knot tying to facilitate plication of gastrointestinal tissues (Hu et al. 2005a), ligation of bleeding vessels (Hu et al. 2005b), and creation of a gastrojejunal anastomosis in swine models (Kantsevov et al. 2005). Park and colleagues have independently demonstrated safe gastrotomy closure with another prototypical suturing device in six chronic pigs and the feasibility of NOTES cholecystectomy or cholecystogastrostomy in acute studies (Park et al. 2005). Swanstrom and colleagues have developed a novel NOTES device that enables the midportion of a flexible endoscope to lock into place, while still allowing freedom of the scope tip to maneuver and perform procedures. In addition to the selectively rigid endoscope guides, a prototype NOTES 18 mm endoscope was used that incorporated a 4 mm video scope and two 5.5 mm accessory ports that accepted endoscopic grasping devices with triangulation capabilities (Swanstrom et al. 2005). These early and cursory reports helped these investigators to recognize the trials and tribulations of NOTES, and resulted in a unified plan to probe the potential of this novel surgical approach in a systematic fashion.

1.7 Barriers to NOTES

Although the promise of NOTES is electrifying to surgeons and endoscopists, as with any new technology, several key issues need to be resolved prior to the incorporation of NOTES into routine clinical practice (Ponsky 2005). Recognizing this need, leaders from the Society of American Gastrointestinal and Endoscopic Surgeons (SAGES) and the American Society of Gastrointestinal Endoscopy (ASGE), formed a consortium cleverly dubbed the Natural Orifice Surgery Consortium for Assessment and Research (NOSCAR). During this “think tank” session of prominent surgical endoscopists, they established taxonomy, delineated the current limitations to NOTES, and produced a unified plan of research to propel NOTES into human practice. (Table 1) (Rattner and Kalloo 2006). Important barriers identified included developing endoscopic techniques for accessing the peritoneal cavity and for closing the gastric defect. To accommodate this, they proposed the potential need for technological developments such as suturing devices and possibly an entirely new surgical platform to facilitate NOTES procedures. Furthermore, they recognized that this unique inside-out approach may introduce untoward physiological ramifications such as increased risk of infection. Their seminal white paper will allow scientists, physicians, and engineers alike to grapple with these numerous limiting aspects of NOTES for many years to come.

1.8 The Current Status of NOTES

The excitement, potential and curiosity emanating from the field of natural orifice surgery has flourished over the past several years and an increasing number of studies are

Table 1. NOSCAR Potential Barriers to Clinical Practice for NOTES

Access to peritoneal cavity
Gastric (intestinal closure)
Prevention of infection
Development of suturing device
Development of anastomotic (nonsuturing) device
Spatial orientation
Development of a multitasking platform to accomplish procedures
Control of intraperitoneal hemorrhage
Management of iatrogenic intraperitoneal infections
Physiologic untoward events
Compression syndromes
Training other providers

Rattner and Kalloo 2006

currently being published. NOTES remains a challenging endeavor when limited to the armamentarium of contemporary endoscopic equipment. All components of a natural orifice approach to surgery, including access, procedure and closure, are affected by the technical limitations of the current instrumentation. Manipulating organs, tissue, and surgical adjuncts such as suture and biomaterials requires articulation and triangulation of instruments independent of endoscopic movement. Most commercially available endoscopic devices used to manipulate tissue are dependent on endoscopic movement for proper positioning and have limited degrees of freedom. Movement of the instruments within the scope is limited because they must be inserted through a channel in the endoscope, constraining the plane of end effector movement to the long axis of the endoscope shaft and resulting in very limited articulation independent of the scope tip.

The inability to apply traction, lack of multiplanar instrumentation and working channels that limit the size of instruments are other obstacles. At least two groups are evaluating the use of magnets as a means to apply countertraction (Ryou and Thompson 2009, Raman et al. 2009). Others are developing miniature robots to be deployed into the peritoneal cavity through a NOTES approach. The robot's arms can be fitted with a variety of end-effectors and is operated remotely (Lehman et al. 2009). Prior to widespread acceptance by surgeons, future NOTES devices will require improvements in articulation capabilities, reflecting the same evolution that laparoscopic equipment has undergone during the past decade. Unfortunately, the success of NOTES depends on the development of better instrumentation, but commercial investment in new technology is dependent on the probable success of NOTES. Initial NOTES applications are limited by this paradox.

Most of the recent reports of NOTES continue to be procedure-based investigations demonstrating the feasibility of pure NOTES in an animal model, expanding the potential surgical applications. Onders used a transgastric approach in a pig model and demonstrated the feasibility of abdominal exploration for ICU patients (Onders et al. 2007c). Also using a porcine model, Onders further demonstrated the potential of NOTES for diaphragmatic mapping and placement of pacing wires applicable to ALS (amyotrophic lateral sclerosis) patients (Onders et al. 2007a). Miedema reported on the feasibility of transgastric NOTES for placement of mesh used for hernia repair in a survival study in pigs (Miedema et al. 2009). A transvaginal approach was used by Perretta to demonstrate the success of adrenalectomy via NOTES (Perretta et al. 2009). Freeman, in a group of dogs, successfully completed transgastric NOTES oophorectomy (Freeman et al. 2009). Also in a canine model, Sherwinter proposed the utility of NOTES for inguinal hernia repair (Sherwinter and Eckstein 2009) while Lomanto used pigs to demonstrate feasibility of abdominal wall hernia repair via a transvaginal route (Lomanto et al. 2009). A porcine model was used by Zacharopoulou to demonstrate retroperitoneal exploration feasibility by NOTES (Zacharopoulou et al. 2009). Nephrectomy was successfully completed in two pigs through a transvaginal approach by Raman (Raman et al. 2009). Leroy, in five minipigs, reports on successful sigmoidectomy using transgastric NOTES (Leroy et al. 2009). Cahill demonstrated the use of NOTES for sentinel node biopsy in six pigs via transvaginal access (Cahill et al. 2009). Despite the technical challenges, NOTES has been demonstrated, in animal models, to be feasible for a wide range of abdominal procedures.

Due to technical limitations of pure NOTES, the use in human cases has been restricted to procedures employing laparoscopic ports to assist the NOTES approach. These “hybrid” techniques have been utilized for a wide range of applications. Three groups report the use of a transvaginal NOTES approach accompanied by laparoscopic ports to successfully complete cholecystectomy in human patients (Noguera et al. 2009, Navarra et al. 2009, Palanivelu et al. 2009). Noguera performed the procedure on 15 patients using two additional ports; one 5 mm and one 3 mm port for instrument insertion (Noguera et al. 2009). Navarra and colleagues reports six successful cases using a single accessory 5 mm port and several transabdominal stay sutures for retraction (Navarra et al. 2009). The third group using a transvaginal approach for cholecystectomy in humans, reports eight cases. Of these, two cases were converted to laparoscopy due to technical difficulties. The remaining six cases were completed successfully using a 3 mm laparoscopic port in just under 150 minutes (Palanivelu et al. 2009), attesting to the technical difficulties still accompanying NOTES procedures. Auyang and colleagues reports performing cholecystectomies in four patients without operative complications using a transgastric approach and a single laparoscopic port (Auyang et al. 2009). For the treatment of gastric cancer, Abe reports of successful full-thickness mucosal resection with laparoscopic assistance in a group of four patients (Abe et al. 2009). Burghardt reports the first case in which a right hemicolectomy was performed via NOTES with a single 5 mm laparoscopic port for assistance (Burghardt et al. 2008). A combined technique of NOTES and laparoscopy was also used in cadavers to demonstrate the feasibility of gastric bypass surgery (Hagen et al. 2008). Four procedures were completed using a transvaginal NOTES approach and transumbilical ports with operative

times ranging from 6-9 hours. Perhaps, rather than a limitation of NOTES, these hybrid procedures should be interpreted as an advancement to laparoscopy. These surgeons recognized that natural orifice access to the peritoneal cavity can be used as an additional or secondary access point, rather than an exclusive access point. The number of conventional laparoscopic incisions is reduced, minimizing the injury caused by the treatment. Hybrid surgical approaches combining the novel concept of NOTES with the standard of minimally invasive surgery offers a whole new surgical paradigm which may help to propel NOTES into clinical practice.

Determining which surgical procedures would benefit from a NOTES approach is arguably the most important consideration at this time. Without an application for this approach, overcoming the other barriers is irrelevant. To date, studies have focused on the feasibility of performing common diagnostic and therapeutic procedures via NOTES, not yet addressing the benefits. As NOTES moves forward, more attention will need to be given to which procedures *should* be done, not just which procedures *can* be done.

Recognizing this, several groups have focused less on the feasibility of a specific application of NOTES and have turned their attention to investigating the *approach* of NOTES. As defined by the NOSCAR group in their white paper (Rattner and Kalloo 2006), important barriers to NOTES implementation include a better evaluation of access and closure techniques and investigating the physiological ramifications such as bacterial burden and inflammatory consequences.

Viscerotomy creation, which enables the endoscope to pass from the lumen of hollow viscera to the abdominal cavity, is inevitably the first step in NOTES. The mouth, anus, vagina and urethra are all potential orifices for NOTES procedures. One study

evaluated endoscopic visibility and time required to access abdominal organs from a transgastric or transcolonic approach and concluded that there were no discernable differences (Kim et al. 2008). Transgastric access techniques have been briefly described using needle-knife, sphincterotome, and balloon dilatation methods (Rosen et al. 2006). However, in the absence of studies comparing access techniques, no conclusive evidence establishes the optimal technique. For investigators using gastrotomy to gain NOTES access, the gastrotomy site within the stomach can have a profound effect on the technical feasibility of the subsequent abdominal surgery. Developing a safe, reliable and efficient access point that allows easy manipulation of target organs requires further investigation.

Ensuring adequate closure of the viscerotomy is also of paramount importance and is regarded by many as the largest hurdle between animal studies and eventual human clinical use. Contemporary closure techniques described include endoscopic suturing (Kantsevov et al. 2005, Park et al. 2005), tissue opposition or clipping (Kalloo et al. 2004, Kratt et al. 2008, Hookey et al. 2009), tissue placcation (McGee et al. 2008a), transmural sutures (von Renteln et al. 2009) and management of the gastrotomy with a PEG tube (Marks et al. 2006, McGee et al. 2008b). Of direct clinical relevance, PEG tube management may facilitate bedside intraperitoneal access in critically ill patients requiring intra-abdominal exploration who concomitantly require long-term feeding access (Marks et al. 2006, Onders et al. 2007b). Few animal studies have described the efficacy of viscerotomy closure in chronic studies. Since even low leak rates of 1% to 2% of transvisceral closures are unacceptable when compared to the established safety of contemporary laparoscopic techniques for minimally invasive abdominal surgery,

ensuring superior closure technique during NOTES will be the lynchpin responsible for the success or failure of the budding surgical paradigm (Rattner and Kalloo 2006). Continued evaluation of existing and newly evolving viscerotomy closure technologies is essential.

The physiological ramifications of NOTES are numerous and worthy of extensive investigation. This unique, inside-out approach to surgery may induce physiological consequences leading to unexpected morbidity despite the apparently less-invasive approach. Intra-peritoneal endoscopic procedures will employ endoscope-driven pneumoperitoneum. While the pathology of high intraperitoneal pressures has been investigated and is understood (Decker 2001), modern endoscopes do not afford the ability to monitor or control intraperitoneal pressure. McGee and colleagues validated the use of the instrument channel on a standard endoscope for monitoring endoscopic pressure, allowing pure NOTES procedures to be investigated until new technology is developed (McGee et al. 2007). Another option used by many groups is to control and monitor pneumoperitoneum using a standard laparoscopic insufflator and Veress needle, as is done in many hybrid procedures (Sánchez-Margallo et al. 2008, Crouzet et al. 2008, Palanivelu et al. 2008).

Another physiological concern of NOTES is sterility. Since no natural orifice is sterile, alimentary contaminants may be catastrophic to the sterile peritoneal cavity, similar to gastric or colonic leakage during conventional surgery. Infectious consequences and means of bacterial reduction need to be studied and validated with quantitative bacteriologic studies. Three studies, to date, have reported on the bacteriologic burden of NOTES procedures. McGee and colleagues investigated the

infectious implications in a 14 day chronic porcine model following transgastric NOTES with PEG tube management for closure (McGee et al. 2008b). The authors report that all animals thrived. Although 61% of the animals reportedly had evidence of infection upon necropsy, there were no clinical consequences apparent at that time. Also in a porcine model, Bachman describes two methods of reducing colonic bacterial burden (Bachman et al. 2009). Despite significant reduction in colonic contamination, the peritoneal cavity was still contaminated following transcolonic NOTES. In the only study reporting on bacterial contamination in human NOTES-related cases, Narula measured the bacteria levels in the peritoneal cavity before and after transgastric access in ten patients (Narula et al. 2008). Similar to the animal studies, despite finding an increase in peritoneal bacterial contamination following the NOTES procedure, there was not a clinically significant response. These few bacteriological studies are not adequate to draw conclusions about the infectious consequences surrounding natural orifice procedures and additional investigation is clearly warranted.

Moreover, eliminating skin incisions does not eliminate the internal tissue destruction and accompanying inflammatory response. McGee (McGee et al. 2008c) and Bingener (Bingener et al. 2009) performed similar studies in a survival porcine model. Each group measured serum cytokine levels, as markers of inflammation, following NOTES and compared the levels to conventional laparoscopic surgery. Neither group found marked discrepancies between the two approaches. These two studies, alone, however, do not provide adequate information to draw strong conclusions regarding the inflammatory consequences of NOTES. The unknown role of immunologic

inflammatory mediators merits future scientific scrutiny to further characterize the physiologic effects of NOTES.

1.9 Specific Aims and Hypotheses

Despite a growing body of work, relatively few investigations have been conducted to address the many questions surrounding the utility of a natural orifice approach to abdominal surgery. The studies conducted to date are, as would be expected early in the adoption of a new surgical paradigm, preliminary and largely focused on the feasibility of NOTES procedures. For this project, three important barriers to NOTES were identified which required investigation in much greater detail and with more scientific rigor. The three specific aims of this project are:

- 1) to evaluate different NOTES access techniques, driven by the hypothesis that differences in access methodology will significantly affect the safety and efficacy of access for natural orifice surgery
- 2) to investigate conventional closure methodologies for transgastric NOTES, with the hypothesis that different closure devices will significantly affect the safety and efficacy of gastrotomy closure for natural orifice surgery
- 3) to compare the inflammatory response induced by NOTES with that induced by conventional surgical approaches, testing the hypothesis that natural orifice surgery causes no greater of an inflammatory response than laparoscopic surgery.

II

ACCESS TO THE PERITONEAL CAVITY VIA NOTES

2.1 Background

Natural Orifice Transluminal Endoscopic Surgery (NOTES) is conceptually feasible. But is it technically practical? A paradigm shift in endoscopy from a less-invasive diagnostic tool to a full-fledged invasive surgical platform will require a giant step in technology. Devices and instruments specifically designed for NOTES procedures do not yet exist. Devices that do exist for endoscopy may not be capable of achieving the goals of NOTES.

NOTES, like any surgery, can be divided into three key sequential steps, access, surgical procedure and closure. Access to the peritoneal cavity is routinely achieved through an open laparotomy or through less-invasive laparoscopic ports. NOTES access will require crossing the endoluminal surface of the gastrointestinal tract to enter the peritoneal cavity. Procedures performed through NOTES may vary widely or may be limited to a few specific applications. It is too early to tell. Closure of the viscerotomy will be necessary to ensure proper healing and prevent leakage of gastrointestinal

contents into the sterile peritoneal cavity. Each of the three steps has unique and limiting technical challenges which will be explored further.

Many of the surgical barriers of NOTES are due to the limitations of the available instruments. Although the field of surgical endoscopy has emerged from the intersection of more invasive endoscopy and lesser invasive surgery, as previously mentioned, venturing further into the realm of NOTES will likely require unique instrumentation. At this time, however, those instruments are not available and in order to investigate NOTES as a potential surgical approach, it is necessary to utilize the tools that are currently available.

2.1.1 Endoscopic Instruments

The Endoscope

Paramount to the birth of NOTES was the creation of the contemporary endoscope, which has provided the substratum on which the fields of gastroenterology and surgical endoscopy were predicated. The endoscope, which is currently used to perform NOTES, is the product of several iterations of technological development during the past century. Bozzini is credited with inventing the first endoscope, the Lichtleiter, in 1805 (Cappell et al. 2000, Bush et al. 1974, Rathert et al. 1974). This rigid, candle-powered endoscope was initially used to perform cystoscopy (Bush et al. 1974). Nearly 60 years would pass before Kussmaul's modifications to the Lichtleiter resulted in a device suitable to perform gastroscopy in sword swallows in 1868 (Modlin et al. 2004). Von Mikulicz later added electric light to the endoscope in 1880, enabling improved visualization of luminal contents (Modlin et al. 2004). Although the principles of fiber optics were demonstrated

in the late 1800s, nearly a half-century would pass before Hirschowitz unveiled the first fiber optic, fully flexible fiberoptic in 1957, which became commercially available three years later (Hirschowitz 1961). In 1963, an external light source and instrument channels were added to the flexible gastroscope. By the 1980s, video cameras replaced fiber optics, and the contemporary endoscope was born.

The modern endoscope (Figure 3) consists of a flexible tube varying in length from approximately 100 cm for gastroscopes to 200 cm for enteroscopes. The diameter of the scope can also vary depending on its application, but it is generally approximately 1.4cm for gastroscopes. The tip of the scope has a forward viewing camera and adjustable light source. Typically, scopes have one or two working channels capable of delivering endoscopic devices or instruments. The scope also has a channel for delivery of air and for aspiration to inflate and deflate the working space as needed for visualization.

The handle of the scope contains the controls for manipulation of the tip of the scope in two planes; up and down, left and right. Forward and backward movements are made by advancing the length of scope. The handle also contains entry into the working channels and controls for air and aspiration.

Two important considerations limit the modern endoscope as an instrument capable of complex surgical maneuvers. First, while the tip of the scope can be articulated, the greater length of the scope cannot. The distal portion of the scope can be angulated to view objects in the surrounding area, however, the length of the scope cannot necessarily be moved in that direction. In other words, if the scope is flexed in a J-shape, advancing the scope moves the tip further from the direction it is viewing, rather than closer to it. Second, the camera and instruments are arranged in-line. Unlike traditional or

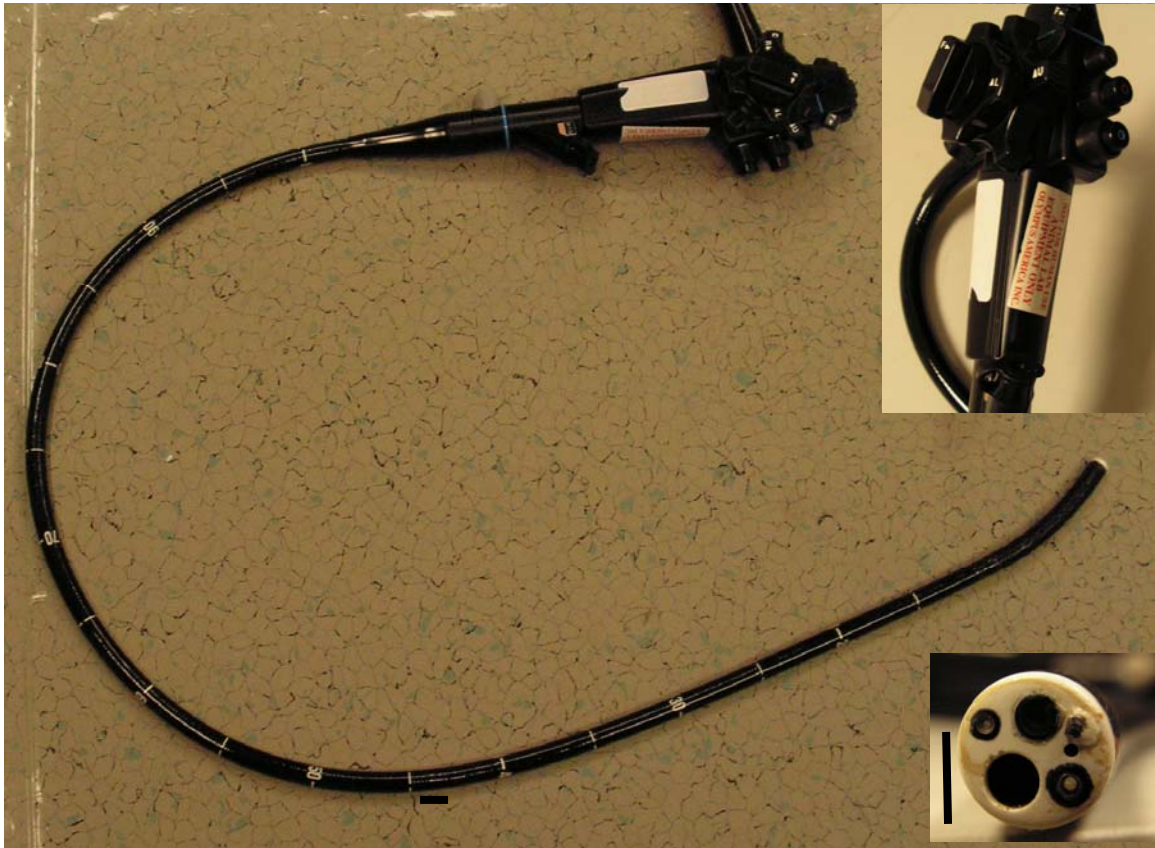


Figure 3. Modern Flexible Endoscope. Insert A, Endoscopic handle with controls and instrument channels. Insert B, Tip of scope showing channels, light, camera and air channel, Scale bar = 1.0 cm

laparoscopic surgery, the image and instruments are situated close together. It is a common principle when working with our hands to triangulate the hands with the eyes. Whether this is for surgery or even tying our shoes, our hands approach from opposite sides and our eyes view from a third direction. The endoscopic view and tools are in the same plane. Both of these features greatly restrict the capabilities of the endoscope for NOTES procedures.

Endoscopic Devices

A myriad of endoscopic devices exist and many are uniquely designed for highly specific procedures. Just as scissors and clamps are essential to open or laparoscopic surgery, these devices also exist in an endoscopic form. Common endoscopic devices also include wires for guiding, snares for grasping, balloons for dilating, nets for retrieving and needles for injecting. As in other surgical approaches, many endoscopic devices can be connected to electrocautery for cutting tissue or controlling hemostasis.

Importantly, however, endoscopic devices, due to the nature of the endoscope and the working distances are inherently long, narrow and delicate, and thus are not capable of purchasing the same amount of tissue or of generating the same force as standard instruments. In these early phases of NOTES incorporation, this limited selection and limited capability of devices contributes to the surgical barriers which must be overcome.

2.1.2 Choosing an Access Site

Potential transluminal access sites include the stomach via the mouth, the rectum or colon via the anus, the vagina or cervix via the vaginal opening, and the urethra or urinary bladder via the urethral opening. Each of these orifices or access routes has its potential and its limitations.

Important considerations in choosing an access route include familiarity, distance, availability, size, sterility, access to the targeted surgical area and safety. Familiarity is a property of the surgeon, not the orifice itself. Gastroenterologists are more comfortable performing gastroscopy just as urologists are more familiar with cystoscopy. One apparent disadvantage of the mouth as the access route is that it is quite a distance from the stomach; the point of peritoneal access. The anus, vagina and urethra, however, each are in close proximity to the transluminal access point, generally just a few centimeters. The main limitation to using vaginal access is that this route is limited to females, while the other routes are universal. The urethra's major limitation is its relatively small size compared to the other three routes. Each of the possible orifices is known to be contaminated with many naturally occurring bacteria and, therefore, each has the potential to lead to contamination of the peritoneal cavity. The extent of associated risks and precautions are unknown at this time. It is uncertain which orifice will provide the best access to the targeted area. The fact that the gallbladder is located closer to the stomach than to the anus does not mean that transgastric access for a cholecystectomy will be preferred. It may be more difficult to flex the scope from that position. It is speculated that, similar to any other surgery, the access point will vary depending on the procedure. The final consideration, safety, was regarded as the most important for

preliminary studies. In order to attempt any procedures or investigations of NOTES, access into the peritoneal cavity had to be, above all else, safe. The proximity of organs, vessels and other vital structures to all access points suggests considerable risk. An incision across the lumen of the vagina, stomach, rectum or bladder, without knowing what structures lay behind the incision, could be catastrophic. One exception to this consequence was identified, however. The stomach has been used for years as a site for insertion of a feeding tube. Known as a PEG (Percutaneous Endoscopic Gastrostomy) tube, this feeding tube is delivered endoscopically by taking advantage of the fact that the inflated stomach rests against the abdominal wall (Gauderer et al. 1980, Ponsky and Gauderer 1981). The anterior portion of the stomach provides a proven safe site for delivering a PEG tube.

2.2 A Comparison of Transgastric Access Techniques

2.2.1 Introduction

NOTES burst into prominence with surgeons and endoscopists a few years ago. The concept of succeeding laparoscopic surgery with an even less invasive approach was an attractive alternative driving a myriad of interest and conjecture. Using natural orifices such as the mouth as the point of entry and crossing the gastrointestinal tract to enter the peritoneal cavity, offered an opportunity to perform surgery without a single skin incision. Potentially, the patient would experience less pain, lower risk of infection, fewer complications and would walk away without any visible scars.

Since Tony Kalloo first proposed the concept of natural orifice surgery in 1997 (Johns Hopkins Medicine, Spring/Summer 2007, The Johns Hopkins University), surgeons from all over the world have pushed the envelope of current technology to attempt incisionless procedures. While pure NOTES remains investigational, hybrid NOTES/laparoscopic procedures have been conducted in humans (Lacy et al. 2008, Dolz et al. 2007, Zorrón et al. 2007, Marescaux et al. 2007, Zornig et al. 2007, Hazey et al. 2008). Due largely to technical difficulties closing a gastrotomy, the early success of NOTES applied clinically has been mainly with cases that can be conducted through transvaginal access (Lacy et al. 2008, Dolz et al. 2007, Zorrón et al. 2007, Marescaux et al. 2007, Zornig et al. 2007). Closure of the colpotomy is much easier and can be done with standard instruments since the distance from the orifice to the incision is much shorter. Obviously, this approach limits the patient cohort to females. As endoscopic devices continue to be developed and refined to provide a reliable closure for the gastrotomy, efficient and reproducible transgastric access will become equally important.

Access, whether it be via a transgastric, transvaginal or transrectal approach, is generally achieved with the use of electrocautery and/or a dilation balloon. The anterior transgastric approach has been presumed to provide safe peritoneal access based on the success of this approach for placement of percutaneous endoscopic gastrotomy (PEG) tubes (Gauderer et al. 1980, Ponsky and Gauderer 1981). However, the validity of this presumption has not been tested. No studies have scientifically evaluated different techniques to ascertain the safety and efficacy of the method. Few groups have described their access technique in detail. Rosen and colleagues performed a pilot study pioneering the use of electrocautery and balloon dilation (Rosen et al. 2006). Sumiyama and

Gostout describe three basic methods of transgastric access; using electrocautery to “cut”, using a balloon to “dilate” or using a submucosal tunneling technique to “offset” the gastrotomy (Sumiyama and Gostout 2008). Modifications for safe access have been demonstrated with preliminary pneumoperitoneum (Ko et al. 2007) and through use of an overtube (Hondo et al. 2007). Kim and colleagues compared access through the anterior stomach wall to access through the rectosigmoid colon to investigate which access site provided the best approach to the abdominal organs (Kim et al. 2008). By their evaluation, for exploration of lower abdominal organs, they found no difference between transgastric and transcolonic access. However, for upper organs, these investigators found a transcolonic approach to be superior.

As NOTES implementation continues to move forward, reliable, safe and efficient access to the peritoneal cavity is paramount. The extracorporeal controls, flaccid shaft and remote instruments make flexible endoscopy an arduous and fastidious discipline. Subtle differences in techniques translate into highly variable operative times. Although no NOTES studies have focused on access techniques, one group’s report of access times ranging from 11-85 minutes exemplifies this high variability (Sporn et al. 2008). Delays in peritoneal access will not only increase the length of anesthesia time, but also be directly related to the amount of air infused into the bowel, compounding the limitations of NOTES procedures. Safe, efficient and reliable access to the peritoneal cavity is vital to the success of NOTES. The objective of this study was to evaluate different NOTES access techniques to test the hypothesis that differences in access methodology would significantly affect the safety and efficacy of transgastric access for natural orifice surgery.

2.2.2 Methods

All procedures were approved by the Case Western Reserve University Institutional Animal Care and Use Committee (IACUC). A total of 18 domestic farm pigs (weight 25-40 kg) were obtained from a local vendor (Pineview Farms, Valley City, Ohio). The animals were food deprived 12 hours before the procedure. Anesthesia was induced with 10 mg/kg of intramuscular tiletamine hydrogen chloride and zolazepam, and was maintained with 2.0% to 2.5% inhaled isoflurane after endotracheal intubation (6.0 mm). The animals were mechanically ventilated with 15 to 20 mL/kg of tidal volume at 12 respirations/min and 100% oxygen. Continuous pulse oximetry was monitored throughout the procedure to help maintain normal physiologic parameters. The same operator, a single assistant and single channel endoscope (GIF 1T130, Olympus America, Center Valley, PA) were used for all studies. Prior to the first method, an exploratory laparotomy was performed to rule out prior pathology and the duodenum was ligated so subsequent exploration was not hindered by inflated bowel. The laparotomy was closed temporarily with non-piercing towel clips. Each method was initiated with identification of the access point by creating a transcutaneous gastric dimple. This excluded delays encountered navigating past the pharyngeal diverticulum of the swine and other irrelevant variables. No instruments were pre-loaded into the endoscope. Completion of each access trial was regarded as the point at which the endoscope was passed into the peritoneal cavity. Time to complete each step in the process was recorded. An overview of the basic steps to anterior transgastric access via endoscopy can be seen in figure 4.

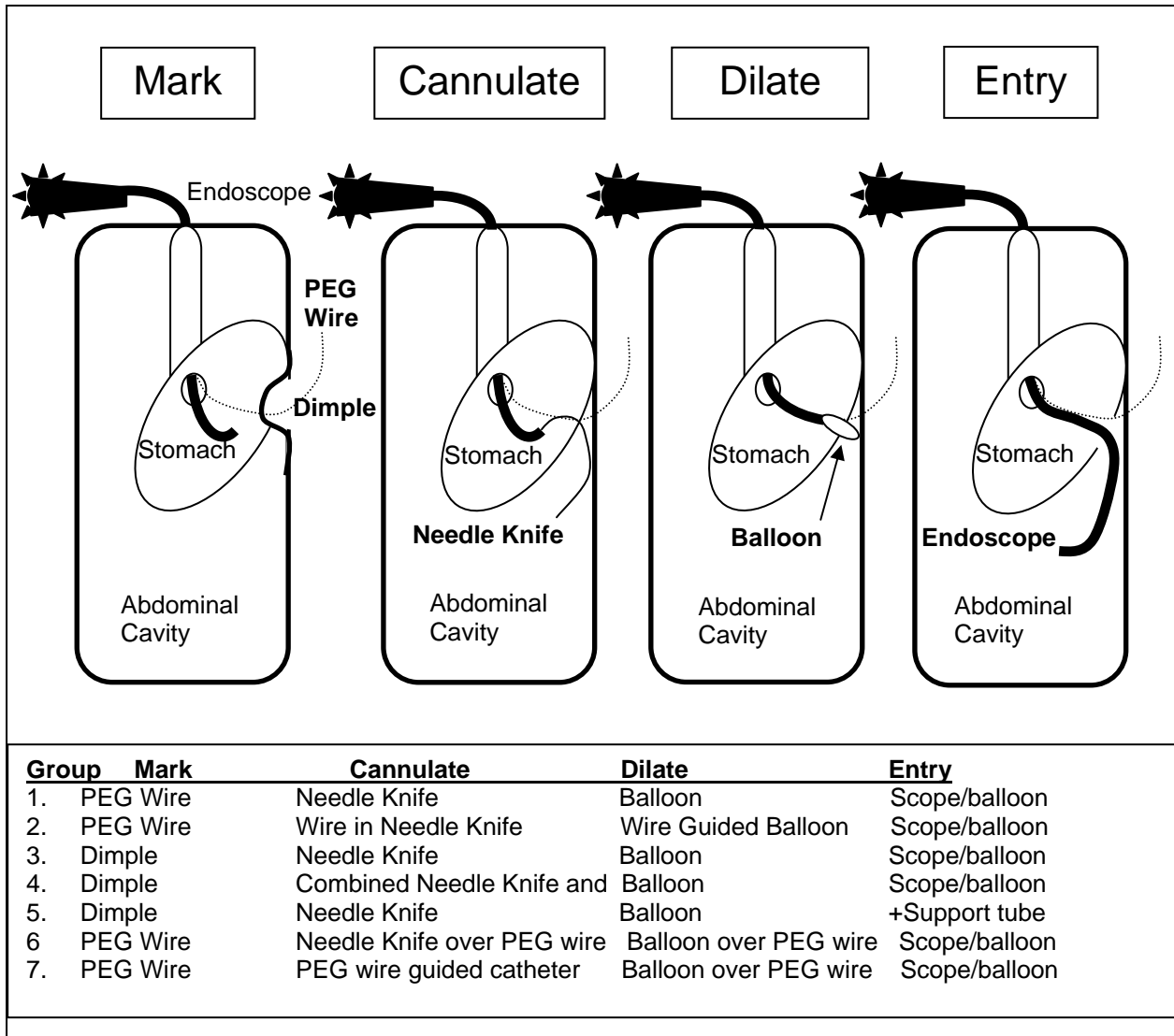


Figure 4. Anterior Transgastric Access Techniques.

Method 1 used the Seldinger technique to place a transabdominal guide wire (0.89 mm) through the anterior wall of the stomach which was snared endoscopically, tracked through the esophagus, exited the mouth and was secured on both ends as done for Percutaneous Endoscopic Gastrostomy (PEG) tubes. The endoscope was reintubated alongside the PEG guide wire. Needle knife (Single Use Triple Lumen Needle Knife KD-441Q, Olympus America) electrocautery was used to create a 2-3 mm gastrostomy adjacent to the guidewire. The hole was cannulated with the needle knife verifying a full-thickness gastrostomy. The needle knife was removed and exchanged with a dilation balloon. The balloon (CRE 20 mm, #5838, Boston Scientific Cork Ltd. Cork, Ireland) was advanced across the gastrostomy until approximately $\frac{3}{4}$ of the balloon was intraperitoneal. The balloon was fully inflated with air and left inflated for 30 seconds. The balloon was deflated slightly and the scope was advanced through the gastrostomy.

Method 2 used the PEG guide wire and used a needle knife for formation of the gastrostomy as in method one. The needle knife was, however, preloaded with a second guide wire. The wire was advanced through the gastrostomy and the needle knife removed. An esophageal dilating balloon with central lumen (CRE Wireguided, 20 mm, #5844, Boston Scientific Cork Ltd. Cork, Ireland) was advanced over the second guide wire, through the scope and used as in method one to gain peritoneal access.

Method 3 did not use the PEG guide wire. Instead, a 1:1 transcutaneous “dimple” was created as in the PEG technique to identify a safe, anterior site, but no wire was placed. Needle knife electrocautery and balloon were used as in method one.

Method 4 identified a safe anterior site as in method three. In this method, the needle knife and balloon were combined into one device. The lumen of the balloon contained a

modified guide-wire which had been deinsulated at the ends to provide a means for electrocautery.

Method 5 extended the technique of method three by including a snug fitting overtube (US Endoscopy prototype). The overtube was just large enough to fit the single channel scope. This support tube was positioned such that the balloon was partially within the tube when inflated, limiting the ability of the balloon to hinge against the scope tip.

Method 6 used a PEG wire as in methods 1 and 2. The PEG wire, however, was tracked through the scope instead of adjacent to it. The PEG wire was then used as a guide for the needle knife and balloon to create the gastrotomy.

Method 7 was similar to method 6, except that instead of a needle knife, the transgastric hole created by the PEG wire was dilated sequentially with two biliary catheters, first a 7 Fr. catheter with a 4 Fr. tip followed by a 10 Fr. catheter with a 6 Fr. tip (SBDC-7 and SBDC-10, Cook Medical, Winston-Salem, NC). Following dilation with the catheters, the balloon was advanced over the PEG wire and the gastrotomy was dilated as done previously.

Total time for the access procedure was recorded as was time for each individual step within the process. The individual steps were designated as: 1) Mark: marking the site for the gastrotomy was measured as the time of transcutaneous dimple until the PEG wire was placed or in the case of techniques three, four and five, which used the dimple as the mark, time was recorded as zero, 2) Cannulation: cannulating the gastrotomy was measured as the time from Mark until a device was passed through the small gastric incision and could be advanced freely in the peritoneal cavity, 3) Dilation: dilation of the gastrotomy was measured as time from Cannulation until the balloon had been correctly

positioned in the gastrotomy and inflated providing dilation of the gastrotomy and 4)

Entry: entry was measured as time from Dilation until the endoscope was passed through the gastrotomy and was free to move in the peritoneal cavity. Time for each step was recorded. Once access was obtained, the laparotomy was reopened and the abdomen was examined in a systematic fashion. Size of gastrotomy was measured by positioning the stomach in a flat plane with no tension on the defect. For the last six trials of each method, the location of the gastrotomy relative to the greater curvature was also noted. Gastrotomies located anterior to the greater curvature were recorded as positive values and gastrotomies located posterior to the greater curvature were recorded as negative values.

The abdominal wall was inspected and injuries were scored on a scale from 0-5. A score of 0 was defined as no injury. A score of 1 was defined as mild injury or tear to the peritoneum and was represented by the injury caused by the PEG wire alone; full-thickness needle puncture. A score of 2 was defined as mild injury beyond the peritoneal layer exemplified by mild electrical injury reaching to abdominal wall muscle. A score of 3 was defined as moderate injury extending into abdominal muscle and represented by moderate electrocautery injury and/or bleeding from the muscle layers. A score of 4 was defined as severe injury or full-thickness abdominal wall penetration by the electrocautery device represented by severe electrical injury and/or bleeding. A score of 5 indicated severe tissue damage requiring repair or further intervention and was represented by the full-thickness abdominal incision required for a 12 mm laparoscopic port.

The stomach itself and all structures surrounding the site of the gastrotomy were inspected for any signs of injury. Injuries were again scored on a scale of 0-5. A score of 0 was defined as no injury present. A score of 1 was defined as mild injury to non-critical structures and was represented by mild bleeding at the gastrotomy or evidence of electrocautery damage to the omentum. A score of 2 was defined as moderate injury to non-critical structures and was exemplified by appreciable alterations to the omentum. A score of 3 was defined as moderate injury to organs or vessels, represented by slight electrocautery damage to organs or bowel or slight bleeding from adjacent vessels. A score of 4 was defined as severe injury to organs or vessels requiring no further intervention and was exemplified as prolonged, controlled bleeding from gastroepiploic vessels or splenic laceration. A score of 5 was defined as severe injury to organs or vessels requiring further intervention and was exemplified by uncontrolled gastroepiploic bleeding or bowel perforation.

An additional experimental group was added for comparison to laparoscopic peritoneal access. Laparoscopic (Lx) access was obtained through a midline incision using a Veress needle inside a radial dilating sleeve (Step, Covidien) followed by insertion of a blunt port (12 mm, Versastep, Covidien). Identification of the site was defined as time zero. Cannulation was the point at which peritoneal insufflation was achieved via the Veress needle. Dilation was the point at which the port was in place and entry was the time at which peritoneal access was confirmed laparoscopically. Abdominal wall and surrounding structure injury was scored equivalently. All scores were agreed upon by at least two independent observers, familiar with porcine anatomy, physiology and the definitions for the assigned scores.

Each of the eight peritoneal access techniques was performed 10 times. All results are reported as mean +/- SD. Sample size calculation was conducted using preliminary time data suggesting a standard deviation equal to 0.55 times the difference in means with alpha set at 0.05 and beta at 0.2 (power = 0.8). Statistical analysis was performed using SigmaStat (SigmaStat, Systat Software, Inc., Point Richmond, CA) after consultation with a biostatistician. Significant differences were defined by $p < 0.05$.

Total time to complete the procedure, defect size and location of the gastrotomy were compared for all techniques using an ANOVA with Tukey post-hoc testing. Comparisons of the time to complete each step within and between groups were analyzed using a two-factor repeated-measures ANOVA with Tukey post-hoc. Comparisons were made only between techniques for which there was an appropriate control group, not indiscriminately. Defect size, injury to abdominal wall and injury to adjacent organs were each evaluated using Kruskal-Wallis non-parametric ANOVA followed by Tukey. Secondary analysis to examine the effects on time of access despite multiple procedures on a single animal, the effects of a learning curve throughout the ten attempts of each method and the influence of the animal on the location of the gastrotomy site were conducted with linear regression. An analysis of the gastrotomy location relative to the time to complete the procedure was also conducted using a correlation function.

2.2.3 Results

Data relating to the safety of each technique can be found in Table II. Injuries to abdominal wall and to adjacent organs are reported in two ways: as an average of the scored value and as the number of occurrences. Adverse events (AE) are defined as any

Table II. Injury assessment following transgastric NOTES access.

Group	Technique	Defect Size (mm)	Abdominal Wall Injury Score (AE, SAE)	Adjacent Organ Injury Score (AE, SAE)	Gastrotomy Location (cm relative to GC ^{††})
1	PEG wire Balloon (X)	16.1 ± 3.9*	1.9 ± 0.7**(10, 0)	0.9 ± 1.7 (3, 1)	-1.63 ± 3.49
2	PEG Wire Balloon (O)	13.8 ± 2.6*	1.0 ± 0.0**(10, 0)	0.3 ± 0.7 (2, 0)	-0.17 ± 2.55
3	Dimple Balloon (X)	14.6 ± 1.4*	0.4 ± 0.7**†(3, 0)	1.0 ± 1.7 (3, 2)	-1.43 ± 2.42
4	Dimple Single Device	15.6 ± 3.0*	0.9 ± 1.3**(4, 0)	0.0 ± 0.0 (0, 0)	1.02 ± 3.64
5	Dimple Support Tube	16.3 ± 3.2*	0.5 ± 0.7**†(4, 0)	0.5 ± 1.6 (1, 1)	-0.43 ± 2.97
6	PEG Guided Cautery	14.1 ± 2.5*	1.2 ± 0.6**(10, 0)	0.0 ± 0.0 (0, 0)	0.60 ± 1.89
7	PEG Guided No Cautery	12.1 ± 2.3*#	1.0 ± 0.0**(10, 0)	0.0 ± 0.0 (0, 0)	0.73 ± 3.19
Lx	Laparoscopy	22.0 ± 1.8	5.0 ± 0.0 (10, 10)	0.0 ± 0.0 (0, 0)	NA

*p<0.001 compared to Lx. #p<0.05 compared to technique 5. **p<0.05 compared to Lx. †p<0.05 compared to technique 1. X indicates balloon without lumen. O indicates balloon with lumen. Lx, Laparoscopy; GC, greater curvature. AE, Adverse Event; SAE, Serious Adverse Event. ††Negative values indicate posterior to greater curvature. Positive values indicate anterior to greater curvature (n = 6).

injury (score of 1 to 5) and serious adverse events (SAE) are defined as injuries with a score of 4 or 5. The location of the gastrotomy is average location relative to the greater curvature, as explained earlier. Positive values represent a location anterior to, and negative values are posterior to the greater curvature. A result of zero, therefore, would indicate a gastrotomy located on the greater curvature.

The size of the gastric defect ranged from 12.1 ± 2.3 mm for group seven to 16.3 ± 3.2 mm for group five, between the seven transgastric NOTES techniques. The defect size for the technique which used no electrocautery (group 7) was found to be significantly smaller ($p = 0.019$) than the defect size created by the technique which used the overtube (group 5). The size of the gastric defect created for each of the seven techniques were significantly smaller than the size of the abdominal wall defect created via a laparoscopic approach ($p < 0.001$).

The abdominal wall injury average score ranged from 0.5 ± 0.7 to 1.2 ± 0.6 for the seven transgastric NOTES techniques. The number of adverse events ranged from 3 to 10 and serious adverse events were zero for all seven groups. Groups three and five had a statistically smaller extent of abdominal wall injury as compared to group one ($p < 0.05$). The abdominal wall injury produced by each of the seven NOTES techniques was significantly less than the abdominal wall injury created by the laparoscopic approach ($p < 0.05$).

The adjacent organ injury average score ranged from 0.0 ± 0.0 for group four to 1.0 ± 1.7 for group three between the seven NOTES techniques. Four serious adverse events were found, two in group three and one in each of group one and five. Each of the four SAE was excessive bleeding from the gastroepiploic artery, on the greater curvature of

Table III. Efficiency indicators for NOTES access

Group	Technique	Mark Site (min)	Cannulate (min)	Dilate (min)	Entry (min)	Total (min)
1	PEG wire Balloon (X)	1.62 ± 0.30*	6.16 ± 3.54*	4.33 ± 5.48*	2.18 ± 3.50	14.29 ± 7.73*
2	PEG Wire Balloon (O)	1.59 ± 0.29*	4.55 ± 2.08*	3.34 ± 1.47*	1.25 ± 1.46	10.73 ± 1.89*
3	Dimple Balloon (X)	0.00 ± 0.00	3.31 ± 2.53*	3.45 ± 2.76*	3.02 ± 2.62	9.78 ± 5.03
4	Dimple Single Device	0.00 ± 0.00	10.71 ± 8.75*	1.13 ± 1.15*	6.20 ± 9.07	18.04 ± 13.74*
5	Dimple Support Tube	0.00 ± 0.00	2.26 ± 1.22*	5.44 ± 4.84*	4.42 ± 6.86	12.12 ± 7.17*
6	PEG Guided Cautery	1.43 ± 0.34*	6.75 ± 5.72*	4.97 ± 1.87*	4.16 ± 5.89	17.32 ± 11.38*
7	PEG Guided No Cautery	1.64 ± 0.59*	8.36 ± 7.96*	11.55 ± 5.02*	4.22 ± 4.27*	25.76 ± 9.67*
Lx	Laparoscopy	0.00 ± 0.00	0.67 ± 0.14	1.09 ± 0.56	0.29 ± 0.17	2.05 ± 0.51

*p<0.05 compared to laparoscopy. Lx, laparoscopy. X indicates balloon without lumen. O indicates balloon with lumen.

the stomach. Five additional adverse events were found, two each within groups one and two, and one in group three. Each of these adverse events was bleeding from the gastrotomy. No injury was found to any organs other than the stomach. No statistical differences were found in adjacent organ injury between the seven techniques and laparoscopy.

The location of the gastrotomy, relative to the greater curvature, ranged from -1.63 ± 3.49 cm for group one to 1.02 ± 3.64 cm for group four. No significant differences and no correlation ($R^2 = 0.058$) was found between the seven techniques and location of the gastrotomy. The location of the gastrotomy was also not found to correlate to the individual animal ($R^2 = 0.002$) and was not a factor in determining the time to access the peritoneal cavity (Pearson correlation = NS).

For total access time, with the exception of technique three, the remaining six NOTES techniques took significantly longer than laparoscopy to access the peritoneal cavity ($p < 0.05$) (Table III; Figure 5). Technique seven, using no electrocautery, took significantly longer to complete than technique three using a dimple instead of a PEG wire ($p < 0.05$). No other statistically significant differences were found between the various techniques in the total time required for peritoneal access.

An analysis within each technique of the time to complete each of the four steps comprising the entire process; mark, cannulate, dilate and entry, revealed a significantly longer time to cannulate compared to the time required to mark the site for technique one, four, six and seven ($p < 0.05$) (Figures 6, 7 and 8). Dilation took significantly longer compared to the time to mark in groups three, five and seven ($p < 0.05$). Entry required significantly longer time than the time to mark the site for technique four ($p < 0.05$).

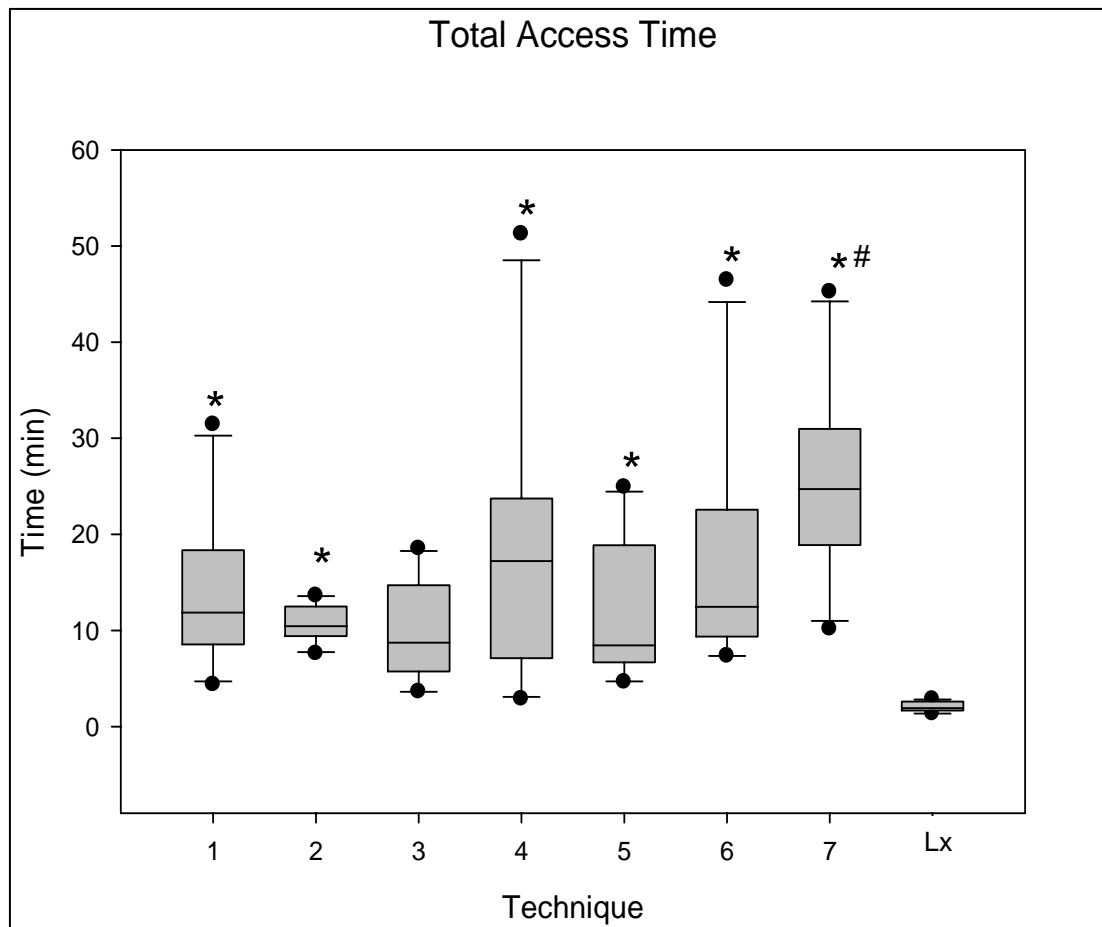


Figure 5. Total time required for peritoneal access. Box defines 25th and 75th percentile. Whiskers define 10th and 90th percentile. Line in box is median. Circles represent high and low values. *p<0.05 compared to Lx. #p<0.05 compared to technique 3.

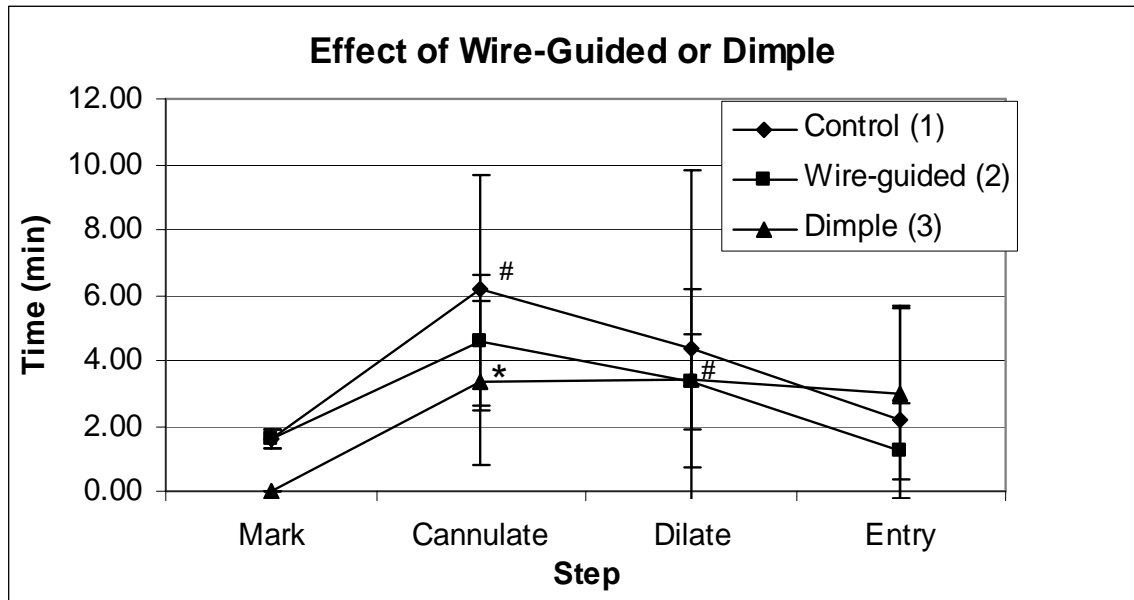


Figure 6. Effect of wire-guided or dimple. * $p < 0.05$ compared to technique 1. # $p < 0.05$ compared to mark.

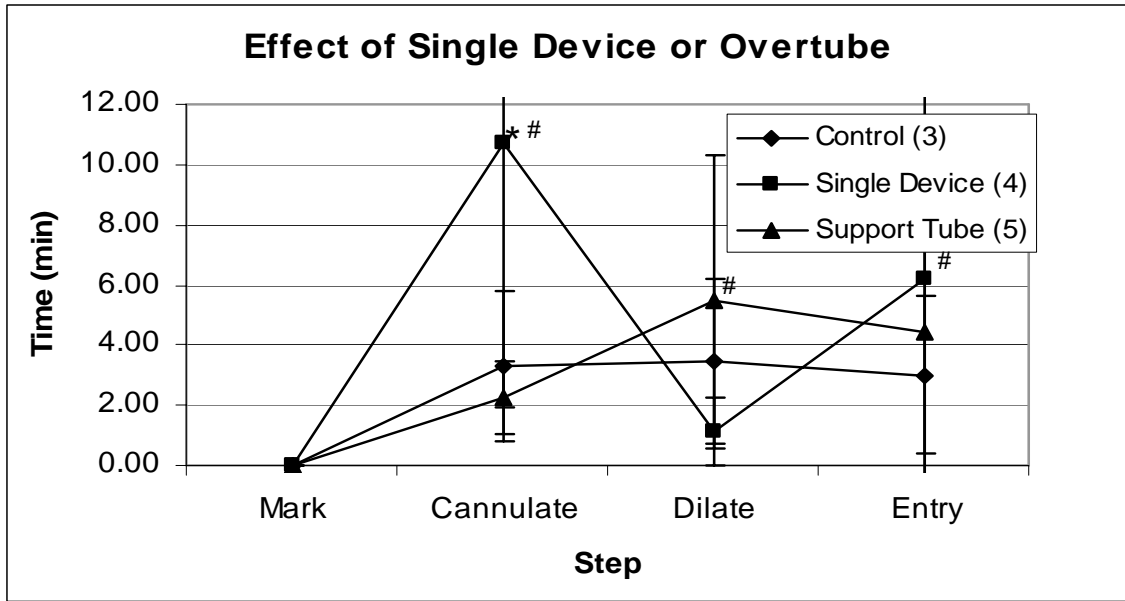


Figure 7. Effect of single device or overtube. * $p < 0.05$ compared to technique 3. # $p < 0.05$ compared to mark.

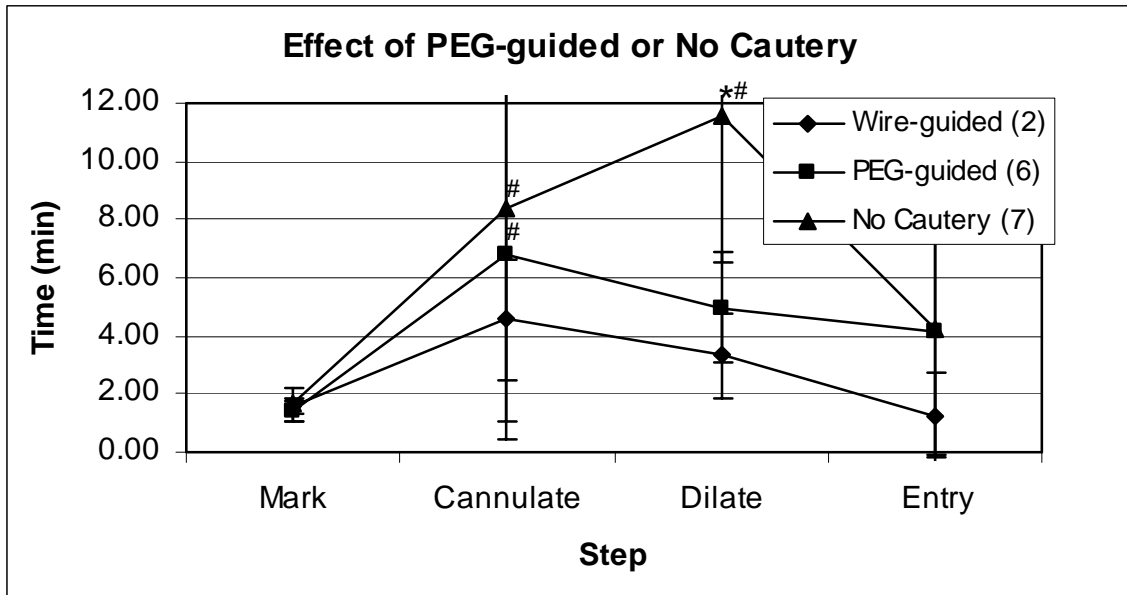


Figure 8. Effect of PEG-guided or no cautery. * $p < 0.05$ compared to technique 2. # $p < 0.05$ compared to mark.

Comparison of individual steps between relevant techniques indicated that cannulation took significantly less time in technique three compared to the time to complete the same step in its control group (technique one) ($p < 0.05$) (Figure 6). Technique four resulted in a significantly longer time for cannulation compared to its control group (technique three) ($p < 0.05$) (Figure 7). Dilation was a prolonged event using technique seven compared to dilation time in group two, the most relevant control ($p < 0.05$) (Figure 8).

Each animal was used for up to four separate, sequential access techniques, but a regression analysis revealed no relationship between the order of each technique and the total time required to complete the overall process ($R^2 = 0.008$). Additionally, a weak relationship was found between the trial number for each technique and the total time required ($R^2 = 0.049$) verifying that the data was not affected by a learning curve.

2.2.4 Discussion

Implementation of NOTES in clinical practice will depend largely on demonstration that a NOTES approach offers a benefit to the patient, not just cosmetically, but physiologically, resulting in overall reduced injury from the treatment. Procedures will continue to be filtered in accordance with the available technology to realize those which are not just feasible, but practical. The primary hurdle facing NOTES is technological advances to facilitate the challenging maneuvers necessary to perform surgery remotely.

This study was designed to evaluate the safety of creating a NOTES anterior gastrotomy and to compare various methodologies of creating the gastrotomy for efficiency. The anterior transgastric approach has been presumed safe due to use of this

site for PEG tube placement and several NOTES access techniques have been demonstrated empirically to be technically feasible, but the safety and efficacy have not been evaluated scientifically. Refining the techniques necessary to merely obtain peritoneal access will be vital to acceptance of this new technology by other surgeons and to facilitate implementation of NOTES procedures into routine clinical use. Additionally, a careful evaluation of access methods can serve as a surrogate to the technical challenges facing the use of endoscopic equipment for NOTES and can help to direct future technological developments.

The size of the gastrotomies created by the seven NOTES access techniques were similar as would be expected since the same size dilation balloon was used for all dilation procedures (Table II). Interestingly, technique five, using the snug fitting overtube did produce a larger hole compared to technique seven which did not use electrocautery prior to dilation and would, therefore be expected to be the least invasive. The size of the gastrotomy was compared to the size of the defect in the abdominal wall produced for a single 12 mm laparoscopic trocar for reference. Although considered to be minimally invasive, a single laparoscopic port produces a considerably larger defect than that required for peritoneal access via NOTES.

Additionally, and not surprisingly, the injury to the abdominal wall produced by the single laparoscopic port was considerably more extensive than abdominal wall injury produced through NOTES access (Table II). Still, this comparison is useful to put into context the minimal nature of the injury caused to the abdominal wall by any of the NOTES access techniques evaluated. Two of the three techniques which did not utilize the PEG wire had significantly less abdominal wall trauma as compared to technique one,

employing a standard PEG wire. The PEG wire was eliminated in favor of a simple dimple to identify the gastrotomy site reasoning that the PEG wire only served to mark the site of the dimple and avoiding the additional step of placing a PEG wire would at least save time and avoid the obligatory full-thickness transmural puncture. However, an important feature of anterior gastric wall access is the expectation that the abdominal wall is approximated to the stomach wall. This is the very reason why this site is presumed safe. The risk of injuring more vital structures is diminished by the substitution of the non-vital tissue of the abdominal wall.

This raises the question of whether techniques which did not employ a PEG wire resulted in greater injury to other structures. The PEG may serve not only to mark the site of the gastrotomy but also to tether the stomach to the abdominal wall. In absence of a PEG wire, the stomach may be more mobile and endoscopic maneuvering to create the gastrotomy may alter the orientation of the stomach relative to the abdominal wall and put more vital structures at greater risk.

This was not the case, however. In fact, in the seventy anterior transgastric access procedures, the only serious adverse event associated with any technique was piercing of the gastroepiploic artery on the greater curvature of the stomach itself and not to any structure beyond the stomach; a consequence that occurred rarely and could not be statistically linked to an individual technique. Furthermore, the location of the gastrotomy itself, relative to the greater curvature was not found to correlate with the specific technique or the individual animal and is possibly a product of the site selection. It is interesting to note, however, that three of the four occurrences of puncture of the gastroepiploic artery did occur in techniques omitting the PEG wire, and although not

statistically significant, each occurrence of gastroepiploic injury was found in the three techniques with the most posterior gastrotomy locations. Collectively these data suggest that placement of a PEG wire may help to maintain a more anterior gastrotomy site and reduce the probability of injury to the gastroepiploic artery.

Average total time for the seven NOTES techniques ranged from approximately ten minutes to 25 minutes (Table III). Access without electrocautery took significantly longer than the fastest technique which used a dimple, needle knife and balloon without the use of any guide wires. The lack of significant difference in total access times between other groups may be attributable to the high variability within almost all techniques (Figure 5). This broad range of access times is reflective of the technical difficulties associated with the use of endoscopy for NOTES and exemplifies the need for a careful examination of subtle differences in methodologies employed. An exception to the high variability in access times was discovered using technique two, which used an additional guide wire in the needle knife and balloon. The relatively small variability for this technique (Figure 5) suggests that it is much more reproducible and may be better suited to demonstrate the NOTES access method to novice surgeons, minimizing the learning curve required for new techniques. Despite a range of techniques evaluated, one technique which was not addressed was hybrid of technique two and three. None of the techniques explored used a dimple followed by a wire-guided needle knife and balloon. Since technique two was most reproducible and technique three was fastest, perhaps the combination of the two would have proved superior to all others. The efficiency of NOTES access was, not surprisingly, much poorer than peritoneal access via a laparoscopic Veress needle technique. At its best, NOTES anterior transgastric access

averaged nearly ten minutes versus just two minutes for the single laparoscopic port (Table III). Whether this five fold increase in time can be extrapolated to the entire surgical procedure is debatable but not unrealistic.

To ascertain which individual step in the access process caused the greatest delays and to better understand the shortcomings of the individual techniques, each step in the process was evaluated for efficiency. Cannulation tended to take the longest of the four individual steps while marking the site was the shortest. Naturally, omitting the step of placing a PEG wire saved time but whether this time savings alone is worth the potential added risk of injury, as noted above, is questionable. Not only did the omission of the PEG wire save time initially for placement but, although not statistically significant, the omission of the PEG wire may have saved additional time throughout the procedure. Technique three, which used the dimple was approximately 1.6 minutes faster to mark the site; the time required to place the PEG. Although PEG wire placement was the only methodological difference between technique three and technique one, the total time difference was approximately 4.5 minutes. An additional three minutes was saved during the steps subsequent to placing the PEG wire. This can potentially be explained by the encumbrances experienced with the scope battling the PEG wire to position the electrocautery and balloon at the PEG wire-marked gastrotomy site.

The use of a guide wire in technique two provided less variability in total procedure time, as mentioned previously, and was the second fastest technique evaluated. Although not statistically significant, this technique reduced the time required to cannulate, dilate and obtain entry compared to technique one which did not use the guide wire (Figure 6). Serving to retain the location of the gastrotomy and to lead the balloon and scope through

the hole, the guide wire technique consistently reduced the time required for each step in the process utilizing the wire.

Since a needle knife is nothing more than a retractable wire within an insulated sheath, combining the needle knife component with the balloon was postulated to reduce the time required to cannulate by reducing the instrument exchanges. A simple device was constructed using a standard guide wire, deinsulated at both ends to allow electrical conduction to the tip. The wire was inserted within the lumen of the balloon. Using this single device technique (technique four) cannulation actually took over ten minutes, longer than any other method (Figure 7). The interpretation for this unexpectedly long cannulation time is likely a fault in the design of the device and not the idea of the device. Unlike a true needle knife, our makeshift device did not govern advancement of the knife allowing the wire to protrude unnecessarily far beyond the tip of the balloon. Furthermore, reuse of the dilation balloon presented an obstacle to visualizing the knife, resulting in less control of the cutting. The balloon, being present upon cannulation, did concomitantly reduce the time required to dilate. A better design of a combined cutting and dilating device may prove valuable to improving access efficiency.

The junction of the inflated balloon and the end of the scope creates a point of flexure hindering entry by allowing the scope to hinge and retroflex instead of passing through the gastrotomy. Technique five utilized a snug fitting overtube. The concept was to align the distal segment of the dilated balloon partially through the gastrotomy while retaining the proximal segment of the balloon within this support tube. Once dilated, the scope, balloon and support tube would form a more rigid structure enabling advancement of the balloon, support tube and scope, as a single unit, through the gastrotomy instead of

the scope potentially looping in the stomach. As can be seen in the data, this support tube concept did not expedite the process (Figure 7). The dilation step actually took longer than other comparable techniques and the time for the entry step was not reduced either. The support tube was difficult to maneuver into position, likely due to the fact that approaching the anterior location of the gastrotomy invariably requires a curved scope tip, whereas the support tube required the scope to be straighter. Fulfilling both conditions required extensive manipulation of the scope, support tube and balloon extending the time required to complete the later steps.

Placement of the PEG wire inevitably creates a hole in the gastric wall. Technique six tested the notion that the PEG wire itself would serve as the necessary guide wire to allow cannulation, dilation and entry. The PEG wire in this case was withdrawn through the endoscopic channel rather than adjacent to the scope. The needle knife and balloon could each be passed over the PEG wire and guided directly to the site of the gastrotomy. An obvious limitation to this technique was that the PEG wire passes through the abdominal wall, thereby preferentially guiding the needle knife to the abdominal wall, not intraperitoneally. To prevent this and facilitate passing the needle knife and balloon intraperitoneally, a loop of PEG wire had to be advanced into the peritoneal cavity prior to cannulation. This proved difficult and time consuming. The PEG wire was advanced from both extracorporeal sites until a loop formed in the peritoneal cavity. The wire preferentially would loop in the stomach. Placing the tip of the scope against the stomach wall and applying suction helped to facilitate looping intraperitoneally. Overall, no advantage was gained by use of this technique (Figure 8).

An extension of the fact that the PEG wire itself creates a pin hole through the stomach wall, technique seven tested the hypothesis that peritoneal access could be achieved without electrocautery. In this technique, as in technique six, the PEG wire was used to guide devices to the gastrotomy site. A modification of this technique to facilitate advancing wire intraperitoneally was the use of a curved metal canula to introduce the PEG wire. This canula was a curved 16G tube with a blunt tip fitted with a sharp metal stylet. The stylet was removed after gastric puncture. After the PEG wire was withdrawn through the scope, the canula could be withdrawn, under direct endoscopic visualization, to be between the stomach and abdominal wall and rotated, dragging PEG wire intraperitoneally. Wire could then be advanced through the canula into the peritoneal cavity facilitating subsequent steps. While this canula expedited advancement of PEG wire intraperitoneally (data not shown), cannulation was still prolonged. Sequential dilations of the gastrotomy were made using increasingly larger catheters until the balloon could be cannulated into the gastrotomy. Cannulation and dilation, together took nearly 20 minutes, almost twice as long as any other technique (Figure 8). Nevertheless, this demonstrated the feasibility of access without risking damage due to electrocauterization.

Limitations to this study include the porcine model used. The pig's stomach is more mobile than the human stomach. It is possible that this mobility could influence the location of the gastrotomy and other factors pertaining to access. Secondly, this study did not account for the influence the access technique would have on closure of the gastrotomy. For example, the PEG wire may serve to guide the closure device to the gastrotomy in the uninflatable stomach and expedite the closure step. Thirdly, dilation

balloons were always reused due to cost. A new, uninflated dilation balloon has a much slimmer profile than does a used balloon. The irregular profile of the used, deflated balloon contributes to unpredictable ability to advance the balloon through the gastrotomy. The times for dilation, therefore, could be expected to be reduced with the use of new balloons.

This study provides data to support the presumption that an anterior transgastric access technique for NOTES procedures is safe but not necessarily anterior. Despite subtle differences in access techniques, seventy access procedures were performed with no complication more serious than bleeding from the gastroepiploic vessels. The data also suggests that dimpling in lieu of a PEG wire, while saving time, may unacceptably risk greater injury. Considering safety and efficacy data, collectively, the use of a PEG wire to mark the site and the use of a second wire to retain the gastrotomy provided a safe, efficient and reproducible technique for anterior transgastric access. Furthermore, comparison to laparoscopy exposed the disparity in the technical challenges facing NOTES procedures, suggesting that new technology and further refinement in methodology will be required for NOTES to be clinically relevant.

2.3 Endoscopic Ultrasound for Identification of Safe Alternate Access Sites

2.3.1 Introduction

Most natural orifice surgeries (NOTES) to date have been performed through the anterior stomach wall, based predominantly on the established safety of PEG placement (Rattner and Kalloo 2006, Ponsky et al. 1983). Although this approach is appropriate for certain operations, it does not afford mechanically efficient access to all anatomic areas

of interest, such as the upper abdomen or the retroperitoneum (Wagh et al. 2005, Swanstrom et al. 2005). Indeed, the white paper identifies the importance of procedure-specific access sites (Rattner and Kalloo 2006) and consequently, several alternate locations such as the rectosigmoid colon, (Pai et al. 2006) vagina, (Scott et al. 2007) and bladder, (Gettman and Blute 2007) have been reported. Several methods of transluminal entry into the peritoneum have been described, all of which require blind puncture through the GI wall, with the inherent risk of injury to adjacent extramural structures. Strategies that mitigate this risk will be essential in the further development of NOTES. One such strategy is the use of Endoscopic Ultrasound (EUS) to identify the presence and location of extraluminal structures at risk for injury during access. Successful use of ultrasound (US) in NOTES access has been reported (Wilhelm et al. 2007, Chak et al. 2006, Fritscher-Ravens et al. 2007). Moreover, for many years EUS has been accepted by authorities as an integral component of endoscopic pseudocyst drainage and pancreatic necrosectomy, two of the index NOTES procedures (Giovannini et al. 2003, Seewald et al. 2005, Charnley et al. 2006). EUS provides real-time anatomic information that can be used to select a safe, procedure-appropriate NOTES access site. With the recent development of a prototype forward-viewing echoendoscope (GIF-UCT160J-AL5; Olympus Medical Systems Corp, Tokyo, Japan) (Voermans et al. 2007), EUS with forward optics can now be performed anywhere in the upper-GI tract and colon. This study was designed to evaluate the utility of EUS in identifying safe alternate access sites for NOTES.

2.3.2 Methods

Endoscopes used in this study were a prototype forward-viewing echoendoscope (GIF-UCT160J-AL5), a standard curved linear array (CLA) echoendoscope (GIFUCT160EUS; Olympus), and a standard single-channel gastroscope (GIF-130; Olympus). Instruments used during the access procedures were a triple-lumen needle-knife catheter (Microvasive Endoscopy, Boston Scientific Corp, Natick, Mass), a 19-gauge EUS-FNA needle (Olympus), a 0.89 mm , 400 cm Jagwire (Boston Scientific), and an 18 mm to 20 mm controlled radial expansion through-the-scope esophageal dilating balloon (Boston Scientific).

All experiments were conducted after approval from the Case Western Reserve University Institutional Animal Care and Use Committee. A total of 12 domestic farm pigs (weight 25-40 kg) were obtained from a local vendor (Pineview Farms, Valley City, Ohio). The animals were removed from wood-chip bedding 72 hours prior to surgery and food was withheld 12 hours before the procedure. Anesthesia was induced with 10 mg/kg of intramuscular tiletamine hydrogen chloride and zolazepam, and was maintained with 1.5% to 2% inhaled isoflurane after endotracheal intubation. The animals were mechanically ventilated with 15 to 20 mL/Kg of tidal volume at 12 respirations/min and 100% oxygen. Continuous pulse oximetry was monitored throughout the procedure to help maintain normal physiologic parameters. After adequate anesthesia, an exploratory laparotomy was performed to evaluate for preexisting anatomic abnormalities. This systematic abdominal exploration was conducted by investigators familiar with porcine abdominopelvic anatomy. Upper abdominal exploration involved thorough evaluation of the stomach, gallbladder, all surfaces of the liver, spleen, pancreas, and Gerota's fascia of

the right and left kidneys. Pelvic exploration involved evaluation of the pelvic side wall, rectum, bladder, uterus and adnexa. Before temporary abdominal-wall closure, evaluation of the small bowel and spiral colon was also performed.

The access procedures were designed to determine the utility of EUS as an adjunct to peritoneal entry through three alternate access sites: the gastric antrum, the posterior stomach wall, and the rectum. These sites were chosen on the basis of their potential future importance in NOTES, as well as the perceived risk of performing blind access through them, such as hepatobiliary complications for transantral access, retroperitoneal structure injury for posterior stomach-wall access, and genitourinary complications for transrectal access. Vascular injury is a perceived risk of access through all three sites. Thirty-two peritoneal access procedures were performed. Sixteen were intended to be safe-access procedures (SAP) and 16 were intended to be unsafe-access procedures (UAP). The intent of the SAPs was to use sonographic guidance to achieve safe intraperitoneal entry by avoiding extraluminal organs and vessels during the initial NOTES puncture. The EUS criteria used to deem an access site “safe” were the lack of an identifiable organ and the absence of a Doppler signal in the intended trajectory of the initial puncture (Figure 9).

UAPs evaluated potential complications of blind access by performing a standard NOTES puncture at sites adjacent to critical extraluminal structures identified by EUS (Figure 10). UAPs targeted the liver, gallbladder, spleen, pancreas, kidney, iliac vessels, or urinary bladder. Access techniques (described below) were identical for SAPs and UAPs. In general, SAP and UAP through a particular access site were performed sequentially in the same pig. SAP was routinely performed first, in the event that the

UAP resulted in a life-threatening complication. One SAP through the posterior stomach wall and one UAP through the gastric antrum were unpaired individual procedures.

Due to limited availability, in 25 of 32 cases, the prototype forward-viewing echoendoscope was used to perform the entire access procedure, whereas, in 7 of 32 cases, the CLA echoendoscope was used for initial puncture and was subsequently substituted for a standard upper endoscope to complete the access procedure. All access procedures were performed by advancing the echoendoscope under direct vision to the luminal area of interest and then deflecting the echoendoscope tip in the general direction of the anticipated incision. Once appropriately positioned in the antrum, rectum, or near the posterior stomach wall, air was suctioned through the echoendoscope to induce complete luminal collapse. Subsequently, EUS was used to identify safe or unsafe access sites within this anatomic region of interest. After the first transrectal access procedure (see below), the pigs were placed in the Trendelenburg's position to minimize the risk of small-bowel perforation. Likewise, for all gastric antral access procedures, the pigs were placed in a reverse Trendelenburg's position. Before transrectal access, the bladder was instilled with methylene blue solution under direct cystoscopic guidance to help identify subsequent bladder puncture.

Peritoneal access was achieved by using one of two techniques. In one technique, visceral puncture was performed by advancing a needle-knife through the gastric or rectal wall after delivering a brief burst of blended electrocautery current. Electrocautery was discontinued immediately upon tactile perception of full-thickness puncture, which was indicated by loss of resistance. In all cases, electrocautery was delivered for less than 0.5



Figure 9. EUS image of a safe access site. Note the lack of an identifiable organ and the absence of a Doppler signal in the intended trajectory of the initial NOTES puncture (Trajectory of Doppler signal delimited by corners).



Figure 10. EUS image of an unsafe access site. Note the black void of the gallbladder within the hepatic parenchyma.

seconds. Upon puncture, a guide wire was freely advanced through the needle-knife into the abdominal cavity. After removing the needle-knife, an esophageal dilating balloon was advanced over the wire and used to radially dilate the incision to an 18 mm circular gastrotomy or colotomy. The endoscope was then advanced through this opening and into the abdominal cavity. In the second technique, a 19-gauge EUS-FNA needle was advanced through the gastric wall under sonographic guidance. Upon puncture into the abdominal cavity, a guide wire was advanced freely through the FNA needle. The FNA needle was removed and access was completed as described above. It should be noted that sonographic visualization of the needle-knife is more challenging than visualization of the FNA needle, particularly during delivery of electrical current, which produces significant EUS artifact. Therefore, during needle-knife access, the position and anticipated trajectory of the needle-knife (compared with extraluminal structures) was identified by sonographically visualizing the prepuncture indentation of the intestinal wall caused by gentle needle-knife-applied pressure.

After obtaining access and then withdrawing the endoscope from the peritoneum, a completion laparotomy was performed. The systematic abdominal exploration described above was repeated after each access procedure. In some cases, surgical closure of the access site was performed after exploration to allow for additional access procedures.

2.3.3 Results

All 32 access procedures resulted in successful peritoneal entry. Of the 16 SAPs, 13 were without complication. In contrast, all 16 UAPs resulted in complications.

Table IV: Complications of unsafe access performed through the gastric antrum

<i>Antrum procedure no</i>	<i>EUS target</i>	Complications
1	Liver and GB	5-mm liver laceration (adjacent GB fossa with bleeding)
2	Liver	8-mm liver laceration with bleeding, 10-mm splenic laceration with bleeding
3	Liver	3-mm liver laceration with bleeding, 3-mm hepatic parenchymal hematoma`
4	Liver and Portal Vein	2-mm liver laceration, GB puncture with bile leak, portal vein puncture with massive hemorrhage
5	GB	GB puncture with bleeding and bile leak
6	GB	GB puncture with bleeding and bile leak

GB, Gallbladder.

Table V: Complications of unsafe access performed through the posterior stomach wall

<i>Posterior Stomach Procedure No.</i>	<i>EUS Target</i>	<i>Complications</i>
1	Pancreas	Pancreatic laceration
2	Pancreas	Gerota's fascia puncture
3	Spleen and left kidney	Splenic laceration with bleeding
4	Left Kidney	Renal puncture with placement of guidewire into collecting system

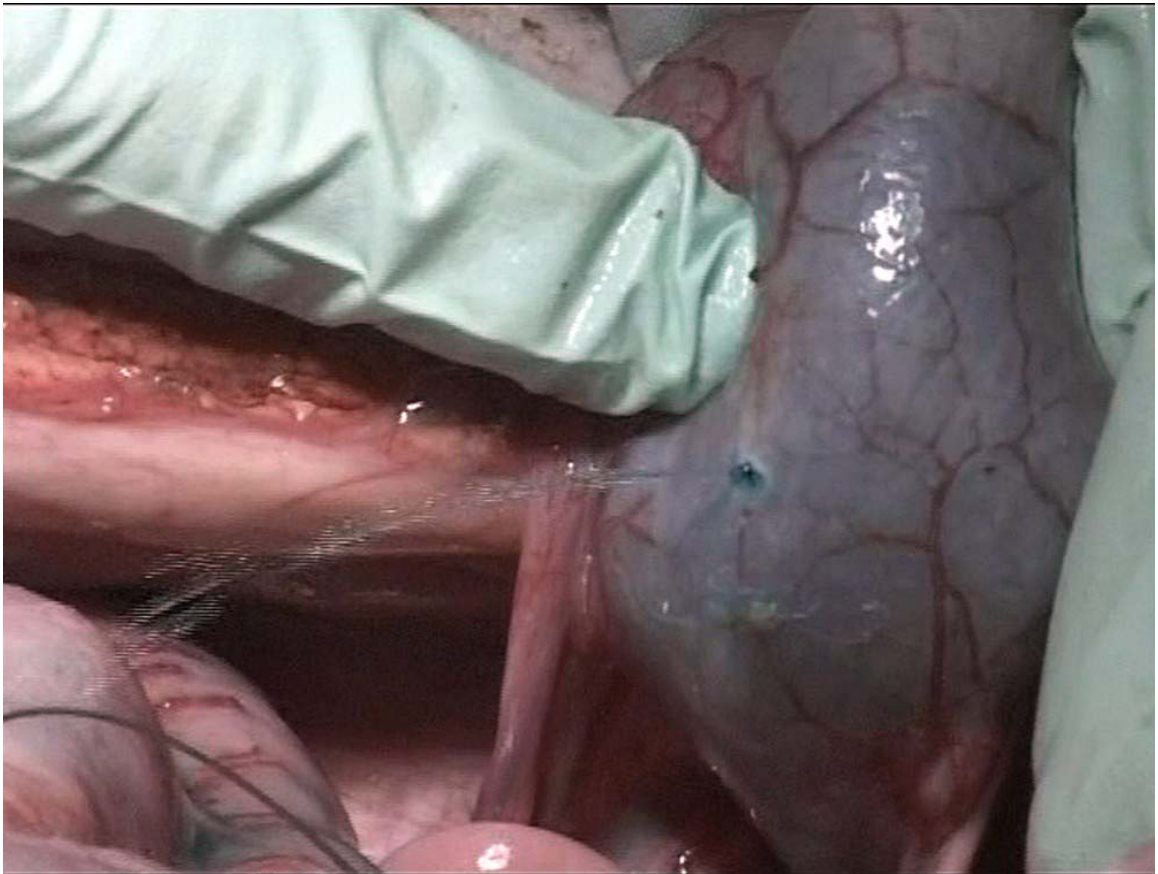


Figure 11. Bladder puncture following an unsafe transrectal access site. Note the urine/methylene blue dye streaming from the puncture site.

Eleven access procedures were performed through the gastric antrum: Five SAPs and Six UAPs. All five SAPs resulted in free peritoneal access, without evidence of complications on laparotomy. All six UAPs resulted in clinically significant complications, including liver laceration, spleen laceration, and gallbladder puncture. The results of the gastric antral UAPs are presented in Table IV.

Nine access procedures were performed through the posterior gastric wall: five SAPs and four UAPs. Two SAPs and all UAPs were performed with the prototype forward-viewing echoendoscope. The remaining three SAPs were performed with the standard CLA echoendoscope. In seven of the nine posterior gastric-wall access procedures, the initial puncture was performed with an EUS-FNA needle as described above. The other two access procedures were performed with a needle-knife. All five SAPs resulted in successful posterior-wall access near the pancreas, without clinically relevant complications. All UAPs, however, resulted in serious complications, including pancreatic laceration and kidney puncture. The results of the posterior gastric wall UAPs are presented in Table V.

Twelve access procedures were performed through the rectum: six SAPs and six UAPs. All UAPs resulted in significant complications, including bladder puncture (Figure 11) and iliac artery injury. The results of the transrectal UAPs are presented in Table VI. Of the six SAPs, three resulted in complications, one major and two minor: there was a small-bowel perforation; a puncture through the left mesosalpinx, without bleeding; and a puncture through the lateral pelvic-wall muscle, also without bleeding (Table VII). All SAPs through the gastric antrum and posterior stomach wall were uncomplicated. As such, these procedures are not listed in tabular form.

Table VI: Complications of unsafe access performed through the rectum

<i>Rectum Procedure No.</i>	<i>EUS Target</i>	<i>Complications</i>
1	Bladder	Bladder puncture with urine leak, fallopian tube laceration
2	Bladder	Bladder puncture with urine leak
3	Bladder	Bladder puncture with urine leak
4	Bladder	Bladder puncture with urine leak
5	Iliac vessels	External iliac artery injury with massive intraperitoneal hemorrhage
6	Iliac vessels	Retroperitoneal puncture within 5-mm of iliac vessels, guidewire into retroperitoneum

Table VII: Complications of safe access performed through the rectum*

<i>Rectum Procedure No.</i>	<i>EUS Target</i>	<i>Complications</i>
1	Safe	Small-bowel perforation
2	Safe	None
3	Safe	Puncture through left mesosalpinx, without bleeding
4	Safe	Puncture through lateral pelvic wall muscle, without bleeding
5	Safe	None
6	Safe	None

*All SAPs performed through the gastric antrum and posterior stomach were without complications.

Positioning of the echoendoscope in the access region of interest and performance of the initial NOTES puncture were technically successful with both echoendoscopes. Because of the oblique-viewing optics, advancement of the CLA echoendoscope through the gastrotomy or colostomy into the peritoneum was technically very challenging and unreliable. As described above, a standard upper endoscope was used for peritoneal entry in cases where the CLA echoendoscope was used for localization and initial puncture. In contrast, the forward-viewing echoendoscope was reliably passed into the peritoneum without difficulty. Overall, procedure times were lower with the forward forward-viewing echoendoscope because of improved maneuverability when guided by forward optics and avoidance of a second endoscopic procedure for peritoneal entry.

2.3.4 Discussion

By using EUS to target at-risk extraluminal organs and vessels, the potential complications of blind NOTES access through sites other than the anterior gastric wall were demonstrated. When EUS was used to identify and avoid such structures, however, access through these sites was substantially safer and less likely to result in a major complication. These findings, although preliminary, suggest that EUS is useful in identifying safe alternate access sites for NOTES. In this study, the presence of an extraluminal structure in the anticipated trajectory of the initial NOTES puncture reliably predicted a serious complication. The absence of such structures, however, did not completely eliminate this risk, particularly for transrectal access. The immediate obstacle in avoiding serious complications with EUS appears to be the interpretation of nonspecific echogenicity that is generally present when identifiable structures are not

visualized (Figure 9). We believe that this ultrasound pattern usually represents low-risk structures, such as the abdominal wall or mesentery. It may, however, represent structures that, if injured, are more likely to result in a serious complication, such as the small intestine. Although the use of gravity to shift the small bowel away from an access site appeared to minimize the risk of perforation, an improved understanding of the significance of this echogenicity will be important in developing safe EUS-guided access strategies. Porcine abdominal anatomy differs quite substantially from that of humans, making the pig a suboptimal model for determining immediate clinical applicability. As such, this study is intended to demonstrate proof of concept that blind access through alternate sites could be prohibitively dangerous. Further research in both animal models and human beings is necessary to determine whether EUS will have widespread or selective applicability in the area of NOTES access. Moreover, strategies that would further improve the safety of EUS-guided access, such as the induction of hydroperitoneum (Wilhelm et al. 2007, Gordts et al. 1998) and the routine use of general anesthesia to eliminate patient movement and temporarily halt respirations before initial puncture, need to be evaluated.

The alternate access sites evaluated in this study may have important future NOTES applications. The rectum has already been reported to be an efficient point of entry for cholecystectomy (Pai et al. 2006). Additional potential applications of transrectal access to the upper abdomen include complex biliary interventions, fundoplication, hiatal hernia repair, and diaphragmatic pacing. Posterior gastric access may provide an important platform for procedures that involve retroperitoneal structures, most notably the pancreas and surrounding vasculature. Transantral access could complement anterior gastric

access for diagnostic applications, such as cancer staging and biopsy of peritoneal carcinomatosis, or it might prove to be a more efficient access site for procedures of the small bowel and pelvis.

Blind NOTES access through the antrum, posterior stomach wall, and rectum can result in catastrophic complications. In contrast, EUS-guided access through these sites substantially reduced but did not completely eliminate this risk. EUS appears promising as an adjunct to NOTES access, particularly as more experience is gained in definitively excluding the presence of at-risk extraluminal structures.

III

CLOSURE: THE SINGLE GREATEST BARRIER TO NOTES

3.1 Introduction

Obtaining reliable closure at the transvisceral point of NOTES access with the current armamentarium of flexible endoscopic instrumentation is challenging, and remains the largest encumbrance limiting the progression of NOTES into the human setting (Rattner and Kalloo 2006, McGee et al. 2007). Crude endoscopic equipment, limited degrees of freedom, and an inability to triangulate multiple end effectors are chief among several factors that combine to make closure of NOTES access points challenging.

Several techniques of NOTES closure, used with varying degrees of success, have been reported in the literature (Kallo et al. 2004, Hu et al. 2005c, Jagannath 2005a, Ikeda et al. 2005, Raju et al. 2006, Hausmann et al. 2006, Pai et al. 2006, Marks et al. 2007, Sumiyama 2007a-c). However, no standard device or technique has demonstrated superiority. Because even low leak rates of 1% to 2% with transvisceral closures are unacceptable compared with the established safety of contemporary laparoscopic techniques for minimally invasive abdominal surgery, ensuring superior closure during NOTES will be the lynchpin for the success or failure of this budding surgical paradigm

(McGee et al.2007). The goal of this study was to evaluate the safety, reproducibility and efficacy of several techniques for closing a standardized gastric defect in a chronic NOTES animal model.

3.2 Methods

All chronic experiments were conducted following approval from the Case Western Reserve University Institutional Animal Care and Use Committee (Protocol #2006-0109). Domestic swine were obtained from a USDA-approved local vendor (Pineview Farms, Valley City, OH) and underwent a 7-day quarantine and acclimation period. During this time, veterinary personnel evaluated each animal to ensure baseline health. All animals were subjected to the same husbandry procedures. Animals were individually caged with woodchip bedding, fed the same diet and had unlimited access to water. Animals were removed from woodchip bedding 72 h prior to the scheduled date of surgery. Animals were fasted from solid food 24 h prior to surgery but allowed to drink water as desired. Following restraint, animals were sedated with 10 mg/kg intramuscular tiletamine HCl and zolazepam (Telazol, Fort Dodge, Animal Health, Fort Dodge, IA). Endotracheal intubation was performed with a endotracheal tube (6.0 mm) and animals were mechanically ventilated at 12 respirations/minute, with a tidal volume of 15-20 mL/Kg and 100% oxygen. Inhaled isoflurane (AErrane, Baxter Healthcare, Deerfield, IL) was administered at 1.5-2.0% for the duration of the surgery. Following intubation, all animals underwent preprocedural 5-L gastric lavage with 0.9% sterile saline via a 40 French stomach evacuator/lavage tube (Lavacuator II, Mallinckrodt, St. Louis, MO) to assist with removing gastric bezoars and debris. Forty swine underwent a standardized

NOTES gastrotomy with subsequent closure of the defect by one of four techniques. Preliminary acute studies were conducted in live swine and in explanted porcine stomachs to develop the chronic model, gain familiarity with the closure devices and refine the closure technique. Sample size calculation was conducted using preliminary time data suggesting a standard deviation equal to 0.55 times the difference in means with alpha set at 0.05 and beta at 0.2 (power = 0.8). Ten pigs each were assigned to one of the following four groups, utilizing four separate and unique techniques for closure of a NOTES gastrotomy.

Group 1: Tissue Plicating Device (TPD) (Figure 12): The TPD (NDO Plicator, NDO Surgical, Mansfield, MA) is an FDA-approved endoscopic device that provides endoluminal treatment of gastroesophageal reflux disease (Chuttani et al. 2002, Chuttani et al. 2003, Pleskow et al. 2007). The reusable flexible device is introduced per oral access into the stomach and used to cinch the gastroesophageal junction (GEJ) under endoscopic visualization by plicating adjacent gastric tissue. Preloaded, polytetrafluoroethylene (PTFE)-pledgeted, polypropylene suture implants are placed with the assistance of an integrated tissue grasper, ensuring full-thickness serosal approximation of the plicated tissue. Plicated augmentation of the GEJ requires 180° retroflexion of the TPD for adequate visualization. The TPD was used in a non retroflexed position in this study to evaluate its ability to close a NOTES gastrotomy.

Group 2: The Tissue Apposition System (TAS) (Figure 13): The TAS (Ethicon Endo-Surgery Inc, Cincinnati, OH), utilizes tissue anchors (TA) to obtain a full-thickness closure. Each suture terminates with a metal bar that can be deployed into tissue with an endoscopic needle.

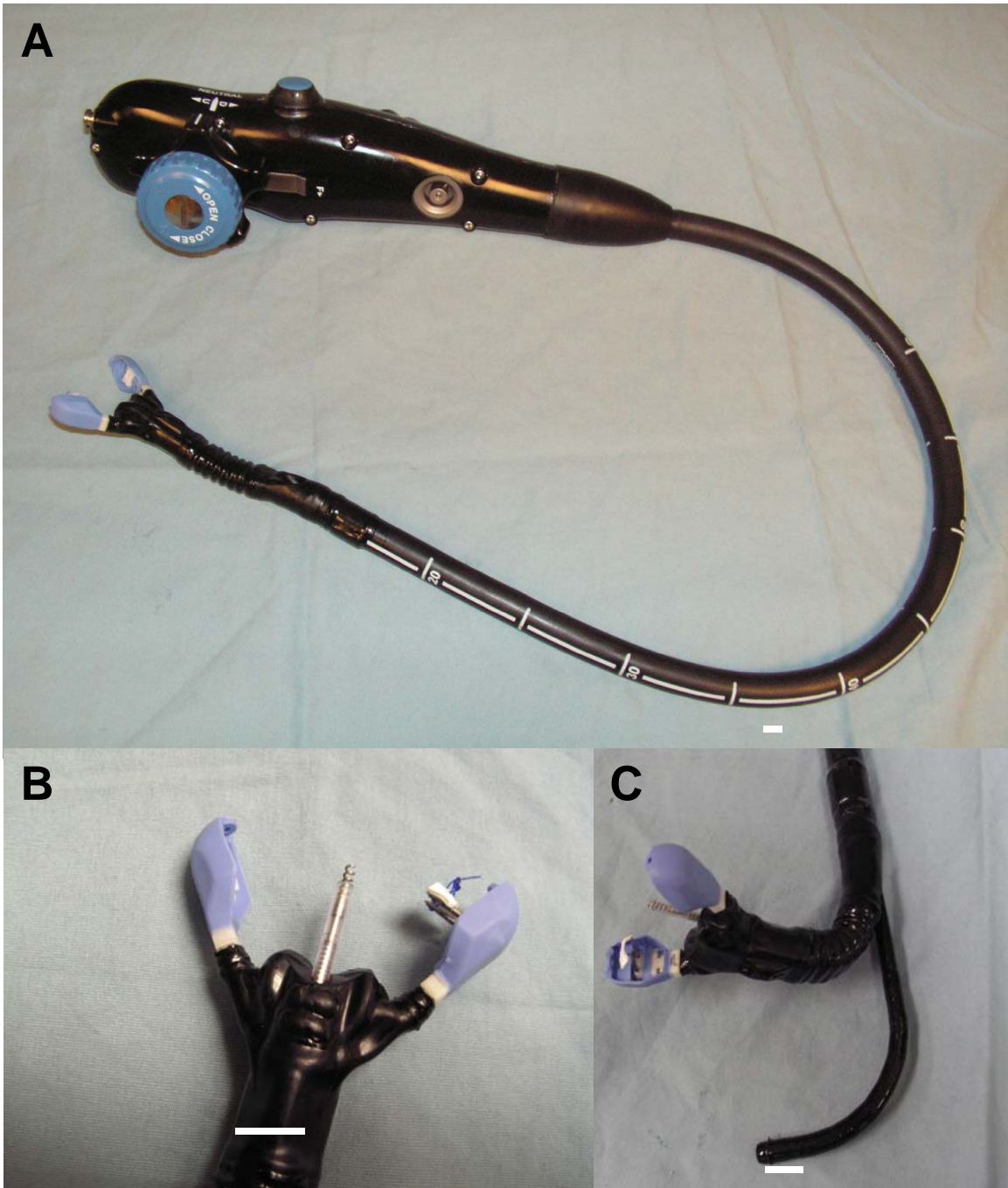


Figure 12. TPD, Tissue Plicating Device. (A) Overview of the entire device. The upper left-hand corner shows the proximal control end of the device. The left-hand center is the distal end of the device with pledgeted suture holders (blue). (B) Enlarged view showing the distal end of the device, with jaws opened in an anteflexed configuration. The corkscrew tissue-grasping device is protruding from the center. (C) Plicator in a retroflexed configuration, with a 5.9 mm thin endoscope passed through the device to provide endoscopic visualization of the jaws during manipulation. Scale bar = 1.0 cm.

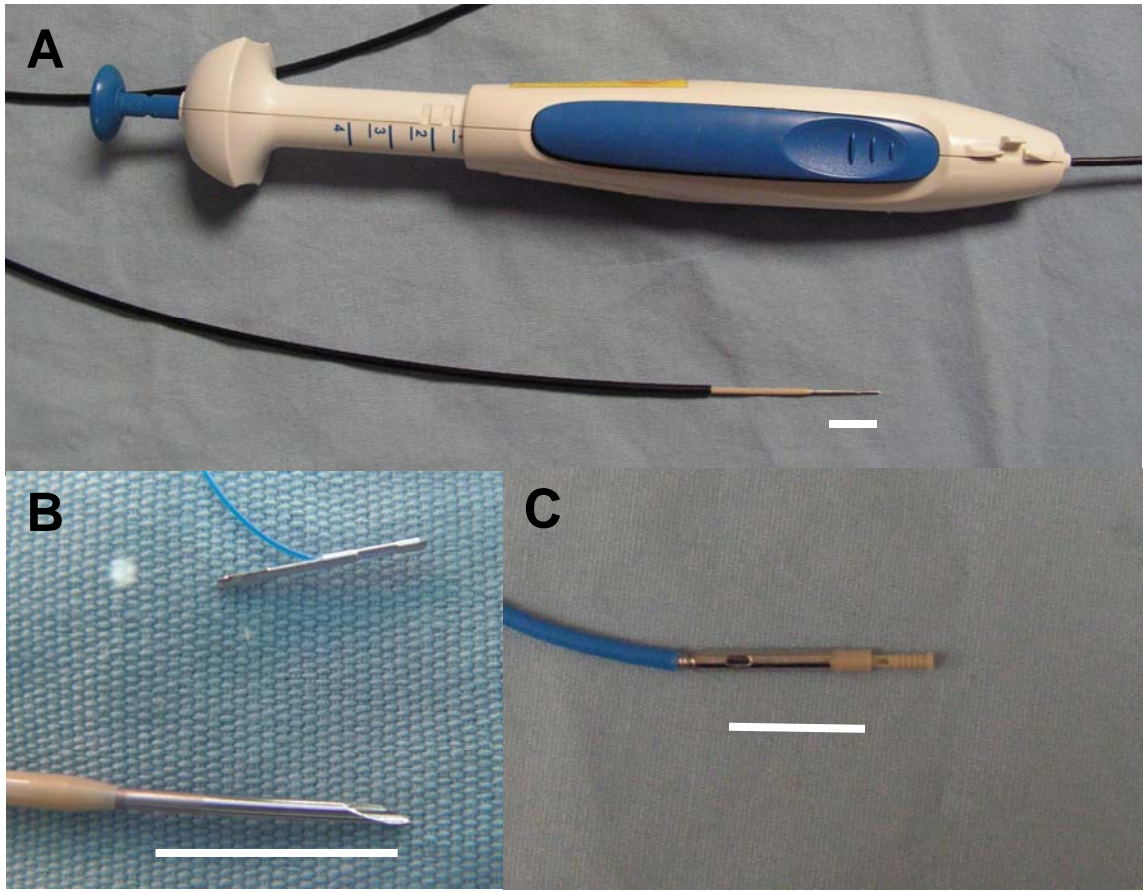


Figure 13. TAS, Tissue Apposition System. (A) Tissue anchor applicator handle and needle. (B) Close-up of tissue anchor and suture and needle. (C) Knotting element. Scale bar = 1.0 cm.

Group 3: Flexible Endoscopic Suturing Device (FESD) (Figure 14): The prototype FESD investigated is similar to the EndoStitch™ laparoscopic suturing device (Covidien, North Haven, CT). It incorporates the needle passing ability of the EndoStitch™ device by placing the functional component at the end of a 120 cm long flexible shaft that is utilized alongside, and moves independently from, an endoscope.

Group 4: Clip/Loop (Figure 15): The Clip/Loop group utilized endoclips (Quickclip II, Olympus, Center Valley, PA) and endoscopic loops (Poly-Loop, Olympus, Center Valley, PA) together to form a junction for closure.

Creation of a Standardized Gastric Defect: Following gastric lavage, NOTES peritoneoscopy was performed using the previously described CASE-T technique (McGee et al. 2007). A standard forward-viewing single-channel video endoscope (Olympus, Center Valley, PA, EVIS Type 100 Q140) was passed via the mouth and gastroscopy was performed. Utilizing transillumination together with external pressure dimpling performed by an assistant, a 12-gauge needle and catheter were passed through the left upper quadrant abdominal wall into the stomach under direct endoscopic visualization. Once the needle tip was intraluminal, a 0.89 mm×400-cm access Jag wire (Boston Scientific, Natick, MA) was passed through the needle and secured intraluminally by an endoscopic snare. The needle and catheter were subsequently removed, and the Jag wire, snare, and endoscope were removed as one unit through the mouth. With the transabdominal access wire coursing from the external abdomen through the abdominal wall into the stomach and out through the mouth, the endoscope was reintroduced alongside the wire into the stomach and the gastric site containing the access wire entrance was visually inspected. A novel modified endoscopic needle knife

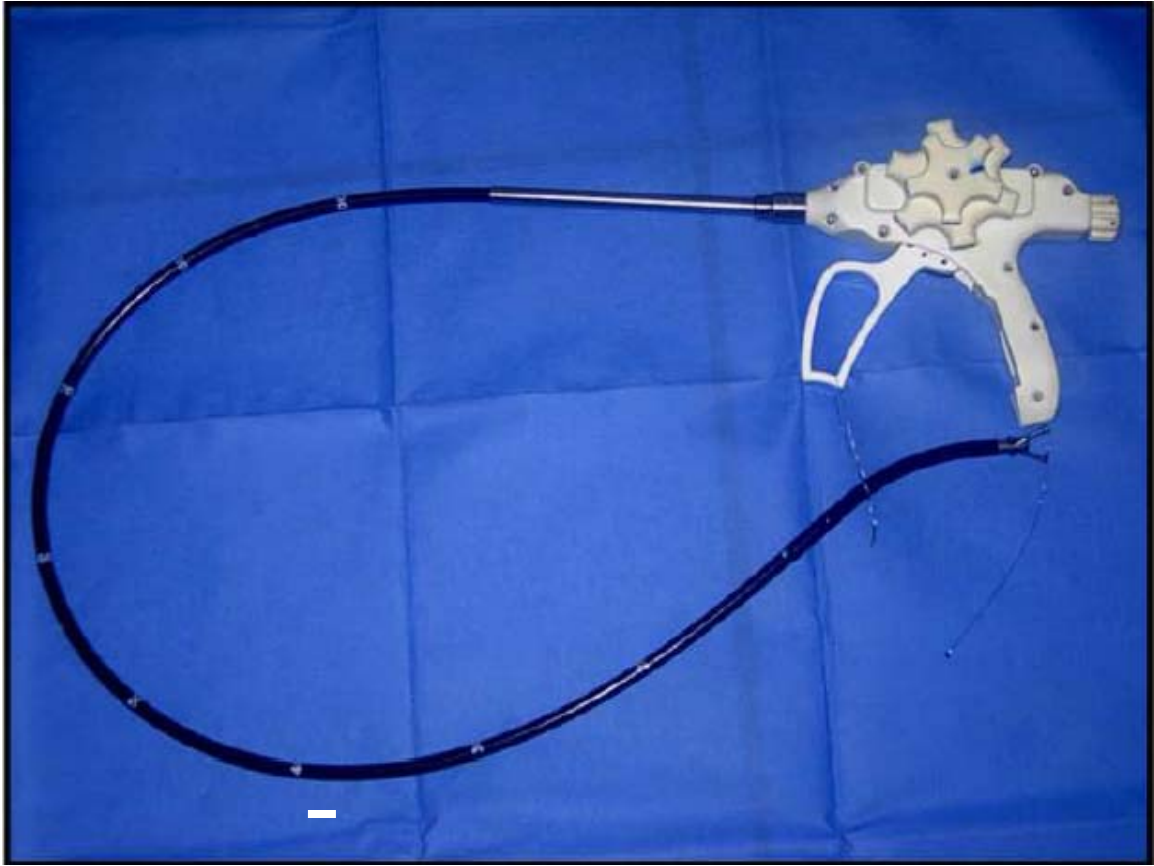


Figure 14. FESD, Flexible endoscopic suturing device displaying multi-directional control wheels for articulation and the functional end loaded with a needle. Scale bar = 1.0 cm.

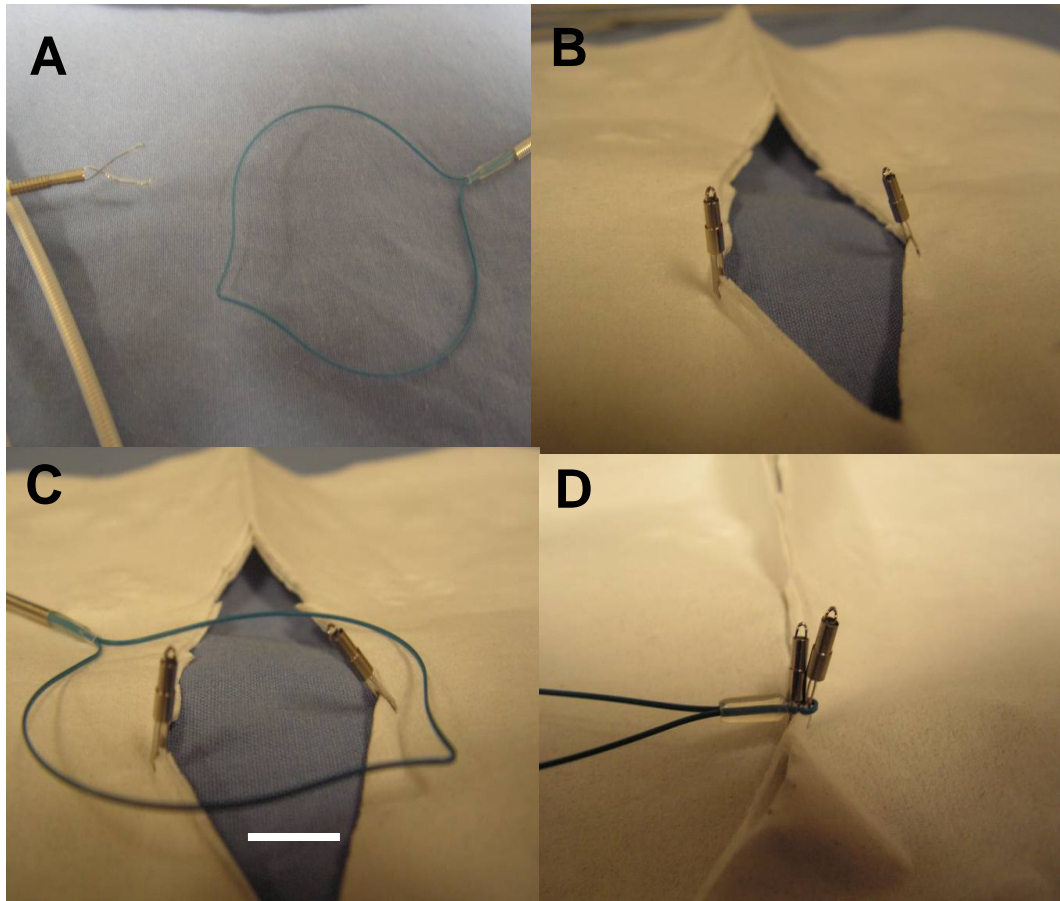


Figure 15. Clip/Loop closure. (A) Open clip and open loop. (B) Two clips affixed to opposing sides of a cut in cloth. (C) Loop positioned around two clips. (D) Loop snared tight pulling clips together and closing hole. Scale bar = 1.0 cm.

electrocautery/access wire incised the adjacent gastric tissue surrounding the wire, resulting in a 3 mm transmural slit enveloping the transabdominal access wire. The needle knife/access wire was then advanced 10 cm through the enlarged gastric defect into the peritoneal cavity. An 18 to 20 mm esophageal/colonic dilating balloon (CRE ESO 18 to 20 mm × 240 cm balloon #5850, Boston Scientific, Cork, Ireland) was passed over the electrocautery/access wire and positioned across the gastrotomy. The balloon was inflated in order to dilate the 3 mm gastrotomy slit to a 12 mm-diameter circular gastrotomy. The balloon and needle knife were then withdrawn from the endoscope and the endoscope tip was directed through the gastric defect into the abdominal cavity. Previous studies have demonstrated that the resultant gastric defect is consistently circular and 12 mm in diameter (Rosen et al. 2006). Systematic NOTES peritoneoscopy was performed to exclude the presence of intra-abdominal injury caused by NOTES access. Upon the completion of NOTES peritoneoscopy, the endoscope was withdrawn from the peritoneal cavity into the stomach. The percutaneous transabdominal access wire was intentionally left coursing from the anterior abdominal wall through the defect and out through the mouth to assist with both endoscopic and subsequent radiographic identification of the closure site. Note that for groups 2 (TAS) and 3 (FESD), placement of suture occurred prior to creation of the gastrotomy, as described later.

Closure of Gastrotomy: Group 1: TPD: A Savary spring-tipped metal guidewire (Cook Medical, Bloomington, IN) was introduced into the stomach through the endoscopic accessory channel, and the endoscope was removed leaving the wire in place. Upon withdrawal of the endoscope, the distance from the GEJ to the incisors was noted. Care was taken to ensure that the Savary wire did not protrude through the NOTES defect

and remained in an intragastric position. The TPD was advanced beyond the measured length of the GEJ over the Savary wire, and the wire was subsequently exchanged for a 5.9 mm-diameter pediatric flexible gastroscope (GIF-XP 160, Olympus) to provide endoscopic visualization (Figure 12). Each defect was closed with a variable number of sequential firings of the device until closure appeared adequate under endoscopic visualization, with the transabdominal access wire left in place. Ideally, the first implant location was selected to bisect the defect, and subsequent implants were placed to close residual gaps. Custom sutures (4 mm) were used to ensure tight closure of the gastric defect. The u-stitch design of the suture implant enabled approximation of the defect edges without entrapment of the transabdominal access wire.

Group 2: TAS: Experience gained from stomach explants and acute studies demonstrated that positioning the tissue anchors (TA) after creating the gastrotomy was difficult due to the inability to insufflate the stomach. TA were, therefore, placed prior to the creation of the gastrotomy. Four individual TA were placed in four quadrants, 1 cm away from the guide wire, using the wire as a focal point for the planned gastrotomy. Care was taken to ensure that TA sutures were paired in opposite quadrants to produce a figure of an “X” when knotted. The sutures were deployed with no more than 0.5 cm of the needle inserted through the gastric wall. Previous acute experience had shown that more than 0.5 cm routinely resulted in attachment of the tissue anchor to the anterior abdominal wall. After all four TA were placed, the endoscope was removed and the sutures tails remained transoral. They were paired and secured to maintain their relative position extracorporeally. At this point, peritoneal access was obtained via standardized gastrotomy.

At the completion of NOTES peritoneoscopy, the endoscope was withdrawn, and the suture tails were back-loaded using a snare in matching pairs into separate channels. The pig was then reintubated with the endoscope. The suture tails were threaded through the knotting element applier (Figure 13) using the knotting element loader. The knotting element applier was advanced over the sutures down through the channel, approximating the opposing pair intragastrically. The suture tails were gently pulled-tight by hand under endoscopic vision. Once the sides of the gastrotomy were apposed, the knotting element was triggered, thereby securing the suture and cutting the tails. As soon as the first suture was knotted, visualization improved allowing for precise placement of the second knotting element across the partially sealed gastrotomy. The second crossed pair of sutures was then approximated and secured. A third suture pair was then placed over the “X” pattern to improve the closure integrity.

Group 3: FESD: As for the TAS group, the technique refined in the acute porcine model was dependent on pre-placement of suture. Following placement of the guide wire, a prototype access tube was placed over the endoscope, and the two were simultaneously reinserted into the mouth, reintubating the esophagus. The distal end of this tube was advanced to the GEJ under direct vision of the endoscope. The dual-channel endoscope was then removed. The FESD and a slim single-channel endoscope (GIF-XP160, Olympus Inc., Center Valley, PA) were simultaneously inserted into the access tube. This tube was further equipped with a stopcock-controlled valve for CO₂ insufflation of the stomach. Using the access wire as a focal point for the planned gastrotomy, two 100cm 2-0 braided absorbable stay sutures (Polysorb™, Covidien, North Haven, CT) secured to EndoStitch™ needles were individually placed intragastrically.

These two sutures were secured in-line through the mucosa on either side of the guidewire. Between one and three running stitches through the mucosa were made in a remote to central fashion approaching the access wire site. Biopsy forceps were occasionally used to lift the mucosa to allow this tissue bite. The tails of these sutures remained extracorporeal and were tagged with hemostats. The needle at the lead end was then dropped to anchor the suture in place. Tension was placed on the tails lifting the mucosa and thereby creating an artificial mucosal ridge/fold leading up to and involving the access wire. Prior experience in non-study explanted stomach models showed improved suturing ability of the gastrotomy when this fold was created. This technique also provided temporary collapse of the gastrotomy which, therefore, improved insufflation. The gastrotomy was then created as described above. After removal of the dual-channel endoscope, the FESD and slim endoscope were then reinserted into the access tube as was done prior to gastrotomy. The FESD, however, was now loaded with a 10 cm length of a prototype barbed suture. The suture used for closure in this investigation was a prototype 2-0 sized absorbable monofilament with barbs (Covidien, North Haven, CT) to allow passage of the suture while preventing it from sliding back. The tail of the suture was also equipped with a preformed anchoring knot for application of tension and cinching with every tissue bite. By this technology, the intention was to allow the suture to be fastened down tightly with each needle passage while not allowing laxity to recur when the needle was released. As few as one needle passage through the tissue can be made to cinch and lock the suture in place.

The tails of the stay sutures were pulled taut to create a mucosal ridge to aid in suturing. Care was taken not to pull too aggressively, as the poorly fixed porcine

stomach can invert into the esophagus. Using a running and overlapping stitch, the gastrotomy defect was closed. The first pass of the suture was pulled completely through the wound until the anchoring knot was snug against the mucosa. Tension was applied to successive passes, allowing the barbs to lock down each individual tissue bite. Once the gastrotomy was closed, the needle was released and left intragastrically. Additional barbed sutures were used at the discretion of the endoscopist if the closure did not appear adequate or if the needle prematurely released from the device. Closure times were recorded from the first passage of FESD through the GEJ to when the last needle was released (if more than one was necessary). The FESD was then removed and an endoscopic scissors were used to cut the tails of the stay sutures.

Group 4: Clip/Loop: Two percutaneously-placed trans-fascial sutures facilitated closure by this method, but were not absolutely necessary. Following placement of the guide wire, but prior to making the gastrotomy, suture (0-monofilament) was passed percutaneously, using laparoscopic suture passers, on either side of the guide wire. Under endoscopic visualization, the suture was passed back to the laparoscopic suture passer and removed percutaneously, creating an intragastric loop on each side of the guide wire. Following the gastrotomy and peritoneoscopy, these two sutures were put under tension to draw the stomach to the abdominal wall, aiding in insufflation of the stomach and visualization for closure. Endoscopic clips were applied in pairs opposite each other at the edges of the gastrotomy (Figure 15). An endoscopic loop was used to snare the two clips, drawing the edges of tissue together. Generally, three pairs of clips were used, but more were added if it appeared that the gastrotomy was not fully closed. The percutaneous sutures were removed.

Postoperative Evaluation of Closure: Once closure appeared complete, multiplanar, real-time, upper GI contrast fluoroscopy was performed under anesthesia in the operative suite. In the prone animal, a large bore orogastric tube was blindly passed into the stomach and 240 mL of water-soluble radiopaque contrast (MDGastroview (diatrizoate meglumine and diatrizoate sodium solution, Mallinckrodt) was infused. The percutaneous transabdominal access wire was left in place to serve as a radiopaque marker of the closure site to assist with fluoroscopic visualization. Anterior–posterior, lateral, and 45° oblique views of the stomach were obtained evaluating for intraperitoneal contrast leakage from the gastrotomy site. Results were immediately interpreted. If leakage was noted, the closure process was continued with additional device placement. For animals with leak-proof closure on contrast fluoroscopy, the transabdominal access wire was removed, anesthesia was terminated and the animal was allowed to recover. All animals received one dose of enrofloxacin antibiotic administered intramuscularly (2.5 mg/kg, Baytril, Bayer Healthcare LLC, Shawnee Mission, KA).

Postoperative Recovery and Screening: Upper GI Contrast Fluoroscopy:

Animals were extubated when clinically appropriate and followed a standardized care path. Animals were permitted to eat and drink without restriction immediately following the surgery. All animals were evaluated daily for the duration of the study by Case veterinary and surgical staff for food intake, pain, bowel and urinary function, and overall wellbeing. Pain medicine was given on a case-by-case basis at the shared discretion of surgical and veterinary personnel. On postoperative days 2 and 7, animals received repeat upper GI contrast fluoroscopic exams (UGI). Conscious sedation was obtained following intramuscular injection of 4 mg/kg of intramuscular tiletamine HCl and

zolazepam (Telazol, Fort Dodge, Animal Health). Animals were sedated to facilitate handling and examination; however, they remained conscious enough to swallow, cough, and maintain a protected airway. Each animal underwent repeated UGI following the aforementioned techniques. The presence or absence of intra-abdominal leakage was recorded for each animal at each time point. Animals returned to their housing facility for recovery following each fluoroscopic exam and were permitted to eat and drink as desired.

Necropsy and Closure Burst Testing: After 14 days, animals were euthanized with intravenous sodium pentobarbital (Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI) and a laparotomy was performed. The gastric closure was evaluated for injury to adjacent organs and adhesions. The peritoneal cavity was explored for abscess formation or other evidence of infection, adhesions, ischemic bowel or any other signs of pathology. Following thorough, systematic intra-abdominal exploration, the stomach underwent pressurized burst testing to evaluate long-term strength of the closure. Pressurized oxygen gradually inflated the stomach through tubing fastened in the esophagus, while intragastric pressure was simultaneously recorded with a digital central venous pressure/arterial blood pressure transducer (Hewlett Packard, Palo Alto, CA, model 68 #M1176a and #M1006b) secured in the duodenum. The stomach was submerged in water and failure was defined at the first sign of bubbling from the serosal surface of the stomach or complete rupture. Burst pressure as well as the location of each failure was recorded. Gastric burst testing was also performed on a control group of 40-kg pigs (n = 10) originally used for another series of nonsurgical experiments.

Endpoints and Data Analysis: In the postoperative period, clinical data were reported by veterinarians in conjunction with surgical staff. Evidence of peritonitis, pain, food intolerance, bowel movements, urination, and activity level were documented for each animal during the postoperative period and reported collectively as binary data relating to ability or failure to thrive. All fluoroscopic imaging was reduced to binary data depending upon the presence or absence of leak. Binary data collected at time of necropsy included evidence of infection (abscesses and/or granulomas) and evidence of injury to adjacent organs from closure. Time required for each closure was either recorded intraoperatively or calculated after each experiment using time coded video recording review and was treated as a continuous variable. Gastric bursting pressure was recorded as continuous numerical data compared between study and control groups. For study animals, the location of bursting failure was classified as occurring at the closure or remotely.

Closure times and gastric burst pressure data was analyzed using one-way ANOVA followed by Tukey post-hoc test, where applicable. For the remainder of the categorical and binary data, statistical comparison was performed with a Chi-square test. For all statistical comparisons, significance was defined as $p < 0.05$.

3.3 Results

Intraoperative: For all groups, gastric NOTES access was easily achieved in all study animals and peritoneoscopy revealed no injury related to obtaining peritoneal access. **Closure Time:** Total time necessary to complete the closure of the gastrotomy for each device evaluated is shown in Table VIII. Closure with the TPD (Group 1) was

statistically longer than for the Clip/Loops (Group 4), 45.00 +/- 16.69 min. vs. 19.21 +/- 11.58 min., respectively ($p = 0.013$). No other statistical differences were found between total closure times.

Within group one, TPD, closure times were further analyzed for each implant. Gastrotomies were closed with a median of 3 ± 0.74 suture implants (range, 2 to 4). Overall, mean time per implant placement was $14:28 \pm 09:00$ min. (range 3:04 to 36:50 min.). Time analysis was performed on only nine animals; videotape failure forced the exclusion of the first animal. The mean time for first, second, and subsequent implant placements was analyzed across all animals. For all animals, the mean time for the first implant was 23:19, whereas the mean time for second implant placement was 12:30 ($p < 0.002$). For closures requiring three implants ($n = 8$) and four implants ($n = 3$), mean time for each implant was 7:30 and 10:00, respectively ($p = 0.59$). The time to fire the first implant took significantly longer than that of the second, third, or fourth implants ($p < 0.005$). Total closure time did not differ significantly between the first and the second half of study animals (52:13 vs. 39:13, $p = 0.27$).

Postoperative: All animals recovered from anesthesia in a comparable manner and appeared to thrive in the early postoperative period. No animals required supplemental analgesics. **Leak:** With one exception, UGI revealed no leak in any pig stomach immediately postoperatively (day 0) or on day two or seven (Table IX). The one exception was the first pig in the TAS group, in which the radiogram demonstrated leak after the initial closure with two pairs of TAS devices. A third overlapping pair was subsequently placed and a repeated fluoroscopy was performed, revealing no leak. For

Table VIII. Total Closure Time

Group	1	2	3	4
Technique	TPD	TAS	FESD	Clip/Loop
Mean (min)	45.00*	25.45	35.59	19.21*
SD (min)	16.69	9.52	21.78	11.58

*p = 0.013

Table IX: Evidence of gastrotomy leak by contrast fluoroscopy

Leak	TPD	TAS	FESD	Clip/Loop
Day 0				
No	10	10	10	10
Yes	0	0	0	0
Day 2				
No	10	10	10	10
Yes	0	0	0	0
Day 7				
No	10	10*	10	10
Yes	0	0	0	0

*One procedure conducted on day 4 due to premature death

all subsequent TAS animals, a third pair was placed prior to fluoroscopy. Chi Square analysis for presence or absence of leak revealed no differences between treatment groups.

Failure to thrive: Failure to thrive was reported for three animals in the TPD group due to weight loss (Table X). Four animals in the TAS group were reported as failure to thrive due to lethargy, reduced interest in food or death ($n = 2$). On day seven, pig four was noted to be lethargic, was not eating and after consult with veterinary staff, was euthanized. Necropsy revealed pneumonia, but its origin and onset could not be definitively determined. Pig five from the TAS group died on postoperative day four. Necropsy revealed infarcted bowel. One animal in the FESD group failed to thrive as evidenced by reduced food intake and lethargy. All animals in the clip/loop group thrived ($p = 0.100$).

Evidence of Infection: Evidence of infection, most commonly the appearance of abscesses, was found in all groups (Table XI). No animal in any group showed any evidence of sepsis or peritonitis. One animal in group one had three subcentimeter, well-circumscribed, walled-off abscesses found in the omentum adjacent to the site of the fistula, the same animal with the gastrocolic fistula described in the next section. In group two, small abscesses or granulomas were found in five animals. The one pig which was euthanized early had lung fibrosis. Remaining animals in group two displayed diffuse flimsy adhesions. Six animals in group three had microabscesses or granulomas. The one animal from this group that failed to thrive also had dense adhesions and multiple abscesses. Remaining

Table X: Failure to thrive following gastrotomy closure

Fail to thrive	TPD	TAS	FESD	Clip/Loop
No	7	6	9	10
Yes	3	4	1	0

p = 0.100

animals had loose flimsy adhesions. Three of the ten animals in group four also displayed microabscesses or granulomas with few adhesions noted. Chi square analysis for evidence of infection demonstrated no statistical significance between groups ($p = 0.098$).

Evidence of injury: Injury due to the closure device was present in one animal in group one (TPD), one animal in group two (TAS) and one animal in group 3 (FESD) (Table XI). In one TPD animal, necropsy revealed an iatrogenic injury introduced during gastric closure. A small piece of colonic serosa was found incorporated into one gastric closure causing the development of a small gastrocolic fistula. The narrow (< 2 mm) fistula tract appeared to involve one corner of a PTFE pledget. The remainder of the animals demonstrated only loose, flimsy omental adhesions to the serosal aspect of each closure site. In one TAS animal, necropsy revealed the T-bar portion of the TAS suture device had penetrated the porcine spiral colon. For group three (FESD), one animal had a hematoma adjacent to the spleen. Presence of injury due to the closure was not detected at necropsy for any animals in group four. No statistical differences were found between groups ($p = 0.550$).

Gastric Burst Pressure: Following one exclusion in each of group one, two and four, 37 stomachs from the test groups and ten untreated control stomachs were evaluated for burst pressure at the conclusion of the 14 day study period (Table XII). The mean pressure necessary to rupture the stomach was 85.1 ± 16 , 93.4 ± 33.4 , 80.5 ± 22.1 , 81.7 ± 23.1 and 85.3 ± 26.9 mm Hg, for groups 1-4 and control, respectively ($p = 0.822$). Six of nine (66.7%) TPD animals and eight of ten (80%) TAS animals failed at a nonsurgical

Table XI. Evidence of infection and injury following gastrotomy closure

	TPD	TAS	FESD	Clip/Loop
Evidence of Infection (p = 0.098)				
No	9	5	4	7
Yes	1	5	6	3
Evidence of Injury (p = 0.550)				
No	9	9	9	10
Yes	1	1	1	0

site remote to the healed closure defect. All ten animals in each of the FESD and Clip/loop group failed remote to the surgical site. No statistically significant differences were found between groups for location of rupture ($p = 0.079$). For study animals failing at the closure defect, the mean burst pressure was 86.8 ± 9.8 mm Hg. There was no statistical difference in mean burst pressure between study animals failing at the closure sites vs. control ($p = 0.907$).

3.4 Discussion

Endolumenal therapies have long afforded alternatives to incision-based surgical approaches, sparing patients the added morbidity and mortality associated with conventional surgery. Endoscopic retrograde cholangiopancreatography, endoscopic colonic polypectomy, and percutaneous endoscopic gastrostomy tube are a few examples of surgical endoscopic procedures that have revolutionized standards of surgical care, and each has become a powerful tool in the armamentarium of general surgeons. In addition to being useful for closure of NOTES access points, tissue approximation and defect closure is an emerging application of contemporary endoscopic technology with a wide range of potential applications including gastric anastomotic leaks and gastric perforations.

A variety of endoscopic tools are available to assist with closure or coverage of endolumenal defects. Endoscopic clips, plugs, stents, tissue fasteners, staplers, and suturing devices have been used to close tissue defects but no one technology has demonstrated superiority, as evidenced by a lack of routine clinical use (Minami et al. 2006, Disibeyaz et al. 2005, Fong et al. 2007, Hausmann et al. 2006, Jagannath et al.

Table XII: Gastric Burst Pressure following gastrotomy closure

<i>Technique</i>	<i>TPD</i>	<i>TAS</i>	<i>FESD</i>	<i>Clip/Loop</i>	<i>Control</i>
Burst Pressure (mm Hg)	85.1 ± 16.1	93.4 ± 33.4	80.5 ± 22.1	81.7 ± 23.1	85.3 ± 26.9

p = 0.822

2005, Pham et al. 2006, Raju et al. 2006, Sclabas et al. 2006, Seaman et al. 2006, Sumiyama et al. 2007a). This current report investigated the safety and efficacy of endolumenal closure techniques for NOTES gastrotomy in a survival animal model.

In this preclinical, survival swine NOTES model, standardized gastric defects were closed using four different endolumenal closure techniques. Intraoperative and postoperative UGI fluoroscopy demonstrated leak-proof closure for all animals intraoperatively and on postoperative days two and seven. Upon ex vivo gastric burst testing, mean failure pressure of closures, for all groups, was commensurate with controls consisting of normal, nonsurgical gastric tissue (Table XII). Most stomachs failed burst testing at a nonsurgical site remote to the gastrotomy, indicating that the strength of the closure equaled or exceeded that of native, nonsurgical gastric tissue. Each of these techniques resulted in full-thickness serosal apposition and binding. Gastric defect closure with each of these modalities resulted in strong, leak-proof closure of gastric defects in the immediate postoperative period.

The clip/loop closure was the only group in which all animals thrived postoperatively. Still, all groups had at least one animal with evidence of infection at necropsy, with abscesses being present in 15 of the 40 animals. It is possible that a longer study period would have revealed additional clinically relevant signs of infection. Importantly, however, it must be noted that the evidence of infection is not exclusively a consequence of the effectiveness of the closure device. Each animal had an equivalent exposure risk during the time between gastrotomy creation and closure.

For the TPD device, there was one subclinical injury noted at necropsy, as one pledget was found incorporated into adjacent colon with evidence of a possible

gastrocolic fistula. Similarly, closure with the TAS device resulted in one injury which was deemed to be clinically insignificant. Additionally, one TAS animal died prematurely due to ischemic bowel, potentially resulting from strangulation secondary to adhesions. One animal in the FESD group was found to have a hematoma adjacent to the spleen. Whether this injury occurred as a result of closure or perhaps during placement of the guidewire for identification of the gastrotomy site could not be definitively determined.

Using time as a surrogate for technical difficulty, endoscopic closure of these defects by any of the four methods should be considered an advanced endoscopic skill. In the hands of two experienced endoscopists working simultaneously, plicated closure of gastric defects took, on average, 45 min. Several technical factors and properties inherent to the TPD add to the time necessary to perform this procedure. Each single-fire suture implant requires an extracorporeal reloading of the device, which adds greatly to the time required to close each defect. Thus, for each implant placed, a guidewire needs to be replaced, the device must be reloaded and reintroduced. Also adding to the length of the TPD procedure were difficulties initially visualizing the defect and apposing tissue. This may not necessarily reflect poor performance of the TPD device but, rather, difficulty inherent to the endoscopic nature of the procedure. The time to place the first implant for each closure took nearly twice as long as that of the second implant. This discrepancy indicates that obtaining initial apposition, visualization and delivery of the first implant is the most challenging step of obtaining closure. Inadequate gastric insufflation, which is caused by intra-abdominal leaks through the open gastrotomy, is probably the single largest factor adding to the time necessary for closure. Once the first implant was placed,

partial closure permitted adequate insufflation and visualization, as evidenced by a marked reduction in time necessary to place subsequent implants.

Improvements in the design of the TPD's integrated tissue grasper may improve closure times by temporarily apposing defect edges prior to implant delivery and improving intragastric insufflation. Additionally, the resultant robust closure is composed of the former defect edges invaginated into the gastric lumen bound with nonabsorbable, pledgeted, full-thickness sutures. One risk of this full-thickness closure is blindly incorporating adjacent organs into the closure. It is likely that the one injury encountered in this series of animals was due to colonic tissue inadvertently incorporated within the gastric closure. It is hypothesized that improvements with the device's tissue grasper may also eliminate the risk of collateral damage by allowing for improved tissue manipulation and selectivity prior to implant delivery.

The FESD device was technically the second most challenging technique, as indicated by the total time required to close the defect. As with the TPD device, the FESD involves a totally distinct device from the endoscope and extracorporeal reloading. The device requires a second skilled endoscopist to manipulate and position it in order to facilitate suturing. In preliminary experiments, the difficulty of engaging tissue without first retracting it into the device or creating a ridge of tissue was recognized. Stay sutures were used to provide a ridge of tissue that enabled suturing. Placement of the stay sutures not only added time, but in some cases also proved to be a hindrance to the movement of the FESD. Ex vivo work also led to the modification of the suture used for closing the gastrotomy to include barbs, ensuring unidirectional movement of the suture. While this simplified the process of tying the suture, the added drag on the suture

resulted in more manipulation of the device, increasing the difficulty of suturing. It was also not uncommon for the suture to release prematurely from the device, requiring an additional extracorporeally reloading. Further enhancements to the FESD such as greater range of motion, finer control of the needle and a tissue grasping mechanism may greatly improve its capabilities.

TAS was the third most technically challenging of the four methods evaluated. As with the previous two devices, TPD and FESD, the novelty of this instrument presented a learning curve which was addressed in ex vivo and acute models where it was recognized that this device presented a unique opportunity to place the T-bar loaded sutures at the site of the gastrotomy prior to creating it. This avoided the poor visualization due to inadequate gastric insufflation after creating the gastrotomy, as mentioned previously. TAS sutures were placed in circumferential locations relative to the guidewire and withdrawn extracorporeally through the mouth and paired. This made creation of the gastrotomy more challenging, however, as the endoscope was often entangled in the four parallel sutures. Unlike the TPD and FESD, an additional benefit to this device was that it is intra-endoscopic, allowing for multiple device use without removal of the endoscope and device to reload. Pre-placement of the sutures as in our technique, however, required multiple reintubations of the scope.

The clip/loop combination proved to be the least technically challenging. Each device was technically simple to operate with a familiar open and closed-hand technique common to many endoscopic devices. Similar to TAS, the clips and loops are intra-endoscopic devices enabling reloading without removal of the scope. Of the four techniques used, this is the only technique which could be completed with a single

intubation of the endoscope. The clips and loops offer the additional benefit of being commercially available and cost-effective for research purposes.

In the absence of standardized techniques to evaluate NOTES closures for leak, contrast fluoroscopy was chosen because of its familiarity, availability, and low cost. Both the surgical and radiological literatures, however, are replete with reports describing the limitations of contrast fluoroscopy. The false negative rates of upper GI contrast following esophagectomy approach 60%, whereas lower GI exams report false positive rates of 12% (Tirnaksiz et al. 2005, Akyol et al. 1992). Multislice CT scanning may improve diagnostic yield; however, the costs of such studies limit experimental use (Power et al. 2007).

Each of the four methods of closure of standardized gastric defects resulted in robust, leak-proof closure in the survival swine model. Only with the clip/loop closure was injury definitively avoided, raising questions about the safety of TPD, TAS and FESD. The relatively long closure times and high variability also expose the inefficiency of these techniques. While technological advancements and device development continues, the clip/loop technique proved useful for investigational NOTES procedures.

IV

THE INFLAMMATORY RESPONSE TO NOTES

4.1 Introduction

NOTES is an experimental paradigm involving the use of flexible endoscopic techniques to perform abdominal surgery by accessing the peritoneal cavity through natural openings such as the mouth. The list of diagnostic and therapeutic procedures which have been demonstrated to be feasible via a NOTES approach continues to grow (Onders et al. 2007b, Onders et al. 2007c, Miedema et al. 2009, Perretta et al. 2009, Freeman et al. 2009, Sherwinter and Eckstein 2009, Lomanto et al. 2009, Zacharopoulou et al. 2009, Raman et al. 2009, Leroy et al. 2009, Cahill et al. 2009). Natural orifice surgery is advertized as providing a less invasive approach to abdominal surgery, offering benefits to patients including less pain, faster recovery, fewer complications and no skin incisions. However, minimal access does not equate to minimally invasive. NOTES is not incisionless. Rather, skin incisions are exchanged for internal incisions to the gastrointestinal lumen. The unknown consequences of transluminal defects along with

the potential peritoneal contamination from intraluminal contents, gastric acids and bile present the potential for a catastrophic inflammatory response. The physiological impact of NOTES should be the primary gauge of the invasiveness of this approach.

Tissue injury, whether caused by trauma, bacteria, chemicals, heat or surgery, results in the complex physiological response of inflammation. Inflammation is characterized by cellular infiltration at the site of injury, followed by release of a cascade of inflammatory mediators such as cytokines and neurohumoral factors (Guyton and Hall 2006). The intensity of the inflammatory response is proportional to the degree of tissue injury (Guyton and Hall 2006), thereby providing a means of assessing the physiological impact of a surgical procedure. Measures of acute phase proteins, such as TNF- α , IL-1 β , IL-6, IL-8, IL-10, C-reactive protein and neurohumoral factors such as cortisol have proved useful as markers of the degree of surgical stress. In fact, many groups have used these markers to characterize the inflammatory response following laparoscopic surgery as compared to open laparotomy. These studies have demonstrated an attenuated acute phase response following laparoscopy, postulated to be due to the less traumatic approach (Matsumoto et al. 2005, Wu et al. 2003, Ure et al. 2002, Miyake et al. 2002, Balague et al. 1999, Collet et al. 1995, Allendorf et al. 1997, Novitsky et al. 2006, Burgos et al. 2005, Wichmann et al. 2005, Delgado et al. 2001, Burpee et al. 2002, Schietroma et al. 2004, Grande et al. 2002, Maruszynski et al. 1995, Torres et al. 2007, Jesch et al. 2006, Yahara et al. 2002, Yim et al. 2000, Nguyen et al. 2002, Jacobi et al. 1998, Kolvenbach et al. 1998, Jess et al. 2000, Bellon et al. 1997).

In contrast, to date, just two studies have been reported characterizing the inflammatory response induced by NOTES (McGee et al. 2008c, Bingener et al. 2009).

Both groups provided valuable data demonstrating that the acute phase inflammatory response induced following NOTES was not catastrophic, as had been anticipated for an approach through a contaminated alimentary tract. However, neither study convincingly demonstrated that the experimental model was sensitive enough to be able to distinguish between surgical approaches. McGee and colleagues measured serum levels of TNF- α , IL-1 β and IL-6 following a NOTES approach compared to conventional surgery and control. IL-1 β and IL-6 levels were mostly undetectable and peak TNF- α levels were indistinguishable between surgical groups and a sham surgery group (McGee et al. 2008c). Bingener's group measured serum TNF- α and IL-1 β but did not demonstrate a typical post-operative inflammatory response profile for either cytokine (Bingener et al. 2009). Several of the aforementioned studies assessing the inflammatory profile following laparoscopic surgery measured peritoneal fluid levels of cytokines following abdominal surgery and reported much higher levels than found in the serum (Matsumoto et al. 2005, Wu et al. 2003). The peritoneal fluid levels of proinflammatory cytokines accompanied by serum markers of C-reactive protein and cortisol may provide a more sensitive measure of the surgical stress induced by NOTES.

Determination of the inflammatory consequences of the NOTES approach is an important consideration in the utility of this novel concept (Rattner and Kalloo 2006). In order for NOTES to be a viable option of performing abdominal surgery the inflammatory response induced should not exceed that produced by laparoscopy or laparotomy. The objective of this study was to measure peritoneal levels of cytokines and systemic markers of inflammation to evaluate the surgical stress induced by NOTES

as compared to conventional surgery, testing the hypothesis that NOTES would evoke no greater of an inflammatory response than laparoscopy.

4.2 Methods

Animals: All experiments were conducted following approval from the the Case Western Reserve University Institutional Animal Care and Use Committee. Female domestic farm pigs (35-45 Kg) were obtained from a USDA-approved vendor (Pineview Farms, Valley City, OH) and underwent a seven day quarantine and acclimation period. Each animal was evaluated by veterinary personnel to ensure baseline health. All animals were subjected to the same husbandry procedures, fed the same diet and had unlimited access to water.

Groups: Animals were assigned to one of four study groups: NOTES peritoneoscopy (NOTES), exploratory laparoscopy (Lx), open laparotomy (Lap) and a sham procedure (Sham) control. Preoperative preparation, anesthesia, placement of an abdominal drain for peritoneal fluid sampling, placement of a venous catheter for blood sampling and post-operative care were identical in all groups.

Preoperative Preparation and Anesthesia: All animals were restricted from food 24 hours prior to the scheduled procedure but allowed unrestricted access to water. Animals were sedated with 10 mg/Kg intramuscular tiletamine HCl and zolazepam (Telazol, Fort Dodge, Animal Health, Fort Dodge, IA) and transported to the operating suite. Endotracheal intubation was performed with a endotracheal tube (6.0 mm) and animals were mechanically ventilated at 12 respirations/minute, with a tidal volume of 15-20 mL/Kg, and 100% oxygen. Inhaled isoflurane (AErrane, Baxter Healthcare Corp.,

Deerfield, IL) was administered at 1.5% for exactly 90 minutes while the animal underwent the predetermined procedure. Enrofloxacin (2.5 mg/kg, intramuscular) (Baytril, Bayer Healthcare LLC, Shawnee Mission, KA) was administered pre-operatively. Hair was removed from the anterior abdominal wall and neck and the skin was prepped with 2% chlorhexidine gluconate and 70% isopropyl alcohol (Chloraprep, Cardinal Health, Leawood, KS). Non-sterile areas were draped with sterile towels and sheets, exposing the mouth, right anterior neck and abdominal wall. Sterile instruments and techniques were used for placement of the drain and catheter.

Placement of Abdominal Drain for Peritoneal Fluid Sampling for Cytokine

Levels: Using the Hasson technique for peritoneal entry 2 cm below the umbilicus, a flat 10 mm Jackson-Pratt abdominal drain was inserted and directed into the pelvis.

Peritoneal fluid was immediately aspirated through the drain (0h) and stored for analysis of cytokine level. The drain was then tunneled subcutaneously to exit the skin in a more protected location, near the right flank and sealed with a stop-cock. The point of entry was closed in two layers, closing the fascia with a #0 glycolide/lactide copolymer suture (Polysorb, Syneture, Norwalk, CT) and the skin with interrupted #4-0 nylon suture (Monosof, Norwalk, CT). The drain was secured to the skin with interrupted sutures (#4-0 Monosof) along the length of the externalized portion of the drain tube.

Placement of Venous Catheter for Blood Sampling for CRP and Cortisol:

Simultaneously with placement of the abdominal drain, a second surgeon performed a cut-down to access the right internal jugular vein through a 5 cm vertical incision. The jugular vein was mobilized over a 3-5 cm length, a venotomy was performed and the catheter (Hickman, CR Bard Inc., Murray Hill, NJ) was inserted and advanced 10 cm

toward the chest. The distal vein was ligated, and the catheter was secured within the vein using #3-0 silk ties (Sof silk, Syneture, Norwalk, CT). The catheter was tested for patency and blood was drawn (0h). The catheter was flushed and filled with heparin between blood draws. The catheter was tunneled subcutaneously to exit the skin on the lateral neck. The incision was closed in two layers using a running #3-0 suture (Polysorb, Syneture, Norwalk, CT) to close the platysma and an interrupted skin closure with #4-0 nylon (Monosof, Syneture, Norwalk, CT). The external portion of catheter was secured to the skin with #2-0 nylon suture (Monosof, Syneture, Norwalk, CT).

Surgery: Animals assigned to the sham procedure received no further intervention. Animals assigned to the Lx group underwent a standardized three port abdominal exploration using sterile instruments and technique. A 12 mm trocar was placed at the umbilicus (superior to the drain position) using the Hassan technique.

Pneumoperitoneum was created with a laparoscopic insufflator to 15 mm Hg through the trocar. Two additional 5 mm ports were placed bilaterally along the linea semilunaris under laparoscopic visualization and a standardized 10 minute abdominal exploration was conducted using laparoscopic bowel graspers for tissue manipulation. At the completion of the exploration, trocars were withdrawn and the fascial umbilical defect was closed with a figure-of-eight #0 absorbable suture (Polysorb, Syneture, Norwalk, CT). Overlying skin was closed for all ports using #4-0 nylon (Monosof, Syneture, Norwalk, CT) in an interrupted fashion.

NOTES equipment and endoscopic instruments underwent high-grade disinfection with 0.55% ortho-phthalaldehyde solution (Cidex OPA Solution, Advanced Sterilization Products, Irvine, CA) for 20 minutes followed by a rinse in 70% isopropyl alcohol,

otherwise the NOTES procedure was not considered to be conducted using sterile technique. Following endoscopic gastric intubation, gastric fluids were aspirated with the endoscope but the stomach was not lavaged or treated with antimicrobial agents.

Animals assigned to the NOTES group underwent standardized gastric NOTES access, as described previously (Chapter III) (McGee et al. 2007). Once the scope was passed intra-abdominally, a standardized 10 minute exploration was conducted using endoscopic biopsy forceps for tissue manipulation. The NOTES gastrotomy was closed with an endoscopic tissue plicating device (NDO Plicator, NDO Surgical, Mansfield, MA), using the technique previously reported (Chapter III).

Animals assigned to Lap underwent a midline laparotomy extending from the xiphoid process 25 cm inferiorly using sterile instruments and technique throughout. The laparotomy was created with a #10 scalpel blade to incise the skin and electro-surgical dissection of the remaining layers. The standardized 10 minutes of exploration was conducted using sterile hands to assist with tissue manipulation. The laparotomy was closed in layers using a running looped #1 glycolide and trimethylene carbonate suture (Maxon, Syneture, Norwalk, CT) to close the fascia and with #4-0 nylon (Monosof, Syneture, Norwalk, CT) in an interrupted fashion to close the skin. Laparotomy incisions were covered with a protective dressing (Tegaderm, 3M, St. Paul, MN).

Recovery, Post-Operative Observation and Fluid Collection: The same recovery protocol was employed in all animals. After 90 minutes of anesthesia, inhaled isoflurane was stopped and once adequate respiration returned the animals were extubated. Repeated blood and peritoneal fluid collections were performed at hours 2 (2h), 4 (4h), and 6 (6h) from the initiation of anesthesia. Subsequent collections occurred on post-

operative days 1 (1d), 2 (2d), and 7 (7d). For pain control, a prophylactic single dose of buprenorphine (5 ug/kg) was injected intra-muscularly at the time of the 2h fluid collection. Animals were permitted to eat and drink immediately following surgery and were observed by a team of veterinary and surgical personnel daily throughout the study period. Following fluid collection on post operative day seven, animals were euthanized. An exploratory laparotomy was performed on non-sham animals to assess for evidence of iatrogenic injury.

Sample Analysis: Blood (serum) samples were analyzed for C-reactive protein (CRP) and cortisol by an independent laboratory (University Hospitals Case Medical Center core laboratory, Cleveland, OH). The CRP assay was based on a particle enhanced turbidimetric immunoassay (PETIA) technique. Latex particles coated with antibody to C-reactive protein aggregate in the presence of CRP in the sample. The increase in turbidity which accompanies aggregation is proportional to the concentration of CRP. The cortisol assay was a competitive immunoassay using chemiluminescent technology. Cortisol in the sample competes with acridinium ester-labeled cortisol for binding to polyclonal antibody in a solid phase. The acridinium-produced chemiluminescence is inversely proportional to the concentration of cortisol in the sample.

Peritoneal fluid was aliquoted and stored at -80°C until analysis. The concentrations of TNF- α , IL-1 β , and IL-6 were measured in the peritoneal fluid using a quantitative “sandwich” enzyme-linked immunosorbent assay (ELISA) kit (R & D System, Minneapolis, MN) according to the manufacturer’s instructions. Samples were dispensed into 96-well microtiter plates containing an immobilized monoclonal antibody specific

for the relevant porcine cytokine. After incubation, unbound protein was rinsed from the wells and then an enzyme-linked antibody was added. After further rinsing to remove unbound enzyme-linked antibody, a substrate solution was added. The substrate solution reacts with the bound enzyme to produce a blue color with an intensity proportional to the amount of cytokine bound. Assays were performed on duplicate samples. Cytokine concentrations were determined spectrophotometrically at 450nm with a plate correction of 540nm by comparing to a standard curve using a four parameter logistic curve-fit. Samples exceeding the linear range of the assay were diluted and reassayed.

Data Analysis: Levels of peritoneal and serum inflammatory markers were analyzed using a two-factor repeated measures ANOVA (one-repeating factor) with Tukey post hoc testing (SimgaStat version 3.5, Systat Software, Inc., Point Richmond, CA). Peak levels were compared to baseline levels within each group and peak levels between groups were compared. Sample size calculation was conducted using preliminary data suggesting a standard deviation equal to 0.55 times the difference in means with alpha set at 0.05 and beta at 0.2 (power = 0.8). p-values less than 0.05 were regarded as significant. All data are reported as mean \pm SEM.

4.3 Results

Four animals were excluded from the study; one in each of the NOTES and Sham group due to bowel injury incurred upon placement of the abdominal drain, one NOTES animal which developed rectal prolapse intraoperatively and one Lx animal which died the day after the procedure from pneumonia. All remaining animals appeared to thrive post-operatively. No observable differences were noted between animals during the

study period. No iatrogenic injuries were found at necropsy and no animals were excluded retrospectively. For the nine animals included in the Lx group, 62/63 peritoneal fluid samples were analyzed for cytokine levels and 63/63 blood samples were analyzed for CRP and cortisol. For the ten animals included in the NOTES group, 69/70 peritoneal fluid samples were analyzed for cytokines and 59/70 blood samples were analyzed for CRP and cortisol. The Lap group contained five animals and all 35 samples were analyzed for cytokines with 34/35 available for CRP and cortisol. The sham group consisted of five animals. 34/35 peritoneal and 23/35 blood samples were available for analysis. Missing peritoneal samples were due to the inability to aspirate from the abdominal drain for that time point. Missing blood samples were due to the inability to aspirate from the indwelling catheter, improper storage of the sample prior to analysis or inability of the core lab to conduct the test.

Cytokines: **TNF- α** levels increased steadily and reached a significantly higher peritoneal concentration by 4 hours compared to 0 hours for both Lx ($3,801.4 \pm 941.5$ pg/mL vs. 55.6 ± 20.2 pg/mL; $p < 0.001$) and NOTES ($2,181.4 \pm 541.0$ pg/mL vs. 33.4 ± 11.9 pg/mL; $p < 0.001$) groups (Figure 16). Although there was a slight increase in peritoneal concentration of TNF- α at 4 hours compared to 0h for Lap (576.1 ± 169.3 pg/mL vs. 52.3 ± 16.2 pg/mL), and Sham (260.9 ± 207.6 pg/mL vs. 35.9 ± 14.8 pg/mL), the rise was not statistically significant compared to 0 hour. TNF- α concentration had returned to baseline levels by day 1 for all groups ($p = 1.000$ vs. 0h for each group).

Baseline levels (0h or 1d) did not differ significantly between groups ($p = 1.000$ for 0h and $p \geq 0.994$ for 1d for all comparisons) Peak (4h) TNF- α levels for Lx (3801.4 ± 941.5 pg/mL) and NOTES ($2,181.4 \pm 541.0$ pg/mL) were significantly higher than

compared to the same time point for either Lap (576.1 ± 169.3 pg/mL) or Sham (260.9 ± 207.6 pg/mL) ($p < 0.001$ for Lx vs. Sham or Lap; $p = 0.007$ for NOTES vs. Sham; $p = 0.037$ for NOTES vs. Lap). Furthermore, TNF- α levels at 4h for Lx were significantly higher compared to levels at 4h for NOTES ($p = 0.007$). Differences in 4h levels of TNF- α between Lap and Sham were not significant ($p = 0.969$).

IL-1 β levels increased significantly reaching peak peritoneal concentrations at 6 hours compared to 0 hours for Lx ($104,468.7 \pm 37,125.1$ pg/mL vs. 4.7 ± 4.7 pg/mL; $p < 0.001$) and NOTES ($62,104.4 \pm 13,348.8$ pg/mL vs. 106.4 ± 88.0 pg/mL; $p = 0.001$) groups (Figure 17). The increase in peritoneal IL-1 β levels at 6 hours compared to 0 hours for Lap ($48,596.5 \pm 28,760$ pg/mL vs. 67.6 ± 35.5 pg/mL; $p = 0.291$) and Sham ($5,098.2 \pm 2,309.6$ pg/mL vs. 16.7 ± 10.8 pg/mL; $p = 1.000$) was not statistically significant. Peritoneal concentrations of IL-1 β had returned to baseline levels by day 2 for all groups ($p = 1.000$ vs 0h).

Baseline levels (0h and 2d) between groups did not differ significantly ($p = 1.000$ for 0h and $p = 1.000$ for 2d for all comparisons). Peak (6h) levels of IL-1 β for Lx ($104,468.7 \pm 37,125.1$ pg/mL; $p < 0.001$) and for NOTES ($62,104.4 \pm 13,348.8$ pg/mL; $p = 0.020$) were significantly higher compared to levels at the same time point in the Sham group ($5,098.2 \pm 2,309.6$ pg/mL). Additionally, the IL-1 β level at 6 hours was significantly higher in the Lx group compared to the Lap group ($p = 0.027$) and approached statistical significance compared to the NOTES group ($p = 0.050$). Peritoneal concentrations of IL-1 β at 6h were not significantly different for Sham compared to Lap ($p = 0.222$).

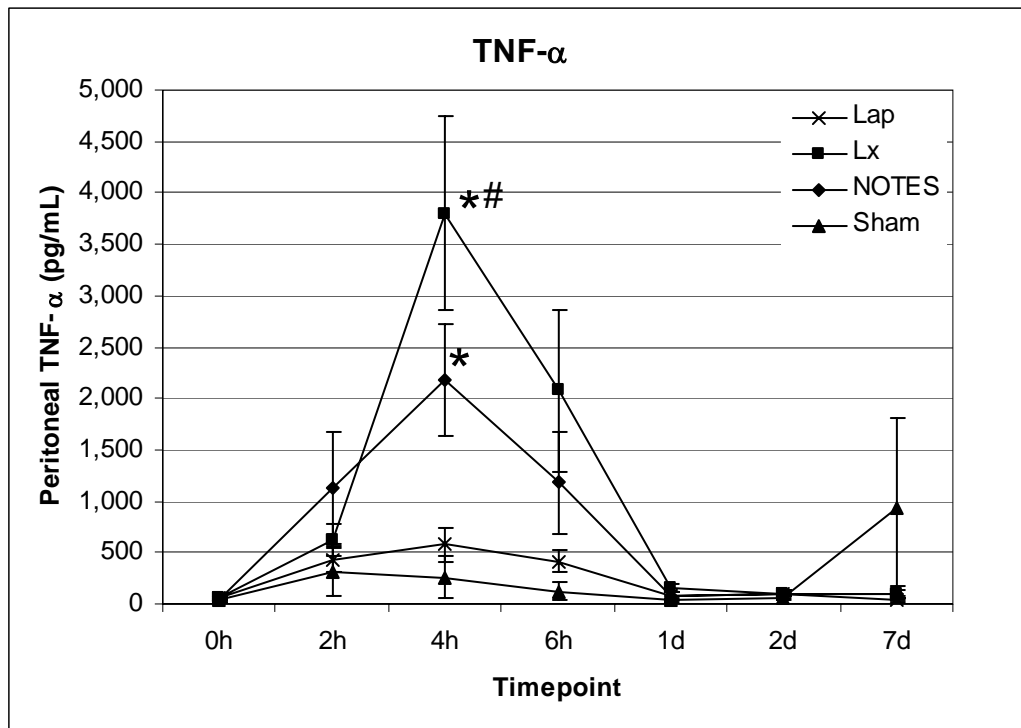


Figure 16. TNF- α levels in peritoneal fluid. Lap, Laparotomy; Lx, Laparoscopy. * $p < 0.05$ compared to 0h within group and compared to Sham or Lap at 4h. # $p < 0.05$ compared to NOTES at 4h.

IL-6 concentrations in the peritoneal fluid increased significantly, reaching peak levels at 6 hours compared to 0 hours for Lx ($12,576.3 \pm 1,709.2$ pg/mL vs. 200.6 ± 53.2 pg/mL; $p < 0.001$), NOTES ($11,275.5 \pm 1,433.6$ pg/mL vs. 435.2 ± 216.2 pg/mL; $p < 0.001$) and Lap ($29,207.4 \pm 4,287.9$ vs. 0.0 ± 0.0 pg/mL; $p < 0.001$) groups (Figure 18). The increase in IL-6 levels at 6 hours in the Sham group ($2,486.6 \pm 1,127.4$ vs. 19.4 ± 17.0 pg/mL; $p = 0.957$) was not statistically significant. Baseline levels of IL-6 were restored by day 1 for all groups ($p \geq 0.855$ vs 0h).

Baseline levels (0h and 1d) did not differ significantly between the four groups ($p = 1.000$ for 0h and $p \geq 0.770$ for 1d). The IL-6 concentration in peritoneal fluid at 6 hours was significantly higher in Lap ($p < 0.001$), Lx ($p < 0.001$) and NOTES ($p < 0.001$) compared to the same time point in the Sham group. Additionally, 6 hour IL-6 levels for the Lap group were significantly higher compared to NOTES ($p < 0.001$) or Lx ($p < 0.001$) at that time. Peak (6h) levels of IL-6 did not differ significantly between NOTES and Lx ($p = 0.893$).

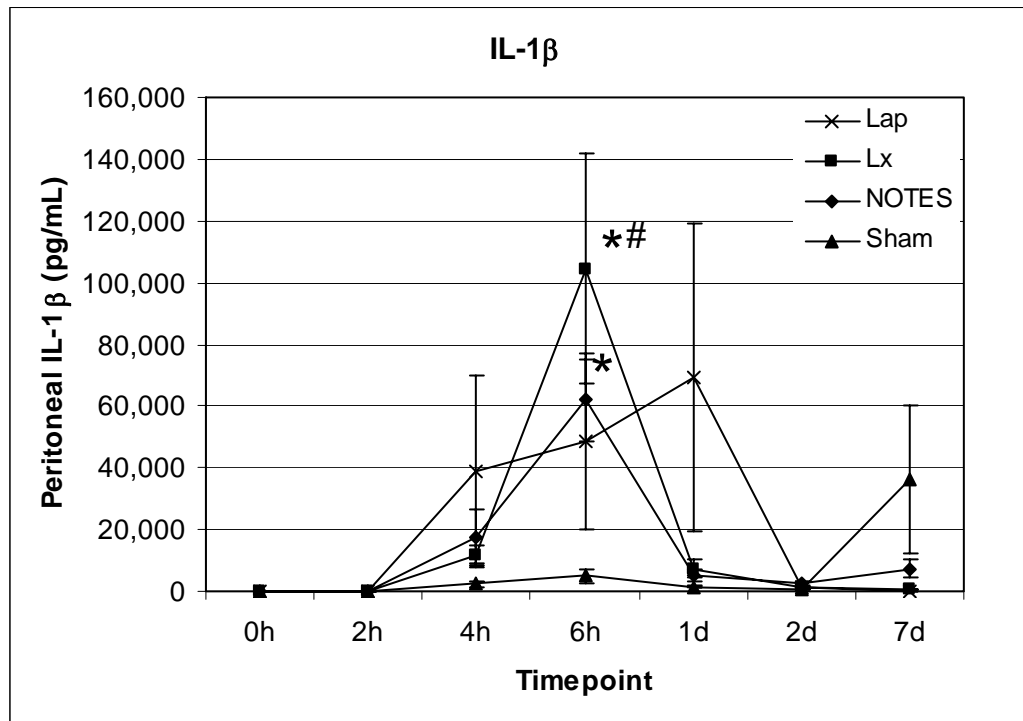


Figure 17. IL-1 β levels in peritoneal fluid. Lap, Laparotomy; Lx, Laparoscopy. * $p < 0.05$ compared to 0h within group and compared to Sham at 6h. # $p \leq 0.05$ compared to NOTES or Lap at 6h.

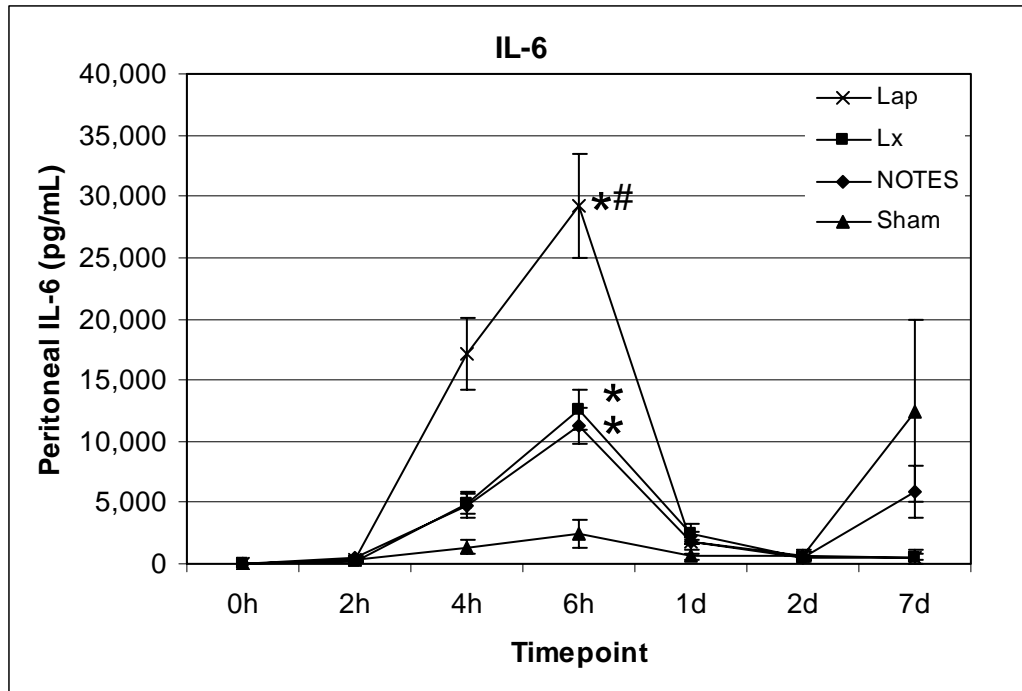


Figure 18. IL-6 levels in peritoneal fluid. Lap, Laparotomy; Lx, Laparoscopy. * $p < 0.05$ compared to 0h within group and compared to Sham at 6h. # $p < 0.05$ compared to NOTES or Lx at 6h.

CRP levels increased significantly by day 1 compared to 0 hour for Lx (1.09 ± 0.06 mg/dL vs. 0.00 ± 0.00 mg/dL; $p < 0.001$), NOTES (0.95 ± 0.07 mg/dL vs. 0.00 ± 0.00 mg/dL; $p = 0.015$) and Sham (1.43 ± 0.15 mg/dL vs. 0.00 ± 0.00 mg/dL; $p = 0.010$) groups (Figure 19). The increase in serum CRP at day 1 compared to 0 hour, however, was not significant for the Lap group (0.80 ± 0.10 mg/dl vs. 0.00 ± 0.00 mg/dL; $p = 0.248$). Differences between groups at baseline or at peak CRP levels (1d) were not statistically significant.

Cortisol concentration in the plasma increased to a maximal level at 4 hours. The increase at 4 hours was significantly higher compared to baseline (0h) for Lx (15.96 ± 0.27 ug/dL vs. 5.18 ± 0.26 ug/dL; $p < 0.001$), NOTES (12.61 ± 0.32 ug/dL vs. 5.74 ± 0.37 ug/dL; $p < 0.001$) and Lap (17.65 ± 1.03 ug/dL vs. 3.40 ± 0.39 ug/dL; $p < 0.001$) groups (Figure 20). The 4h increase in serum cortisol level in the Sham group (9.65 ± 1.27 ug/dL vs. 5.43 ± 0.80 ug/dL; $p = 0.691$) was not significantly elevated compared to 0h. Baseline levels were restored by day 1 for all groups.

Differences between groups in cortisol concentration at baseline (0h and 1d) or at 4h were not significant. However, differences between Lap and either Sham or NOTES approached statistical significance (Lap vs. Sham; $p = 0.069$ and Lap vs. NOTES; $p = 0.051$).

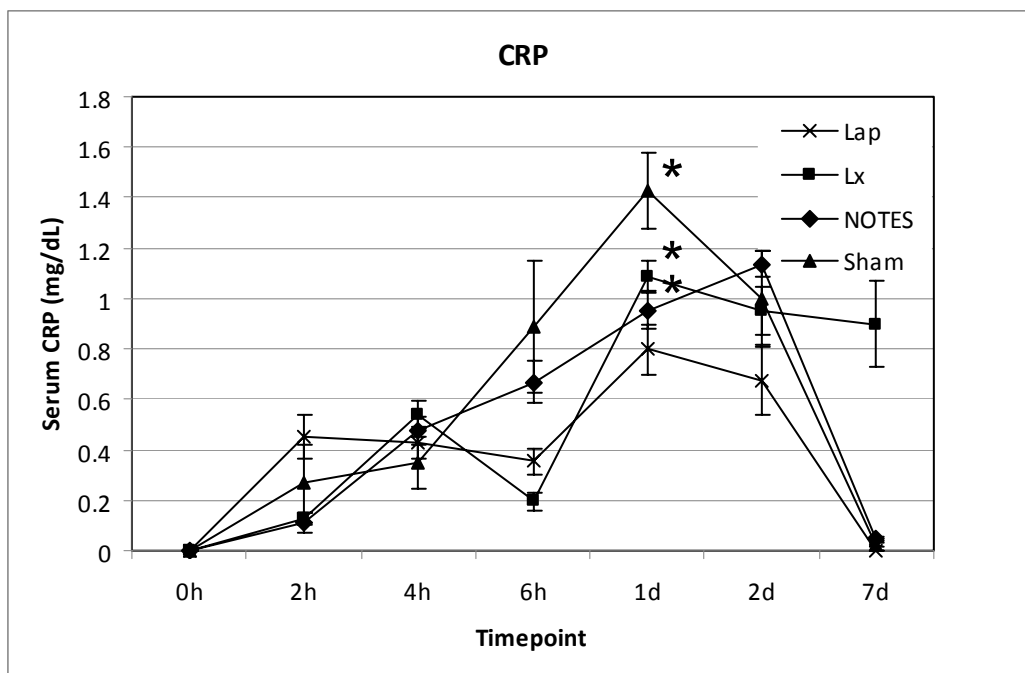


Figure 19. CRP levels in serum. Lap, Laparotomy; Lx, Laparoscopy. * $p < 0.05$ compared to 0h within group.

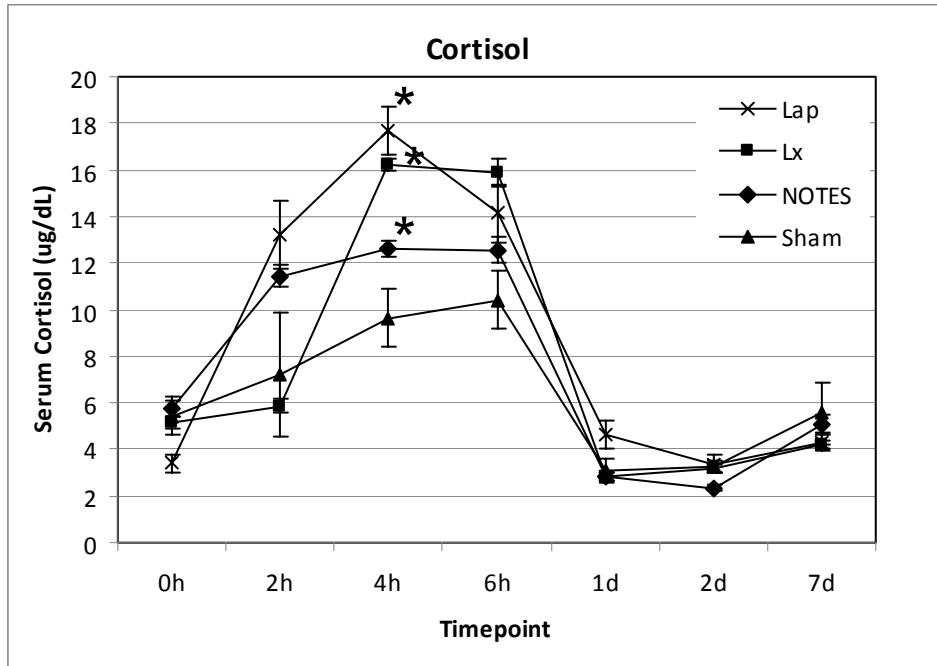


Figure 20. Cortisol levels in serum. Lap, Laparotomy; Lx, Laparoscopy. * $p < 0.05$ compared to 0h within group.

4.4 Discussion

Natural orifice surgery, while hindered by technical challenges, is touted as providing a less invasive approach. Invasiveness of a surgical procedure includes not only the physical injury produced but also the metabolic or physiological changes, as well. One of the important barriers to the advancement of NOTES into clinical practice is an understanding of the inflammatory consequences (Rattner and Kalloo 2006). NOTES, by definition, avoids skin incisions and consequently any incision-related complications. To provide a benefit, therefore, the adverse physiological consequences of NOTES need not be diminished relative to conventional approaches but need only to be equivalent.

Determination of the extent of surgical stress or tissue injury is often assessed using markers of inflammation. Cytokines TNF- α , IL-1 β and IL-6 are members of the group of acute phase proteins which play a vital role in the macrophage-neutrophil response to inflammation (Guyton and Hall 2006). CRP is non-specific marker of inflammation that correlates well with the extent of disease (Goodman 2004). Cortisol is a hormone released by the adrenal gland in response to stress, either physical or neurogenic, resulting in anti-inflammatory effects (Guyton and Hall 2006). Collectively, these markers can provide a means of assessing the magnitude of the acute inflammatory reaction to a surgical intervention. Each of these markers has been widely used in evaluating the physiological responses to surgery (Matsumoto et al. 2005, Wu et al. 2003, Ure et al. 2002, Miyake et al. 2002, Balague et al. 1999, Collet et al. 1995, Allendorf et al. 1997, Novitsky et al. 2006, Burgos et al. 2005, Wichmann et al. 2005, Delgado et al. 2001, Burpee et al. 2002, Schietroma et al. 2004, Grande et al. 2002, Maruszynski et al.

1995, Torres et al. 2007, Jesch et al. 2006, Yahara et al. 2002, Yim et al. 2000, Nguyen et al. 2002, Jacobi et al. 1998, Kolvenbach et al. 1998, Jess et al. 2000, Bellon et al. 1997).

This study performed standardized abdominal exploration in a chronic porcine model by three different surgical approaches; NOTES, laparoscopy or open laparotomy as compared to a sham surgery. Peritoneal fluid and blood samples were collected temporally for quantifying peritoneal TNF- α , IL-1 β and IL-6 as well as serum CRP and cortisol as markers of the inflammatory response induced by each surgical approach. The surgical procedure; ten minutes of exploration, was intentionally simplistic in order to not mask the inflammatory response caused by the approach with that of the procedure.

As anticipated, peritoneal fluid concentrations of cytokines became elevated two to three orders of magnitude greater than levels previously reported in serum (McGee et al. 2008c) and allowed for detectable levels of all cytokines including IL-1 β and IL-6 in all groups. The sham group received the identical treatment to all other groups except for the surgical approach and abdominal exploration. Sham animals had a modest inflammatory response with peak peritoneal cytokine and serum cortisol levels not reaching a statistical difference from baseline levels. Serum CRP levels, however, did increase above baseline levels on post-operative day 1. These data suggest that the distinct increases in inflammatory markers seen in other groups were not a result of anesthesia or placement of the drain and catheter required to obtain fluid samples. The increases in inflammatory markers seen in Lx, Lap and NOTES groups can be attributed to the approach and/or surgical procedure, validating the utility of this model for assessing surgical stress.

The Lap group had the greatest total incision length but did not show the greatest inflammatory response. Following open laparotomy, peritoneal TNF- α , IL-1 β and serum CRP levels did not increase significantly above baseline levels. However, IL-6 and cortisol did increase significantly relative to baseline and returned to basal levels by day 1. In fact, IL-6 levels at the 6 hour time point were significantly higher than levels found in the other three groups.

Animals in the Lx group, undergoing standard three port laparoscopy, experienced a significant rise in all markers of inflammation assessed in this study. Peritoneal TNF- α , IL-1 β , IL-6 and serum CRP and cortisol levels, all rose significantly relative to baseline levels. Peak levels for TNF- α and IL-1 β in the Lx group were significantly higher than peak levels in the other three groups. Peak levels of IL-6, CRP and cortisol following Lx, however, could not be distinguished from peak levels following NOTES.

A NOTES approach, as with laparoscopy, resulted in a significant rise compared to baseline levels in all markers of inflammation assessed in this study. The peak levels of TNF- α and IL-1 β following NOTES, however, were significantly lower than that induced by Lx. NOTES also resulted in significantly lower peak levels of IL-6 relative to the level resulting from open laparotomy. CRP and cortisol increases following NOTES were indistinguishable from increases produced in other groups.

These data demonstrated that, for all markers of inflammation measured, the response induced by NOTES did not exceed the response induced by Lx. If each of these markers was an absolute measure of the invasiveness of the procedure, however, this data would have also theoretically demonstrated that the inflammatory response induced following

Lap would have exceeded that produced following NOTES and/or Lx. This was not the case for TNF- α , IL-1 β or CRP.

Considering the complexity of the inflammatory response, departures from ideal data are not unexpected. Although, as mentioned earlier, other studies have generally concluded that the inflammatory response following laparotomy is greater than that following laparoscopy, for any given marker, results are not uniform. For example, Matsumoto and colleagues conducted a similar study comparing the inflammatory response following nephrectomy via laparotomy, laparoscopy and hand-assisted laparoscopy (Matsumoto et al. 2005). Although they noted differences between hand-assisted and the other two groups, they found no differences in levels of peritoneal TNF- α , IL-1 β , IL-6 or serum cortisol at 4 hours between laparotomy and laparoscopy. Likewise, Ure and colleagues using a chronic pig model for investigating the use of CO₂ gas instead of air for obtaining pneumoperitoneum demonstrated significant elevations of peritoneal IL-6 with laparotomy compared to laparoscopy, but found no differences in TNF- α and IL-1 β levels (Ure et al. 2002). Effective assessment of the physiological consequences of a surgical procedure requires the collective evaluation of several markers of inflammation.

A second, perhaps more intriguing explanation for the relatively smaller rise in TNF- α and IL-1 β levels following laparotomy than would be expected by the invasiveness of the approach, is if the local measurement of these mediators of inflammation are more indicative of the level of peritoneal trauma. As described earlier, the faux procedure of ten minutes of peritoneal exploration was conducted for the lap group using gentle manual manipulation of the bowel and organs whereas in the Lx and NOTES groups,

graspers were used. It was noted during the procedures that following grasping the bowel, a white patch often remained on the bowel for several minutes. This white area could represent ischemic damage or tissue injury leading to an enhanced inflammatory response in these two groups. A better experimental design, in this case, would have been to exclude the ten minutes of exploration altogether to truly assess the inflammatory response of the approach, independent of the procedure.

This chronic porcine model, to evaluate surgical stress using peritoneal and systemic markers of inflammation, proved to be a reliable model, as evidenced by the lack of a significant inflammatory response in the sham group. Overall, laparoscopy produced the greatest inflammatory response of the four groups. The inflammatory response following NOTES was significantly less than laparoscopy for TNF- α and IL-1 β . For all peritoneal and serum markers evaluated, peak levels following NOTES did not exceed those following laparoscopy. The data supports the conclusion that NOTES induces an inflammatory response that is no greater than that induced following laparoscopy.

V

CLOSING REMARKS

5.1 Summary

Minimizing the injury necessary to provide treatment should be the goal of all medical practitioners. From numbing the skin before an injection to reducing the size of the incision for organ removal, the objective is the same. As endoscopy has evolved from a diagnostic technique to a therapeutic approach and surgery has continued to look toward less invasive approaches, the two fields have merged into the unique discipline of surgical endoscopy, epitomized by NOTES. While minimally invasive surgery has been synonymous with laparoscopy for years, the birth of natural orifice surgery has given a second meaning to this phrase.

This revolutionary surgical concept of using a natural orifice to gain access to the abdominal cavity exhilarated surgeons, endoscopists, gastroenterologists and urologists, prompting a wealth of preliminary research demonstrating the feasibility of a wide range of procedures (Onders et al. 2007b, Onders et al. 2007c, Miedema et al. 2009, Perretta et al. 2009, Freeman et al. 2009, Sherwinter and Eckstein 2009, Lomanto et al. 2009, Zacharopoulou et al. 2009, Raman et al. 2009, Leroy et al. 2009, Cahill et al. 2009). Success in animal studies influenced some to venture into modified or hybrid procedures

in humans, combining a natural orifice access route with conventional laparoscopic access ports (Noguera et al. 2009, Navarra et al. 2009 Palanivelu et al. 2009, Auyang et al. 2009, Abe et al. 2009). A few studies questioned the physiological consequences of a natural orifice approach, investigating the infectious implications and inflammatory response (McGee et al 2008b, McGee 2008c, Bachman et al. 2009, Narula et al. 2008, Bingener et al. 2009).

This project adds to the growing body of work in the field of NOTES. Data from the access study supports the safe nature of a transgastric approach and provides potential for the use of endoscopic ultrasound for safe access at alternate sites. An evaluation of several closure modalities demonstrated diverse methods each providing a robust closure. Additionally, markers of inflammation were found to be elevated no higher in NOTES, despite crossing the non-sterile gastrointestinal lumen, than in laparoscopy. The technical challenges, however, were recognized, as well, with simple surgical procedures being prolonged due to the limited endoscopic capabilities.

The consensus of the literature, to date, is that NOTES is feasible, safe and, surprisingly, less of a physiological burden than expected. But feasibility is not equivalent to practicality. Before NOTES will be accepted into routine clinical use, numerous aspects of this new surgical paradigm still need evaluation, such as technological developments, cost, demonstration of a true benefit, training of practitioners and patient perception of this unusual approach.

5.2 Technological Developments

Improved instrumentation to simplify the technical challenges may be key to acceptance by surgeons. As discussed in chapter II, pertaining to the access data, the transgastric access techniques served as a surrogate to the technical difficulties faced when using flexible endoscopy for simple surgical procedures. Encumbrances with current endoscopes include the inability to lock the scope in a flexed position, the inability to triangulate instrumentation for retraction and exposure, the small size of the instrument channels, inability to alter the field of view relative to the instruments, lack of control of pneumoperitoneum and poor suction and irrigation capabilities, not to mention the limitations of the endoscopic devices themselves (Bardaro and Swanstrom 2006).

Redesigning the endoscope for the intended use of NOTES may vastly reduce these problems. Several prototype devices are being developed including the R-Scope (Olympus America, Center Valley, PA), the Cobra and TransportTM (USGI Medical, San Juan Capistrano, CA). The R-Scope was evaluated in our laboratory in an unpublished study to test the efficacy of this scope for assessing bowel pathology in an ICU setting. The R-scope has a standard outer diameter of 1.5 cm and contains two working channels each 3.8 mm. The distal end of each channel is equipped with a deflector control on the handle which allows articulation of the endoscopic instruments in perpendicular planes. While this modification to a standard scope adds a bit of triangulation to the device, the movements are still minimal. The TransportTM offers considerably more modifications which seem well-suited for NOTES procedures. This device is only slightly larger than a standard scope at 1.6 cm, but contains four channels, one large enough to accommodate a slim 6 mm scope and a second 4 mm channel capable of handling larger devices.

Additionally, the scope is capable of 180° retroflexion and can be locked in a rigid conformation. The device can also be attached to a laparoscopic insufflator for control of pneumoperitoneum (Bardaro and Swanstrom 2006). The Cobra is designed with three independent distal arms to provide triangulation, as afforded in laparoscopic surgery (Bardaro and Swanstrom 2006). Continued development and testing is required to determine whether these devices can alleviate the shortcomings of current endoscopes.

5.3 Cost of NOTES

No analysis of the cost associated with NOTES procedures has yet been reported. Since pure NOTES procedures are yet to be conducted, the current costs of hybrid NOTES/laparoscopic procedures should be equivalent to the laparoscopic costs, not accounting for the potentially longer procedural length. As procedures become truer to NOTES, depending less on accessory percutaneous ports, they are still likely to be conducted in the operating room (OR) and costs may remain similar. Once confidence is gained with the success of NOTES procedures, the goal is to move the procedure from the OR to the endoscopy suites or to outpatient clinics. Then the cost of procedures could be greatly reduced, avoiding the exorbitant OR charges. The current cost of a PEG tube placement performed in the OR is \$2,600 compared to \$2,200 if done in the endoscopy suite (University Hospitals of Cleveland, Financial Office). We must, however, keep in mind that cost is always relative to benefit. If the benefit to the patient is great enough, issues of the cost of the procedure are more likely to be resolved.

5.4 Demonstration of a True Benefit

Whether NOTES will offer a benefit to the patient is not just speculative, since NOTES, by definition, will result in no scars and no complications from skin incisions. Still, these benefits must outweigh any additional consequences. Until NOTES can be performed routinely in patients, the full spectra of benefits may not be realized. NOTES, once technically reproducible, will likely take a while to gain widespread use and acceptance, as was the case for laparoscopy. The benefit of smaller incisions with laparoscopy compared to open procedures was evident, as with NOTES compared to laparoscopy, but whether that alone would be sufficient for the procedure to be adopted was less evident. Evaluating the benefits of laparoscopy has proven difficult with the multitude of uncontrolled variables inherent to surgical procedures. Laparoscopy has gained gradual acceptance within the medical community as outcomes prove to be no worse than with open surgery. Laparoscopy has, generally, been demonstrated to require a shorter recovery period with less pain, but little difference has been shown in morbidity or mortality (Meyer et al. 1993, Balique et al. 1993, Habib et al. 1997, Murray et al. 2006, McCormack et al. 2003, Medeiros et al. 2008). Additionally, the cost of laparoscopic procedures exceeds the cost of open procedures due to more sophisticated equipment requirements and longer operative times (Meyer et al. 1993, Habib et al. 1997, Murray et al. 2006, Medeiros et al. 2008). NOTES may follow a similar path of acceptance, with outcomes not necessarily needing to be superior to laparoscopy, but rather, proving no worse than laparoscopy, along with the added benefits of reduction in recovery time, less pain and no visible scars.

5.5 Training NOTES practitioners

Who will perform NOTES procedures? As mentioned previously, NOTES appeared at the intersection of less invasive surgery and more invasive endoscopy. Will the procedures be performed by general surgeons, endoscopists, both or neither; will they require a new hybrid practitioner? The knowledge base for NOTES will need to include gastrointestinal (GI) anatomy, physiology and pathology as well as abdominal anatomy, physiology and pathology. Likewise, the practitioner will need to be skilled in both endoscopic diagnostic and therapeutic techniques as well as open and laparoscopic surgery. Current training for gastroenterologists includes three years of internal medicine residency, three years of GI fellowship and one optional year of advanced endoscopy. General surgeons complete five years of surgical residency with an optional fellowship, potentially including advanced endoscopy. It is evident that the skills which surgeons lack, gastroenterologists possess, and vice versa. One proposed training paradigm for future NOTES interventionalists would include three years of general surgery residency focused on GI diseases and three years combined GI and advanced endoscopy training. For now, a combined effort of surgeons and gastroenterologists is recommended.

5.6 Patient Perception

Another important consideration for the advancement of NOTES into clinical practice is the perception of this inside-out approach to the patient. Will patients be able to accept the potential added risk associated with this novel approach for the advertised benefits? In a small, yet to be published study at our institution, the health utility measure for NOTES was determined in a group of 16 patients with acute cholecystitis. Health utility

evaluations are not surveys but are tools based in utility theory that provide a quality of life measure that is comparable across disease states. Health utility is valued on a scale of 0 (state equivalent to death) to 1 (state of perfect health). Therefore, the greater risk a patient is willing to accept, the lower the score. The health utility values for all NOTES complications ranged from 0.71-0.89. For comparison, the health utility value for acute cholecystitis by conventional surgery is 0.77. These data suggest that patients are equally willing to accept the potential risks of NOTES for the benefits gained.

Furthermore, aesthetic disdain for scars alone should not be underestimated. Cosmetic surgery, laser treatments, creams and medicated bandages are all available suggesting a strong desire on the part of the health care consumer to hide or rid themselves of scars. With our ever growing awareness of body image, it is likely that a number of people will be willing to accept slightly greater risks for the rewards of NOTES.

5.7 NOTES By-products

Proponents of NOTES will continue struggling with the aforementioned challenges, battling the hurdles to drive the success or failure of NOTES. Regardless of the future of NOTES, this innovative concept has led to shifts in other surgical paradigms. A reduced apprehension to crossing the gastrointestinal tract and the development of NOTES closure devices may allow the endoscopist to venture further into the surgical realm by attempting endolumenal repair and bowel anastomoses, from the inside. Also inspired by the NOTES inside-out approach, treatments for gastroesophageal reflux disease (GERD), obesity (gastric reduction) and esophageal (Fritscher-Ravens et al. 2009) or gastric cancer

(Elmunzer et al. 2008), once done exclusively through laparoscopic or open surgical approaches, have now been reinvented to be conducted from an oral approach. Additionally, resurgence of the goal to minimize the injury caused by the treatment has lead to the birth of single incision laparoscopic surgery (SILS). Surgeons are now invigorated with the prospects of performing familiar laparoscopic procedures through a single, small abdominal incision (Bucher et al. 2009) requiring mere modifications to existing laparoscopic instruments, rather than a whole new platform as required for NOTES. Whether the day arrives where a gallbladder is routinely removed through the mouth is unclear. The novel approach of natural orifice surgery has, however, already had an impact on the direction of existing surgical procedures.

BIBLIOGRAPHY

1. Abe N, Takeuchi H, Yanagida O, Masaki T, Mori T, Sugiyama M, Atomi Y. Endoscopic full-thickness resection with laparoscopic assistance as hybrid NOTES for gastric submucosal tumor. *Surg Endosc.* 2009 Jan 30 (PMID: 19184206).
2. Akyol AM, McGregor JR, Galloway DJ, George WD. Early postoperative contrast radiology in the assessment of colorectal anastomotic integrity. *Int J Colorectal Dis* 1992;7:141–143.
3. Alishahi S. Randomized clinical trial of the effect of pneumoperitoneum on cardiac function and haemodynamics during laparoscopic cholecystectomy (*Br J Surg* 2004; 91:848–854). *Br J Surg.* 2005;92: 378–379.
4. Allen ML, Hoschtitzky JA, Peters MJ, Elliott M, Goldman A, James I, Klein NJ. Interleukin-10 and its role in clinical immunoparalysis following pediatric cardiac surgery. *Crit Care Med* 2006;34:2658-65.
5. Allendorf JD, Bessler M, Whelan RL, Trokel M, Laird DA, Terry MB, Treat MR. Postoperative immune function varies inversely with the degree of surgical trauma in a murine model. *Surg Endosc* 1997;11:427-30.
6. Angele MK, Faist E. Clinical review: immunodepression in the surgical patient and increased susceptibility to infection. *Crit Care Med* 2002;6(Suppl 1):298-305.
7. Araujo Filho I, Honorato Sobrinho AA, Rego AC, Garcia AC, Fernandes DP, Cruz TM, Costa TC, Medeiros AC. Influence of laparoscopy and laparotomy on gasometry, leukocytes and cytokines in rat abdominal sepsis model. *Acta Cir Bras* 2006;21:74-9.
8. Are C, Kutka M, Talamini M, Hardacre J, Mendoza-Sagaon M, Hanley E, Toung T. Effect of laparoscopic antireflux surgery upon renal blood flow. *Am J Surg.* 2002;183: 419–423.
9. Auyang ED, Hungness ES, Vaziri K, Martin JA, Soper NJ. Human NOTES Cholecystectomy: Transgastric Hybrid Technique. *J Gastrointest Surg.* 2009 Feb 7 (PMID: 19198959).
10. Bachman SL, Sporn E, Furrer JL, Astudillo JA, Calaluce R, McIntosh MA, Miedema BW, Thaler K. Colonic sterilization for natural orifice transluminal endoscopic surgery (NOTES) procedures: a comparison of two decontamination protocols. *Surg Endosc.* 2009 Jan 1 (PMID: 19118416).

11. Baker EA, El-Gaddal S, Williams L, Leaper DJ. Profiles of inflammatory cytokines following colorectal surgery: relationship with wound healing and outcome. *Wound Repair Regen* 2006;14:566-72.
12. Balague C, Targarona EM, Pujol M, Filella X, Espert JJ, Trias M. Peritoneal response to a septic challenge. Comparison between open laparotomy, pneumoperitoneum laparoscopy, and wall lift laparoscopy. *Surg Endosc* 1999;13:792-6.
13. Balique JG, Guinot R, Mondesert C, Mortier L. Retrospective comparative study of 600 appendectomies carried out by laparotomy (Mac Burney) or laparoscopy. A plea for laparoscopy. *Chirurgie*. 1993-1994;119(9):504-10.
14. Bardaro SJ, Swanström L. Development of advanced endoscopes for Natural Orifice Transluminal Endoscopic Surgery (NOTES). *Minim Invasive Ther Allied Technol*. 2006;15(6):378-83.
15. Baselski VS, Wunderink RG. Bronchoscopic diagnosis of pneumonia. *Clin Microbiol Rev* 1994;7:533-58.
16. Basgul E, Bahadir B, Celiker V, Karagoz AH, Hamaloglu E, Aypar U. Effects of low and high intra-abdominal pressure on immune response in laparoscopic cholecystectomy. *Saudi Med J* 2004;25:1888-91.
17. Bellon JM, Manzano L, Bernardos L, Ga-Honduvilla N, Larrad A, Bujan J, Alvarez-Mon M. Cytokine levels after open and laparoscopic cholecystectomy. *Eur Surg Res* 1997;29:27-34.
18. Bingener J, Krishnegowda NK, Michalek JE. Immunologic parameters during NOTES compared with laparoscopy in a randomized blinded porcine trial. *Surg Endosc*. 2009 Jan;23(1):178-81.
19. Bowrey DJ, Blom D, Crookes PF, Bremmer CG, Johansson JL, Lord RV, Hagen JA, DeMeester SR, DeMeester TR, Peters JH. Risk factors and the prevalence of trocar site herniation after laparoscopic fundoplication. *Surg Endosc*. 2001;15:663-666.
20. Bratzler DW, Houck PM. Antimicrobial prophylaxis for surgery: an advisory statement from the National Surgical Infection Prevention Project. *Am J Surg*. 2005;189:395-404.
21. Bucher P, Pugin F, Morel P. Transumbilical Single Incision Laparoscopic Sigmoidectomy for benign disease. *Colorectal Dis*. 2009 Mar 6. PMID: 19320667.

22. Buggy D. Can anaesthetic management influence surgical-wound healing? *Lancet*. 2000;356:355-357.
23. Burghardt J, Federlein M, Müller V, Benhidjeb T, Elling D, Gellert K. Minimal invasive transvaginal right hemicolectomy: report of the first complex NOS (natural orifice surgery) bowels operation using a hybrid approach. *Zentralbl Chir*. 2008 Dec;133(6):574-6.
24. Burgos FJ, Linares A, Pascual J, Marcen R, Villafruela J, Zamora J, Cuevas B, Correa C, Gomez V. Modifications of renal blood flow and serum interleukin levels induced by laparoscopic and open living donor nephrectomies for kidney transplant: an experimental study in pigs. *Transplant Proc* 2005;37:3676-8.
25. Bush RB, Leonhardt H, Bush IV, Landes RR. Dr. Bozzini's Lichtleiter: a translation of his original article (1806). *Urology*. 1974;3:119-123.
26. Cahill RA, Asakuma M, Perretta S, Dallemagne B, Marescaux J. Gastric lymphatic mapping for sentinel node biopsy by natural orifice transluminal endoscopic surgery (NOTES). *Surg Endosc*. 2008 Sep 24 (PMID: 18813997).
27. Cappell MS, Waye JD, Farrar JT, Sleisenger MH. Fifty landmark discoveries in gastroenterology during the past 50 years. A brief history of modern gastroenterology at the millennium: part II. Gastrointestinal motility, nutrition, and diseases of the lower gastrointestinal tract, liver, and pancreas. *Gastroenterol Clin North Am*. 2000;29:513-550, viii.
28. Carlson MA, Ludwig KA, Condon RE. Ventral hernia and other complications of 1,000 midline incisions. *South Med J*. 1995;88:450-453.
29. Castellanos G, Pinero A, Serrano A, Llamas C, Fuster M, Fernandez JA, Parrilla P. Translumbar retroperitoneal endoscopy: an alternative in the follow-up and management of drained infected pancreatic necrosis. *Arch Surg*. 2005;140:952-955.
30. Chak A, McGee M, Faulx A. EUS guided natural orifice transluminal endoscopic surgery (NOTES) approach to the retroperitoneum [abstract]. *Gastrointest Endosc* 2006;63:AB264.
31. Charnley RM, Lochan R, Gray H, O'Sullivan CB, Scott J, Oppong KE. Endoscopic necrosectomy as primary therapy in the management of infected pancreatic necrosis. *Endoscopy* 2006;38:925-8.

32. Cheadle WG, Turina M. Infection and organ failure in the surgical patient: a tribute to seminal contributions by Hiram C. Polk, Jr, M.D. *Am J Surg* 2005;190:173-7.
33. Chuttani R, Kozarek R, Critchlow J, Lo S, Pleskow D, Brandwein S, Lembo T. A novel endoscopic full-thickness plicator for treatment of GERD: an animal model study. *Gastrointest Endosc* 2002;56:116–122.
34. Chuttani R, Sud R, Sachdev G, Puri R, Kozarek R, Haber G, Pleskow D, Zaman M, Lembo A. A novel endoscopic full-thickness plicator for the treatment of GERD: a pilot study. *Gastrointest Endosc* 2003;58:770–776.
35. Conio M, Ponchon T, Blanche S, Filiberti R. Endoscopic mucosal resection. *Am J Gastroenterol.* 2006;101:653-663.
36. Crouzet S, Haber GP, Kamoi K, Berger A, Brethauer S, Gatmaitan P, Gill IS, Kaouk JH. Natural orifice transluminal endoscopic surgery (NOTES) renal cryoablation in a porcine model. *BJU Int.* 2008 Dec;102(11):1715-8.
37. Cruse PJ, Foord R. The epidemiology of wound infection:a 10-year prospective study of 62,939 wounds. *Surg Clin North Am.* 1980;60:27-40.
38. Cullen DJ, Coyle JP, Teplick R, Long MC. Cardiovascular, pulmonary, and renal effects of massively increased intra-abdominal pressure in critically ill patients. *Crit Care Med.* 1989;17: 118–121.
39. de la Fuente SG, Demaria EJ, Reynolds JD, Portenier DD, Pryor AD. New developments in surgery: natural orifice transluminal endoscopic surgery (NOTES). *Arch Surg* 2007;142:295-7.
40. Decker D, Tolba R, Springer W, Lauschke H, Hirner A, von Ruecker A. Abdominal surgical interventions: local and systemic consequences for the immune system—a prospective study on elective gastrointestinal surgery. *J Surg Res* 2005;126:12-8.
41. Decker G. Abdominal Compartment Syndrome. *J Chir (Paris)*, 2001 Oct;138(5):259-60.
42. Delgado S, Lacy AM, Filella X, Castells A, Garcia-Valdecasas JC, Pique JM, Momblan D, Visa J. Acute phase response in laparoscopic and open colectomy in colon cancer: randomized study. *Dis Colon Rectum* 2001;44:638-46.

43. Burpee SE, Kurian M, Murakame Y, Benevides S, Gagner M. The metabolic and immune response to laparoscopic versus open liver resection. *Surg Endosc* 2002;16:899-904.
44. DiPiro JT, Martindale RG, Bakst A, Vacani PF, Watson P, Miller MT. Infection in surgical patients: effects on mortality, hospitalization, and postdischarge care. *Am J Health Syst Pharm.* 1998;55:777-781.
45. Disibeyaz S, Parlak E, Koksak AS, Cicek B, Koc U, Sahin B. Endoscopic treatment of a large upper gastrointestinal anastomotic leak using a prolene plug and cyanoacrylate. *Endoscopy* 2005;37:1032–1033.
46. Dolz C, Noguera JF, Martín A, Vilella A, Cuadrado A. Transvaginal cholecystectomy (NOTES) combined with minilaparoscopy. *Rev Esp Enferm Dig.* 2007 Dec;99(12):698-702.
47. dos Santos CC, Zhang H, Slutsky AS. From bench to bedside: bacterial growth and cytokines. *Crit Care* 2002;6:4-6.
48. Duepre HJ, Senagore AJ, Delaney CP, Fazio VW. Does means of access affect the incidence of small bowel obstruction and ventral hernia after bowel resection? Laparoscopy versus laparotomy. *J Am Coll Surg.* 2003; 197:177-181.
49. Dunn MD, McDougall EM. Renal physiology: laparoscopic considerations. *Urol Clin North Am.* 2000;27: 609–614.
50. Ebong S, Call D, Nemzek J, Bolgos G, Newcomb D, Remick D. Immunopathologic alterations in murine models of sepsis of increasing severity. *Infect Immun* 1999;67:6603-10.
51. El-Dawlatly AA, Al-Dohayan A, Abdel-Meguid ME, El-Bakry A, Manaa EM. The effects of pneumoperitoneum on respiratory mechanics during general anesthesia for bariatric surgery. *Obes Surg.* 2004;14: 212–215.
52. Ellis, Harold, [A history of surgery](#), Greenwich Medical Media, 2002.
53. Elmunzer BJ, Trunzo JA, Marks JM, Poulouse BK, Chak A, Schomisch SJ, Bailey JJ, Ponsky JL. Endoscopic full-thickness resection of gastric tumors using a novel grasp-and-snare technique: feasibility in ex vivo and in vivo porcine models. *Endoscopy.* 2008 Nov;40(11):931-5.
54. Eloubeidi MA, Seewald S, Tamhane A, Brand B, Chen VK, Yasuda I, Cerfolio RJ, Omar S, Topalidis T, Wilcox CM, Soehendra N. EUS-guided FNA of the left adrenal gland in patients with thoracic or GI malignancies. *Gastrointest Endosc.* 2004;59:627-633.

55. Enander LK, Gustavsson S. Colonoscopic appendectomy: report of two cases. *Acta Chir Scand.* 1979;145:575-576.
56. Fazel A. An endoscopic perspective on pancreatic pseudocysts. *Curr Gastroenterol Rep.* 2005;7:107-113.
57. Fong DG, Pai RD, Thompson CC. Transcolonic endoscopic abdominal exploration: a NOTES survival study in a porcine model. *Gastrointest Endosc* 2007;65:312–318.
58. Freeman LJ, Rahmani EY, Sherman S, Chiorean MV, Selzer DJ, Constable PD, Snyder PW. Oophorectomy by natural orifice transluminal endoscopic surgery: feasibility study in dogs. *Gastrointest Endosc.* 2009 Feb 26 (PMID: 19249772).
59. Fritscher-Ravens A, Cuming T, Jacobsen B, Seehusen F, Ghanbari A, Kahle E, von Herbay A, Koehler P, Milla P. Feasibility and safety of endoscopic full-thickness esophageal wall resection and defect closure: a prospective long-term survival animal study. *Gastrointest Endosc.* 2009 Feb 26. PMID: 19249776.
60. Fritscher-Ravens A, Mylonaki M, Pantes A, Topalidis T, Thonke F, Swain P. Endoscopic ultrasound-guided biopsy for the diagnosis of focal lesions of the spleen. *Am J Gastroenterol.* 2003;98:1022-1027.
61. Fritscher-Ravens A, Patel K, Ghanbari A, Kahle E, von Herbay A, Fritscher T, Niemann H, Koehler P. Natural orifice transluminal endoscopic surgery (NOTES) in the mediastinum: long-term survival animal experiments in transesophageal access, including minor surgical procedures. *Endoscopy* 2007;39:870-5.
62. Fuentes JM, Hanly EJ, Aurora AR, De Maio A, Shih SP, Marohn MR, Talamini MA. CO2 abdominal insufflation pretreatment increases survival after a lipopolysaccharide-contaminated laparotomy. *J Gastrointes Surg* 2006;10:32-8.
63. Galizia G, Prizio G, Lieto E, Castellano P, Pelosio L, Imperatore V, Ferrara A, Pignatelli C. Hemodynamic and pulmonary changes during open, carbon dioxide pneumoperitoneum, and abdominal wall-lifting cholecystectomy: a prospective, randomized study. *Surg Endosc.* 2001;15: 477–483.
64. Gauderer MW, Ponsky JL, Izant RJ Jr. Gastrostomy without laparotomy: a percutaneous endoscopic technique. *J Pediatr Surg.* 1980;15:872-875.

65. Gettman MT, Blute ML. Transvesical peritoneoscopy: initial clinical evaluation of the bladder as a portal for natural orifice transluminal endoscopic surgery. *Mayo Clin Proc* 2007;82:843-5.
66. Giovannini M, Binmoeller K, Seifert H. Endoscopic ultrasound-guided cystgastrostomy. *Endoscopy* 2003;35:239-45.
67. Gordts S, Campo R, Rombauts L, Brosens I. Transvaginal hydrolaparoscopy as an outpatient procedure for infertility investigation. *Hum Reprod* 1998;13:99-103.
68. Grande M, Tucci GF, Adorisio O, Barini A, Rulli F, Neri A, Franchi F, Farinon AM. Systemic acute-phase response after laparoscopic and open cholecystectomy. *Surg Endosc* 2002;16:313-6.
69. Gundersen Y, Vaagenes P, Thrane I, Bogen IL, Haug KH, Reistad T, Opstad PK. Response of circulating immune cells to major gunshot injury, haemorrhage, and acute surgery. *Injury* 2005;36:949-55.
70. Guyton A, Hall J. Textbook of medical physiology. 11th Edition. WB Saunders Co. 2006.
71. Habib E, Elhadad A, Mekkaoui M, Brassier D, Hallak Y. Cost of appendectomy: laparoscopy versus laparotomy. A retrospective study of two series of 114 cases. *Ann Chir.* 1997;51(6):637-46.
72. Hagen ME, Wagner OJ, Swain P, Pugin F, Buchs N, Cadeddu M, Jamidar P, Fasel J, Morel P. Hybrid natural orifice transluminal endoscopic surgery (NOTES) for Roux-en-Y gastric bypass: an experimental surgical study in human cadavers. *Endoscopy*. 2008 Nov;40(11):918-24.
73. Hanly EJ, Fuentes JM, Aurora AR, Bachman SL, De Maio A, Marohn MR, Talamini MA. Carbon dioxide pneumoperitoneum prevents mortality from sepsis. *Surg Endosc* 2006;20:1482-7.
74. Hausmann U, Feussner H, Ahrens P, Heinzl J. Endoluminal endosurgery: rivet application in flexible endoscopy. *Gastrointest Endosc* 2006;64:101-3.
75. Hazy J, Needleman B, Melvin W, Mikami D, Narula V. Transgastric instrumentation and bacterial contamination of the peritoneal cavity. *Surg Endosc* 2007;21(S1):S112.
76. Hazy JW, Narula VK, Renton DB, Reavis KM, Paul CM, Hinshaw KE, Muscarella P, Ellison EC, Melvin WS. Natural-orifice transgastric

- endoscopic peritoneoscopy in humans: Initial clinical trial. *Surg Endosc.* 2008 Apr;22(4):961-6.
77. Hazey JW, Narula VK, Renton DB, Reavis KM, Paul CM, Hinshaw KE, Muscarella P, Ellison EC, Melvin WS. Natural-orifice transgastric endoscopic peritoneoscopy in humans: Initial clinical trial. *Surg Endosc.* 2008 Jan;22(1):16-20.
78. Heagy W, Nieman K, Hansen C, Cohen M, Danielson D, West MA. Lower levels of whole blood LPS-stimulated cytokine release are associated with poorer clinical outcomes in surgical ICU patients. *Surg Infect* 2003;4:171-80.
79. HIMAL HS. Minimally invasive (laparoscopic) surgery. *Surg Endosc.* 2002 Dec;16(12):1647-52.
80. Hirschowitz BI. Endoscopic examination of the stomach and duodenal cap with the fiberscope. *Lancet.* 1961;1:1074-1078.
81. Hochberger J, Lamade W. Transgastric surgery in the abdomen: the dawn of a new era? *Gastrointest Endosc.* 2005;62:293-296.
82. Hollerbach S, Willert J, Topalidis T, Reiser M, Schmiegel W. Endoscopic ultrasound-guided fine-needle aspiration biopsy of liver lesions: histological and cytological assessment. *Endoscopy.* 2003;35:743-749.
83. Holmes JH 4th, Connolly NC, Paull DL, Hill ME, Guyton SW, Ziegler SF, Hall RA. Magnitude of the inflammatory response to cardiopulmonary bypass and its relation to adverse clinical outcomes. *Inflamm Res* 2002;51:579-86.
84. Hondo FY, Giordano-Nappi JH, Maluf-Filho F, Matuguma SE, Sakai P, Poggetti R, Mori ND, Fontes B, Birolini D, Ishioka S. Transgastric access by balloon overtube for intraperitoneal surgery. *Surg Endosc.* 2007 Oct;21(10):1867-9.
85. Hookey LC, Khokhotva V, Bielawska B, Samis A, Jalink D, Hurlbut D, Mercer D. The Queen's closure: a novel technique for closure of endoscopic gastrotomy for natural-orifice transluminal endoscopic surgery. *Endoscopy.* 2009 Feb;41(2):149-53.
86. Hu 2005a: Hu B, Chung SC, Sun LC, Kawashima K, Yamamoto T, Cotton PB, Gostout CJ, Hawes RH, Kalloo AN, Kantsevov SV, Pasricha PJ. Eagle Claw II: a novel endosuture device that uses a curved needle for major arterial bleeding: a bench study. *Gastrointest Endosc.* 2005;62:266-270.

87. Hu 2005b: Hu B, Chung SC, Sun LC, Lau JY, Kawashima K, Yamamoto T, Cotton PB, Gostout CJ, Hawes RH, Kalloo AN, Kantsevov SV, Pasricha PH. Endoscopic suturing without extracorporeal knots: a laboratory study. *Gastrointest Endosc.* 2005;62:230-233.
88. Hu 2005c: Hu B, Chung SC, Sun LC, Kawashima K, Yamamoto T, Cotton PB, Gostout CJ, Hawes RH, Kalloo AN, Kantsevov SV, Pasricha PJ. Transoral obesity surgery: endolumenal gastroplasty with an endoscopic suture device. *Endoscopy* 2005;37:411-4.
89. Ikeda K, Fritscher-Ravens A, Mosse CA, Mills T, Tajiri H, Swain CP. Endoscopic full-thickness resection with sutured closure in a porcine model. *Gastrointest Endosc* 2005;62:122-9.
90. Inui K. Natural orifice transluminal endoscopic surgery: a step toward clinical implementation? *Gastrointest Endosc* 2007;65:694-5.
91. Ivatury RR, Sugerman HJ, Peitzman AB. Abdominal compartment syndrome: recognition and management. *Adv Surg.* 2001;35: 251–269.
92. Jacobi CA, Ordemann J, Zieren HU, Volk HD, Bauhofer A, Halle E, Muller JM. Increased systemic inflammation after laparotomy vs laparoscopy in an animal model of peritonitis. *Arch Surg* 1998;133:258-62.
93. Jagannath 2005a: Jagannath SB, Kantsevov SV, Vaughn CA, Chung SS, Cotton PB, Gostout CJ, Hawes RH, Pasricha PJ, Scorpio DG, Magee CA, Pipitone LJ, Kalloo AN. Peroral transgastric endoscopic ligation of fallopian tubes with long-term survival in a porcine model. *Gastrointest Endosc.* 2005;61:449-453.
94. Jagannath 2005b: Jagannath SB, Niiyama H, Chung SS, Cotton PB, Gostout CJ, Hawes RH, Pasricha PJ, Magee CA, Vaughn CA, Barlow D, Shimonaka H, Kalloo AN Endoscopic gastrojejunostomy with survival in a porcine model. *Gastrointest Endosc* 2005; 62:287–292.
95. Jesch NK, Kuebler JF, Nguyen H, Nave H, Bottlaender M, Teichmann B, Braun A, Vieten G, Ure BM. Laparoscopy vs minilaparotomy and full laparotomy preserves circulatory but not peritoneal and pulmonary immune responses. *J Pediatr Surg* 2006;41:1085-92.
96. Jess P, Schultz K, Bendtzen K, Nielsen OH. Systemic inflammatory responses during laparoscopic and open inguinal hernia repair: a randomised prospective study. *Eur J Surg* 2000;166:540-4.

97. Kalloo AN, Singh VK, Jagannath SB, Niiyama H, Hill SL, Vaughn CA, Magee CA, Kantsevov SV. Flexible transgastric peritoneoscopy: a novel approach to diagnostic and therapeutic interventions in the peritoneal cavity. *Gastrointest Endosc.* 2004;60:114-117.
98. Kamei K, Nimura Y, Nagino M, Aono K, Nakashima I. Surgical stress reduces mortality from endotoxin shock. *Langenbecks Arch Surg* 2002;386:512-7.
99. Kantsevov SV, Hu B, Jagannath SB, Vaughn CA, Beitler DM, Chung SS, Cotton PB, Gostout CJ, Hawes RH, Pasricha PJ, Magee CA, Pipitone LJ, Talamini MA, Kalloo AN. Transgastric endoscopic splenectomy: is it possible? *Surg Endosc.* 2006;20:522-525.
100. Kantsevov SV, Jagannath SB, Niiyama H, Chung SS, Cotton PB, Gostout CJ, Hawes RH, Pasricha PJ, Magee CA, Vaughn CA, Barlow D, Shimonaka H, Kalloo AN. Endoscopic gastrojejunostomy with survival in a porcine model. *Gastrointest Endosc.* 2005;62:287-292.
101. Kim CY, Chun HJ, Kim JY, Jang JS, Kwon YD, Park S, Keum B, Seo YS, Kim YS, Jeon YT, Lee HS, Um SH, Lee SW, Choi JH, Kim CD, Ryu HS. Accessibility of peritoneal organs according to the routes of approach in NOTES. *Korean J Gastroenterol.* 2008 Nov;52(5):281-5.
102. Kirkland KB, Briggs JP, Trivette SL, Wilkinson WE, Sexton DJ. The impact of surgical-site infections in the 1990s: attributable mortality, excess length of hospitalization, and extra costs. *Infect Control Hosp Epidemiol.* 1999;20:725-730.
103. Ko CW, Shin EJ, Buscaglia JM, Clarke JO, Magno P, Giday SA, Chung SS, Cotton PB, Gostout CJ, Hawes RH, Pasricha PJ, Kalloo AN, Kantsevov SV. Preliminary pneumoperitoneum facilitates transgastric access into the peritoneal cavity for natural orifice transluminal endoscopic surgery: a pilot study in a live porcine model. *Endoscopy,* 2007 Ect;39(10):849-53.
104. Kolvenbach R, Deling O, Schwierz E, Landers B. Reducing the operative trauma in aortoiliac reconstructions—a prospective study to evaluate the role of video-assisted vascular surgery. *Eur J Vasc Endovasc Surg* 1998;15:483-8.
105. Kozarek RA, Brayko CM, Harlan J, Sanowski RA, Cintora I, Kovac A. Endoscopic drainage of pancreatic pseudocysts. *Gastrointest Endosc.* 1985;31:322-327.

106. Kratt T, Küper M, Traub F, Ho CN, Schurr MO, Königsrainer A, Granderath FA, Kirschniak A. Feasibility study for secure closure of natural orifice transluminal endoscopic surgery gastrotomies by using over-the-scope clips. *Gastrointest Endosc.* 2008 Nov;68(5):993-6.
107. Lacy AM, Delgado S, Rojas OA, Almenara R, Blasi A, Llach J. MA-NOS radical sigmoidectomy: report of a transvaginal resection in the human. *Surg Endosc.* 2008 Jul;22(7):1717-23.
108. Lehman AC, Dumpert J, Wood NA, Visty AQ, Farritor SM, Varnell B, Oleynikov D. Natural Orifice Translumenal Endoscopic Surgery with a miniature in vivo surgical robot. *Surg Endosc.* 2009 Apr 3. PMID: 19343425.
109. Leroy J, Cahill RA, Perretta S, Forgione A, Dallemagne B, Marescaux J. Natural orifice transluminal endoscopic surgery (NOTES) applied totally to sigmoidectomy: an original technique with survival in a porcine model. *Surg Endosc.* 2009 Jan;23(1):24-30.
110. Lindberg F, Bergqvist D, Bjorck M, Rasmussen I. Renal hemodynamics during carbon dioxide pneumoperitoneum: an experimental study in pigs. *Surg Endosc.* 2003;17: 480–484.
111. Lomanto D, Dhir U, So JB, Cheah WK, Moe MA, Ho KY. Total transvaginal endoscopic abdominal wall hernia repair: a NOTES survival study. *Hernia.* 2009 Feb 26 (PMID: 19242774).
112. Malik A, Mellinger JD, Hazey JW, Dunkin BJ, MacFadyen BV Jr. Endoluminal and transluminal surgery: current status and future possibilities. *Surg Endosc* 2006;20: 1179-92.
113. Marescaux J, Dallemagne B, Perretta S, Wattiez A, Mutter D, Coumaros D. Surgery without scars: report of transluminal cholecystectomy in a human being. *Arch Surg.* 2007 Sep;142(9):823-6; discussion 826-7.
114. Marks J, Rosen M, McGee M, Rosen M, Onders R, Chak A, Faulx A, Ignani A, Schomisch S, Ponsky J. A novel technique for management of endoscopic gastrotomy following natural orifice transvisceral endoscopic surgery. *Surg Endosc.* 2006;20:S287.
115. Marks JD, Marks CB, Luce JM, Montgomery AB, Turner J, Metz CA, Murray JF. Plasma tumor necrosis factor in patients with septic shock. Mortality rate, incidence of adult respiratory distress syndrome, and effects of methylprednisolone administration. *Am Rev Respir Dis* 1990;141:94-7.

116. Marks JM, Ponsky JL, Pearl JP, McGee MF. PEG “Rescue”: a practical NOTES technique. *Surg Endosc* 2007;21:816-9.
117. Martin C, Boisson C, Haccoun M, Thomachot L, Mege JL. Patterns of cytokine evolution (tumor necrosis factor-alpha and interleukin-6) after septic shock, hemorrhagic shock, and severe trauma. *Crit Care Med* 1997;25:1813-9.
118. Maruszynski M, Pojda Z. Interleukin 6 (IL-6) levels in the monitoring of surgical trauma. A comparison of serum IL-6 concentrations in patients treated by cholecystectomy via laparotomy or laparoscopy. *Surg Endosc* 1995;9:882-5.
119. Matsumoto ED, Margulis V, Tunc L, Taylor GD, Duchene D, Johnson DB, Pearle MS, Cadeddu JA. Cytokine response to surgical stress: comparison of pure laparoscopic, hand-assisted laparoscopic, and open nephrectomy. *J Endourol* 2005;19:1140-5.
120. McCormack K, Scott NW, Go PM, Ross S, Grant AM; EU Hernia Trialists Collaboration. Laparoscopic techniques versus open techniques for inguinal hernia repair. *Cochrane Database Syst Rev*. 2003;(1):CD001785.
121. McGee 2008a: McGee MF, Marks JM, Onders RP, Chak A, Jin J, Williams CP, Schomisch SJ, Ponsky JL. Complete endoscopic closure of gastrotomy after natural orifice transluminal endoscopic surgery using the NDO Plicator. *Surg Endosc*. 2008 Jan;22(1):214-20.
122. McGee 2008b: McGee MF, Marks JM, Onders RP, Chak A, Rosen MJ, Williams CP, Jin J, Schomisch SJ, Ponsky JL; Case Advanced Surgical Endoscopy Team [CASE-T]. Infectious implications in the porcine model of natural orifice transluminal endoscopic surgery (NOTES) with PEG-tube closure: a quantitative bacteriologic study. *Gastrointest Endosc*. 2008 Aug;68(2):310-8.
123. McGee MF, Rosen MJ, Marks J, Chak A, Onders R, Faulx A, Ignagni A, Schomisch S, Ponsky J. A reliable method for monitoring intraabdominal pressure during natural orifice transluminal endoscopic surgery. *Surg Endosc*. 2007 Apr;21(4):672-6.
124. McGee 2008c: McGee MF, Schomisch SJ, Marks JM, Delaney CP, Jin J, Williams C, Chak A, Matteson DT, Andrews J, Ponsky JL. Late phase TNF-alpha depression in natural orifice transluminal endoscopic surgery (NOTES) peritoneoscopy. *Surgery*. 2008 Mar;143(3):318-28.

125. McKernan JB, Champion JK. Access techniques: Veress needle--initial blind trocar insertion versus open laparoscopy with the Hasson trocar. *Endosc Surg Allied Technol.* 1995 Feb;3(1):35-8.
126. Medeiros LR, Stein AT, Fachel J, Garry R, Furness S. Laparoscopy versus laparotomy for benign ovarian tumor: a systematic review and meta-analysis. *Int J Gynecol Cancer.* 2008 May-Jun;18(3):387-99.
127. Meireles O, Kantsevov SV, Kalloo AN, Jagannath SB, Giday SA, Magno P, Shih SP, Hanly EJ, Ko CW, Beitler DM, Marohn MR. Comparison of intraabdominal pressures using the gastroscope and laparoscope for transgastric surgery. *Surg Endosc* 2007;21:998-1001.
128. Menger MD, Vollmar B. Surgical trauma: hyperinflammation versus immunosuppression? *Langenbecks Arch Surg* 2004;389:475-84.
129. Menzies D, Ellis H. Intestinal obstruction from adhesions—how big is the problem? *Ann R Coll Surg Engl.* 1990;72:60-63.
130. Meyer C, de Manzini N, Rohr S, Thiry CL, Perim-Kalil FC, Bachellier-Billot C. 1000 cases of cholecystectomy: 500 by laparotomy versus 500 by laparoscopy. *J Chir (Paris).* 1993 Dec;130(12):501-6.
131. Miedema BW, Bachman SL, Sporn E, Astudillo JA, Thaler K. Transgastric placement of biologic mesh to the anterior abdominal wall. *Surg Endosc.* 2009 Mar 5 (PMID: 19263146).
132. Millikan KW. Incisional hernia repair. *Surg Clin North Am.* 2003;83:1223-1234.
133. Minami S, Gotoda T, Ono H, Oda I, Hamanaka H. Complete endoscopic closure of gastric perforation induced by endoscopic resection of early gastric cancer using endoclips can prevent surgery (with video). *Gastrointest Endosc* 2006;63:596–601.
134. Mingoli A, Puggioni A, Sgarzini G, Luciani G, Corzani F, Ciccarone F, Baldassarre E, Modini C. Incidence of incisional hernia following emergency abdominal surgery. *Ital J Gastroenterol Hepatol.* 1999;31:449-453.
135. Mirvis SE. Applications of magnetic resonance imaging and three-dimensional computed tomography in emergency medicine. *Ann Emerg Med.* 1989 Dec;18(12):1315-21.
136. Modlin IM, Kidd M, Lye KD. From the lumen to the laparoscope. *Arch Surg.* 2004;139:1110-1126.

137. Moehrlen U, Schwoebel F, Reichmann E, Stauffer U, Gitzelmann CA, Hamacher J. Early peritoneal macrophage function after laparoscopic surgery compared with laparotomy in a mouse model. *Surg Endosc* 2005;19:958-63.
138. Montz FJ, Holschneider CH, Munro MG. Incisional hernia following laparoscopy: a survey of the American Association of Gynecologic Laparoscopists. *Obstet Gynecol.* 1994;84:881-884.
139. Moore AF, Hargest R, Martin M, Delicata RJ. Intraabdominal hypertension and the abdominal compartment syndrome. *Br J Surg.* 2004;91: 1102–1110.
140. Munoz C, Carlet J, Fitting C, Misset B, Bleriot JP, Cavaillon JM. Dysregulation of in vitro cytokine production by monocytes during sepsis. *J Clin Invest* 1991;88:1747-54.
141. West MA, Heagy W. Endotoxin tolerance: a review. 2002;30:S64-73.
142. Murray A, Lourenco T, de Verteuil R, Hernandez R, Fraser C, McKinley A, Krukowski Z, Vale L, Grant A. Clinical effectiveness and cost-effectiveness of laparoscopic surgery for colorectal cancer: systematic reviews and economic evaluation. *Health Technol Assess.* 2006 Nov;10(45):1-141, iii-iv.
143. Narula VK, Happel LC, Volt K, Bergman S, Roland JC, Dettorre R, Renton DB, Reavis KM, Needleman BJ, Mikami DJ, Ellison EC, Melvin WS, Hazey JW. Transgastric endoscopic peritoneoscopy does not require decontamination of the stomach in humans. *Surg Endosc.* 2008 Oct 15 (PMID: 18855060).
144. National Center for Health Statistics. National Survey of Ambulatory Surgery. Hyattsville, Md: Centers for Disease Control and Prevention, National Center for Health Statistics; 1996.
145. Navarra G, Rando L, La Malfa G, Bartolotta G, Pracanica G. Hybrid transvaginal cholecystectomy: a novel approach. *Am J Surg.* 2009 Feb 26 (PMID: 19249734).
146. Neudecker J, Sauerland S, Neugebauer E, Bergamaschi R, Bonjer HJ, Cuschieri A, Fuchs KH, Jacobi C, Jansen FW, Koivusalo AM, Lacy A, McMahon MJ, Millat B, Schwenk W. The European Association for Endoscopic Surgery clinical practice guideline on the pneumoperitoneum for laparoscopic surgery. *Surg Endosc.* 2002;16: 1121–1143.

147. Nezhat C, Nezhat F, Seidman DS. Incisional hernias after operative laparoscopy. *J Laparoendosc Adv Surg Tech A*. 1997;7:111-115.
148. Nguyen NT, Anderson JT, Budd M, Fleming NW, Ho HS, Jahr J, Stevens CM, Wolfe BM. Effects of pneumoperitoneum on intraoperative pulmonary mechanics and gas exchange during laparoscopic gastric bypass. *Surg Endosc*. 2004;18: 64–71.
149. Nguyen NT, Goldman CD, Ho HS, Gosselin RC, Singh A, Wolfe BM. Systemic stress response after laparoscopic and open gastric bypass. *J Am Coll Surg* 2002;194:557-66; discussion 566-7.
150. Nguyen NT, Wolfe BM. The physiologic effects of pneumoperitoneum in the morbidly obese. *Ann Surg*. 2005;241: 219–226.
151. Nichols RL. Preventing surgical site infections: a surgeon’s perspective. *Emerg Infect Dis*. 2001;7:220-224.
152. Noguera J, Dolz C, Cuadrado A, Olea J, Vilella A, Morales R. Hybrid transvaginal cholecystectomy, NOTES, and minilaparoscopy: analysis of a prospective clinical series. *Surg Endosc*. 2009 Apr;23(4):876-81.
153. Nomikos IN, Katsouyanni K, Papaioannou AN. Washing with or without chloramphenicol in the treatment of peritonitis: a prospective, clinical trial. *Surgery* 1986;99:20-5.
154. Novitsky YW, Czerniach DR, Kaban GK, Bergner A, Gallagher KA, Perugini RA, Litwin DE. Immunologic effects of hand-assisted surgery on peritoneal macrophages: comparison to open and standard laparoscopic approaches. *Surgery* 2006;139:39-45.
155. O’Dwyer PJ, Courtney CA. Factors involved in abdominal wall closure and subsequent incisional hernia. *Surgeon*. 2003;1:17-22.
156. Onders 2007a: Onders R, Elmo MJ, Katirji B, Schiltz R, Ignangi. Safe combined placement of the laparoscopic diaphragm pacing system (DPS) and percutaneous endoscopic gastrotomy (PEG) tube: results of a phase one study in amyotrophic lateral sclerosis [abstract]. *Surg Endosc* 2007;21:S348.
157. Onders 2007b: Onders R, McGee MF, Marks J, Chak A, Schiltz R, Rosen MJ, Ignagni A, Faulx A, Elmo MJ, Schomisch S, Ponsky J. Diaphragm pacing with natural orifice transluminal endoscopic surgery: potential for difficult-to-wean intensive care unit patients. *Surg Endosc*. 2007 Mar;21(3):475-9.

158. Onders 2007c: Onders RP, McGee MF, Marks J, Chak A, Rosen MJ, Ignagni A, Faulx A, Schomisch S, Ponsky J. Natural orifice transluminal endoscopic surgery (NOTES) as a diagnostic tool in the intensive care unit. *Surg Endosc.* 2007 Apr;21(4):681-3.
159. Oshinsky GS, Smith AD. Laparoscopic needles and trocars: an overview of designs and complications. *J Laparoendosc Surg.* 1992 Apr;2(2):117-25.
160. Ost MC, Tan BJ, Lee BR. Urological laparoscopy: basic physiological considerations and immunological consequences. *J Urol.* 2005;174: 1183–1188.
161. Pai RD, Fong DG, Bundga ME, Odze RD, Rattner DW, Thompson CC. Transcolonic endoscopic cholecystectomy: a NOTES survival study in a porcine model (with video). *Gastrointest Endosc* 2006;64:428-34.
162. Palanivelu C, Rajan PS, Rangarajan M, Parthasarathi R, Senthilnathan P, Prasad M. Transvaginal endoscopic appendectomy in humans: a unique approach to NOTES--world's first report. *Surg Endosc.* 2008 May;22(5):1343-7.
163. Palanivelu C, Rajan PS, Rangarajan M, Prasad M, Kalyanakumari V, Parthasarathi R, Senthilnathan P. NOTES: Transvaginal Endoscopic Cholecystectomy in Humans-Preliminary Report of a Case Series. *Am J Gastroenterol.* 2009 Mar 10 (PMID: 19277024).
164. Park JW. Interventional cardiology versus minimally invasive cardiac surgery. *Eur J Cardiothorac Surg.* 1999 Nov;16 Suppl 2:S117-8.
165. Park PO, Bergstrom M, Ikeda K, Fritscher-Ravens A, Swain P. Experimental studies of transgastric gallbladder surgery: cholecystectomy and cholecystogastric anastomosis (videos). *Gastrointest Endosc.* 2005;61:601-606.
166. Perretta S, Allemann P, Asakuma M, Dallemagne B, Marescaux J. Adrenalectomy using natural orifice transluminal endoscopic surgery (NOTES): A transvaginal retroperitoneal approach. *Surg Endosc.* 2009 Mar 5 (PMID: 19263136).
167. Pham BV, Raju GS, Ahmed I, Brining D, Chung S, Cotton P, Gostout CJ, Hawes RH, Kalloo AN, Kantsevov SV, Pasricha PJ. Immediate endoscopic closure of colon perforation by using a prototype endoscopic suturing device: feasibility and outcome in a porcine model (with video). *Gastrointest Endosc* 2006;64:113–119.

168. Pitombo MB, Lupi OH, Gomes RN, Amancio R, Refinetti RA, Bozza PT, Castro-Faria-Neto HC. Inflammatory response and bacterial dissemination after laparotomy and abdominal CO2 insufflation in a murine model of peritonitis. *Surg Endosc* 2006;20:1440-7.
169. Pleskow D, Rothstein R, Kozarek R, Haber G, Gostout C, Lembo A. Endoscopic full-thickness plication for the treatment of GERD: long-term multicenter results. *Surg Endosc* 2007;21:439-444.
170. Ponsky J, Gauderer M, Stellato T. Percutaneous endoscopic gastrostomy. Review of 150 cases. *Arch Surg* 1983;118:913-4.
171. Ponsky JL, Gauderer MW. Percutaneous endoscopic gastrostomy: a nonoperative technique for feeding gastrostomy. *Gastrointest Endosc*. 1981;27:9-11.
172. Ponsky JL. Gastroenterologists as surgeons: what they need to know. *Gastrointest Endosc*. 2005;61:454.
173. Power N, Atri M, Ryan S, Haddad R, Smith A. CT assessment of anastomotic bowel leak. *Clin Radiol* 2007;62:37-42.
174. Puzziferri N, Austrheim-Smith IT, Wolfe BM, Wilson SE, Nguyen NT. Three-year follow-up of a prospective randomized trial comparing laparoscopic versus open gastric bypass. *Ann Surg*. 2006;243:181-188.
175. Raju GS, Ahmed I, Xiao SY, Brining D, Poussard A, Tarcin O, Shibukawa G, Dawson K, Knight G, Tanguay R, Hull J. Controlled trial of immediate endoluminal closure of colon perforations in a porcine model by use of a novel clip device (with videos). *Gastrointest Endosc* 2006;64:989-97.
176. Raman JD, Bergs RA, Fernandez R, Bagrodia A, Scott DJ, Tang SJ, Pearle MS, Cadeddu JA. Complete Transvaginal NOTES Nephrectomy Using Magnetically Anchored Instrumentation. *J Endourol*. 2009 Feb 4 (PMID: 19196056).
177. Rathert P, Lutzeyer W, Goddwin WE. Philipp Bozzini (1773-1809) and the Lichtleiter. *Urology*. 1974;3:113-118.
178. Rattner D, Kalloo A. ASGE/SAGES Working Group on Natural Orifice Transluminal Endoscopic Surgery. October 2005. *Surg Endosc* 2006;20:329-33.

179. Ray NF, Larsen JW Jr, Stillman RJ, Jacobs RJ. Economic impact of hospitalizations for lower abdominal adhesiolysis in the United States in 1988. *Surg Gynecol Obstet.* 1993;176:271-276.
180. Rolanda C, Lima E, Pego JM, Henriques-Coelho T, Silva D, Osorio L, Moreira I, Carvalho JL, Correia-Pinto J. Third-generation cholecystectomy by natural orifices: transgastric and transvesical combined approach (with video). *Gastrointest Endosc* 2007;65:111-7.
181. Rosen M, McGee M, Marks J, Chak A, Onders R, Faulx A, Ignangi A, Schomisch S, Ponsky J. Optimizing peritoneal access for natural orifice transvisceral endoscopic surgery (NOTES) [abstract]. *Surg Endosc.* 2006;20:S365.
182. Roumen RM, Hendriks T, van der Ven-Jongekrijg J, Nieuwenhuijzen GA, Sauerwein RW, van der Meer JW, Goris RJ. Cytokine patterns in patients after major vascular surgery, hemorrhagic shock, and severe blunt trauma. Relation with subsequent adult respiratory distress syndrome and multiple organ failure. *Ann Surg* 1993;218:769-76.
183. Ryou M, Thompson CC. Magnetic retraction in natural-orifice transluminal endoscopic surgery (NOTES): addressing the problem of traction and countertraction. *Endoscopy* 2009 Feb;41(2):143-8.
184. Safadi BY, Marks JM, Ponsky JL. Percutaneous Endoscopic Gastrostomy. *Gastrointest Endosc Clin N Am.* 1998 Jul;8(3):551-568.
185. Said M, Ledochowski M, Dietze O, Simader H. Colonoscopic diagnosis and treatment of acute appendicitis. *Eur J Gastroenterol Hepatol.* 1995;7:569-571.
186. Sánchez-Margallo FM, Asencio JM, Tejonero MC, Pérez FJ, Sánchez MA, Usón J, Pascual S. Technical feasibility of totally natural orifice cholecystectomy in a swine model. *Minim Invasive Ther Allied Technol.* 2008;17(6):361-4.
187. Sanowski RA, Kozarek RA, Partyka EK. Evaluation of a flexible endoscope for laparoscopy. *Am J Gastroenterol.* 1981;76:416-419.
188. Schachtrupp A, Lawong G, Afify M, Graf J, Toens C, Schumpelick V. Fluid resuscitation preserves cardiac output but cannot prevent organ damage in a porcine model during 24 h of intraabdominal hypertension. *Shock.* 2005;24: 153–158.

189. Schachtrupp A, Toens C, Hoer J, Klosterhalfen B, Lawong AG, Schumpelick V. A 24-h pneumoperitoneum leads to multiple organ impairment in a porcine model. *J Surg Res*. 2002;106: 37–45.
190. Schietroma M, Carlei F, Franchi L, Mazzotta C, Sozio A, Lygidakis NJ, Amicucci G. A comparison of serum interleukin-6 concentrations in patients treated by cholecystectomy via laparotomy or laparoscopy. *Hepatogastroenterology* 2004;51:1595-9.
191. Schmidt A, Bengtsson A, Tylman M, Blomqvist L. Proinflammatory cytokines in elective flap surgery. *J Surg Res* 2007;137:117-21. van der Poll T. Immunotherapy of sepsis. *Lancet Infect Dis* 2001;1:165-74.
192. Sclabas GM, Swain P, Swanstrom LL. Endoluminal methods for gastrotomy closure in natural orifice transenteric surgery (NOTES). *Surg Innov* 2006;13:23-30.
193. Scott DJ, Tang SJ, Fernandez R, Bergs R, Goova MT, Zeltser I, Kehdy FJ, Cadeddu JA. Completely transvaginal NOTES cholecystectomy using magnetically anchored instruments. *Surg Endosc* 2007;21:2308-16.
194. Seaman DL, Gostout CJ, de la Mora Levy JG, Knipschild MA. Tissue anchors for transmural gut-wall apposition. *Gastrointest Endosc* 2006;64:577–581.
195. Seewald S, Groth S, Omar S, Imazu H, Seitz U, de Weerth A, Soetikno R, Zhong Y, Sriram PV, Ponnudurai R, Sikka S, Thonke F, Soehendra. Aggressive endoscopic therapy for pancreatic necrosis and pancreatic abscess: a new safe and effective treatment algorithm (videos). *Gastrointest Endosc* 2005;62:92-100.
196. Seifert H, Wehrmann T, Schmitt T, Zeuzem S, Caspary WF. Retroperitoneal endoscopic debridement for infected peripancreatic necrosis. *Lancet*. 2000;356:653-655.
197. Sherwinter DA, Eckstein JG. Feasibility study of natural orifice transluminal endoscopic surgery inguinal hernia repair. *Gastrointest Endosc*. 2009 Feb 26 (PMID:19249775).
198. Society of American Gastrointestinal Endoscopic Surgeons. Guidelines for diagnostic laparoscopy. SAGES guidelines. *Surg Endosc*. 1999;13: 202–203.
199. Sporn E, Miedema BW, Astudillo JA, Bachman SL, Loy TS, Davis JW, Calaluce R, Thaler K. Gastrotomy creation and closure for NOTES using a gastropexy technique. *Gastrointest Endosc*. 2008 Nov;68(5):948-53.

200. Strohm WD, Phillip J, Hagenmuller F, Classen M. Ultrasonic tomography by means of an ultrasonic fiberendoscope. *Endoscopy*. 1980;12:241-244.
201. Sumiyama 2007a: Sumiyama K, Gostout C, Techniques for Transgastric Access to the Peritoneal Cavity; *Gastrointest Endosc Clin N Am* 2008; 18;235-244.
202. Sumiyama 2007b: Sumiyama K, Gostout CJ, Rajan E, Bakken TA, Deters JL, Knipschild MA. Endoscopic full-thickness closure of large gastric perforations by use of tissue anchors. *Gastrointest Endosc* 2007;65:134-9.
203. Sumiyama 2007c: Sumiyama K, Gostout CJ, Rajan E, Bakken TA, Knipschild MA, Marler RJ. Submucosal endoscopy with mucosal flap safety valve. *Gastrointest Endosc* 2007;65:688-94.
204. Sumiyama K, Gostout CJ, Rajan E, Bakken TA, Knipschild MA. Transesophageal mediastinoscopy by submucosal endoscopy with mucosal flap safety valve technique. *Gastrointest Endosc* 2007;65:679-83.
205. Swain P. A justification for NOTES: natural orifice transluminal endosurgery. *Gastrointest Endosc* 2007;65:514-6.
206. Swanstrom L, Kozarek R, Pasricha P, Gross S, Birkett D, Park PO, Saadat V, Ewers R, Swain P. Development of a new access device for transgastric surgery. *J Gastrointest Surg* 2005;9:1129-36.
207. Targarona EM, Rodriguez M, Camacho M, Balague C, Gich I, Vila L, Trias M. Immediate peritoneal response to bacterial contamination during laparoscopic surgery. *Surgical Endosc* 2006;20:316-21.
208. Tirnaksiz MB, Deschamps C, Allen MS, Johnson DC, Pairolero PC. Effectiveness of screening aqueous contrast swallow in detecting clinically significant anastomotic leaks after esophagectomy. *European surgical research. Eur Surg Res* 2005;37:123–128.
209. Toens C, Schachtrupp A, Hoer J, Junge K, Klosterhalfen B, Schumpelick V. A porcine model of the abdominal compartment syndrome. *Shock*. 2002;18: 316–321.
210. Torres A, Torres K, Paszkowski T, Staskiewicz GJ, Maciejewski R. Cytokine response in the postoperative period after surgical treatment of benign adnexal masses: comparison between laparoscopy and laparotomy. *Surg Endosc* 2007;21:1841-8.

211. Vittimberga FJ Jr, Foley DP, Meyers WC, Callery MP. Laparoscopic surgery and the systemic immune response. *Ann Surg* 1998;227:326-34.
212. Voermans R, Eisendrath P, Bruno M, Le Moine O, Deviere J, Fockens P; ARCADE group. Initial evaluation of a novel prototype forward-viewing US endoscope in transmural drainage of pancreatic pseudocysts (with videos). *Gastrointest Endosc* 2007;66:1013-7.
213. Volk HD, Reinke P, Krausch D, Zuckermann H, Asadullah K, Muller JM, Docke WD, Kox WJ. Monocyte deactivation—rationale for a new therapeutic strategy in sepsis. *Intensive Care Med* 1996;(22 Suppl 4):S474-81.
214. von Renteln D, Eickhoff A, Kaehler G, Riecken B, Caca K. Endoscopic closure of the natural orifice transluminal endoscopic surgery (NOTES) access site to the peritoneal cavity by means of transmural resorbable sutures: an animal survival study. *Endoscopy*. 2009 Feb;41(2):154-9.
215. Wagh M, Merrifield B, Thompson C. Endoscopic transgastric abdominal exploration and organ resection: initial experience in a porcine model. *Clin Gastroenterol Hepatol* 2005;3:892-6.
216. Wegener M, Pfaffenbach B, Adamek RJ. Endosonographically guided transduodenal and transgastral fine-needle aspiration puncture of focal pancreatic lesions. *Bildgebung*. 1995;62:110-115.
217. Wichmann MW, Huttel TP, Winter H, Spelsberg F, Angele MK, Heiss MM, Jauch KW. Immunological effects of laparoscopic vs open colorectal surgery: a prospective clinical study. *Arch Surg* 2005;140:692-7.
218. Wilhelm D, Meining A, von Delius S, Fiolka A, Can S, Hann von Weyhern C, Schneider A, Feussner H. An innovative, safe and sterile sigmoid access (ISSA) for NOTES. *Endoscopy* 2007;39:401-6.
219. Yahara N, Abe T, Morita K, Tangoku A, Oka M. Comparison of interleukin-6, interleukin-8, and granulocyte colony-stimulating factor production by the peritoneum in laparoscopic and open surgery. *Surg Endosc* 2002;16:1615-9.
220. Yamamoto T, Umegae S, Kitagawa T, Matsumoto K. Intraperitoneal cytokine productions and their relationship to peritoneal sepsis and systemic inflammatory markers in patients with inflammatory bowel disease. *Dis Colon Rectum* 2005;48:1005-15.
221. Yavuz Y, Ronning K, Lyng O, Marvik R, Gronbech JE. Effect of increased intraabdominal pressure on cardiac output and tissue blood flow

assessed by color-labeled microspheres in the pig. *Surg Endosc.* 2001;15:149–155.

222. Yim AP, Wan S, Lee TW, Arifi AA. VATS lobectomy reduces cytokine responses compared with conventional surgery. *Ann Thorac Surg* 2000;70:243-7.
223. Zacharopoulou C, Nassif J, Allemann P, Dallemagne B, Perretta S, Marescaux J, Wattiez A. Exploration of the retroperitoneum using the transvaginal natural orifice transluminal endoscopic surgery technique. *J Minim Invasive Gynecol.* 2009 Mar-Apr;16(2):198-203.
224. Zornig C, Emmermann A, von Waldenfels HA, Mofid H. Laparoscopic cholecystectomy without visible scar: combined transvaginal and transumbilical approach. *Endoscopy.* 2007 Oct;39(10):913-5.
225. Zorrón R, Filgueiras M, Maggioni LC, Pombo L, Lopes Carvalho G, Lacerda Oliveira A. NOTES. Transvaginal cholecystectomy: report of the first case. *Surg Innov.* 2007 Dec;14(4):279-83.