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Early Diagnosis of Colonic Anastomotic Leak With Peritoneal Endoscopy

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ABSTRACT

Background and Objectives: At present, we do not have a reliable method for the early diagnosis of colorectal anastomotic leakage (AL). We tested peritoneal flexible endoscopy through a port placed in the abdominal wall in the early postoperative course, as a new diagnostic method for detection of this complication and evaluated the suggested method for safety, feasibility, and accuracy.

Methods: Ten swine were randomized into 2 groups: group A, colorectal anastomosis without leakage; and group B, colorectal anastomosis with leakage. A button gastrostomy feeding tube was inserted percutaneously into the peritoneal cavity. Colorectal anastomosis (with or without defect) was created 48 hours after the first operation. The swine were examined by peritoneal flexible endoscopy 8 and 24 hours after the colonic operation, by a consultant surgeon who was blinded to both the presence and the allocated location of the of the anastomotic defect.

Results: None of the animals showed signs of illness 48 hours after the intraperitoneal gastrostomy tube placement. More than half of the anastomosis circumference was identified in 60 and 10% of the animals at endoscopy 8 and 24 hours, respectively, after the anastomosis was created. Excessive adhesion formation was observed in all animals, irrespective of AL. The sensitivity and specificity of endoscopy in detecting peritonitis 24 hours after AL were both 60%.

Conclusions: Peritoneal endoscopy is a safe and simple procedure. Visualization of the peritoneal cavity in the early postoperative course was limited due to adhesion formation. Further studies are needed to clarify the accuracy of the procedure and to address additional methodological concerns.

Key Words: Anastomotic leakage, Colorectal anastomosis, Early diagnosis, Peritoneal flexible endoscopy, Randomized trial.

INTRODUCTION

Anastomotic leakage (AL) in colorectal surgery is a major complication that is associated with increased morbidity, mortality, impaired functional results, and reduced survival after cancer surgery.[1,2] The current workup for the diagnosis of colorectal AL is based on detection of the presence of symptoms and signs of anastomotic insufficiency confirmed by radiological imagining, endoscopic examination, or surgical exploration.[3] There are several drawbacks to this diagnostic setup. Clinical symptoms and signs of AL can be vague and indistinct, precluding early diagnosis of clinically significant anastomotic dehiscence and resulting in a delay in the diagnosis.[4] Both computed tomographic (CT) scanning[5–7] and a water-soluble contrast enema[4,7] have been reported to have a sensitivity as low as <50%. Some studies have implied that the high false-negative rate of radiologic diagnostic techniques in clinically suspected AL can postpone surgical exploration, resulting in an adverse postoperative course.[5,6,8] Current imaging methods, if performed routinely in the postoperative course, have the disadvantage of diagnosing clinically silent AL, which does not require active therapeutic intervention.[9,10] There is a significant overlap of radiological features in patients with and without AL, especially in the early postoperative period, limiting specificity of radiological methods.[8,10–12]

The purpose of this study was to test whether peritoneal flexible endoscopy, through a port placed intraperitoneally through transabdominal insertion, is a safe, feasible, and accurate method for early diagnosis of colorectal AL in a porcine animal model.

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MATERIALS AND METHODS

Study Design

The design was a 3-day survival study in swine. The animals were randomized into 2 groups of 5 each: group A, colorectal anastomosis without AL; and group B, colorectal anastomosis with AL.

All animals initially underwent insertion of a button gastrostomy tube into the peritoneal cavity. Then, 48 hours after the initial operation, the swine had 5 cm of large bowel resected, and the colorectal anastomosis was created, with or without defect. The animals were examined by peritoneal flexible endoscopy 8 and 24 hours after creation of the colorectal anastomosis by a consultant surgeon. The consultant surgeon was blinded to both AL presence and localization allocation.

The time necessary to perform the endoscopy, the part of the anastomosis that was seen during the endoscopy, and the presence of signs of peritonitis were recorded.

Animals and Surgical Procedure

The study was approved by The Danish Animal Experiments Inspectorate, Cases 2012/561-146 and 2013-15-2934-00767/ACHOV, in accordance with the Proclamation of the Danish Animal Welfare Act 1343 (April 12, 2007).

We used 10 female domestic Landrace Yorkshire Duroc swine with a mean body weight of 69 kg (range, 62.5–72.5).

On the initial day, all the animals, while under general anesthesia, had a button gastrostomy feeding tube (BGFT) (28 French × 1.5 cm; Bard Access Systems, Salt Lake City, Utah) inserted into the peritoneal cavity. A midline minilaparotomy was performed, and under direct visualization, the BGFT was inserted next to the second distal right teat. The external portion of the BGFT was protected by a transparent waterproof film (Opsite; Smith&Nephew, London, United Kingdom).

All animals were observed during the next 2 days for food intake, behavior changes, temperature, stool production, and signs of illness by trained animal technicians supervised by veterinarians.

Forty-eight hours later, the animals underwent a second operation through a midline incision while under general anesthesia. The animals were then randomized into 2 groups using computer-generated numbers (www.randomizer.org): group A, 5 cm of distal colon resected, and an end-to-end, 2-layer colorectal anastomosis created with continuous absorbable sutures (Biosyn 4-0; Covidien, Mansfield, Massachusetts); group B, same surgery, but with a defect left in a quarter of the anastomosis (defect diameter, 18–22 mm). We measured the semicircumference of the colon to determine the size of the defect. The size of the anastomotic defect was selected based on the results from previous animal studies in which the swine model of AL had been used. It was designed to induce symptomatic AL and diffuse peritonitis.

The cross section of the anastomosis was divided into 4 quadrants, and the position of the anastomotic defect was randomly assigned to one of the quadrants with the same computer-based random number generator as used for the animal groups. Anastomoses in both groups were marked on the outer side of the large bowel with 3 sutures. Two PDS 2-0, 10-cm-long sutures (Ethicon, Somerville, New Jersey) were placed on the dorsal mesenteric side of the anastomotic suture line and 1 on each side of the mesocolon. A single prolene 2-0, 10-cm-long suture was placed on the opposite antimesenteric side of the anastomotic suture line (**Figure 1**).

The animals were given preoperative antibiotic prophylaxis with intravenous streptocillin solution (benzylpenicillin procaine 200 mg and dihydrostreptomycin sulfate 250 mg) 1 mL/10 kg body weight, 30 minutes before the resection and anastomosis procedure.

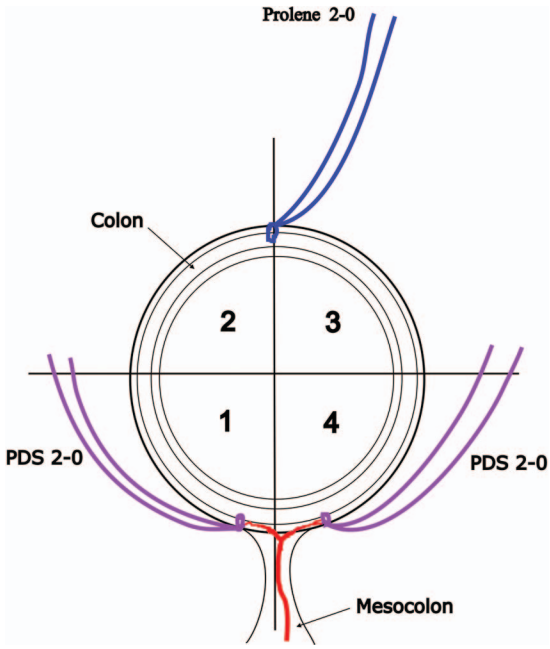

Figure 1. Depiction of an anastomosis cross section.

Endoscopic exploration of the peritoneal cavity (BF-P160 EVIS EXERA; EVIS EXERA II video system; Olympus, Tokyo, Japan) was performed through the BGFT with a video bronchoscope 8 and 24 hours after the second operation. The procedure was performed by a surgical consultant who was blinded to the randomized allocation. Pneumoperitoneum was established through the BGFT, and a Veress needle was then introduced under direct visual guidance. The Veress needle was used to maintain intraperitoneal pressure of 12 mm Hg during the endoscopy. The examiner inspected both the peritoneal cavity for signs of peritonitis and the anastomosis for dehiscence. We defined 4 categories on the basis of the anastomotic circumference visible during the examination: anastomosis not visualized, 0; no semicircumferences visible in full length, <½; half of circumferences visible in full length and half only partially visible, >½; and entire anastomosis visible, 1.

After the second operation, the animals were observed for food intake, behavior changes, temperature, and stool production. All animals were euthanized on the third day after insertion of the BGFT, and a necropsy was performed. All anastomoses were inspected for signs of dehiscence, and the peritoneal cavity for the signs of peritonitis.

Anesthesia

Premedication with intramuscular (IM) injection of 1 mL/10 kg zoletil mixture (250 mg dry zolazepam+ tiletamine, 6.25 mL xylazine 20 mg/mL, 1.25 mL ketamine 100 mg/mL, and 2.5 mL butorphanol 10 mg/mL) was followed by endotracheal intubation. Anesthesia was maintained with intravenous infusion of propofol at 10 mg/kg per hour and fentanyl at 0.5 mL/kg per hour throughout the operation. The animals were mechanically ventilated with a mixture of 50% oxygen and atmospheric air.

Postoperative pain was managed with a fentanyl transdermal patch 25 μg/h, Finadyne (MSD Animal Health, Dublin Ireland) 1 mL/22 kg IM 1 time a day, and buprenorphine 1 mL/20–25 kg IM 3 times a day.

Data Analysis

AL was defined in accordance with the definition of the International Study group for Rectal Cancer[13] as a visible defect in the anastomosis line with communication between the intra- and extraluminal compartments.

Peritonitis was defined as the presence of 1 or all of the following: diffuse peritoneal adhesions, diffuse fibrin deposits, and cloudy ascites on peritoneal endoscopy. The adhesions were graded as localized or diffuse, based on their extent. Localized adhesions were defined as limited to organs adjacent to the anastomosis. Diffuse adhesions were defined as extending over 2 or more quadrants of the peritoneal cavity.

The sensitivity and specificity of the method were calculated for the signs of peritonitis and perforation seen during the endoscopic examination and for those found after the necropsy.

RESULTS

None of the swine showed signs of illness 48 hours after BGFT placement. However, one animal had a moderate amount of cloudy ascites on the day of resection and anastomosis. Ascites was not further examined for the presence of bacterial contamination. The swine did not exhibit signs of illness in the next 24 h. There were no visible signs of peritonitis on peritoneoscopy 8 hours after operation (swine with AL).

Twenty peritoneal endoscopies were performed, with a mean examination time of 28.5 minutes (range, 9–65). The distribution of the portion of the anastomotic circumference that was identifiable on endoscopy 8 and 24 hours after the creation of the colorectal anastomosis is presented in **Figure 2**. The sensitivity and specificity of the endoscopy for detecting peritonitis 24 hours after creation of the AL were both 60%.

Adhesions were observed in 90% of the animals, at both 8 and 24 hours after the operation. We identified diffuse adhesions in 10 and 40% of swine at 8 and 24 hours, respectively (**Table 1**). The predominance of diffuse adhesions in the swine without AL was registered 8

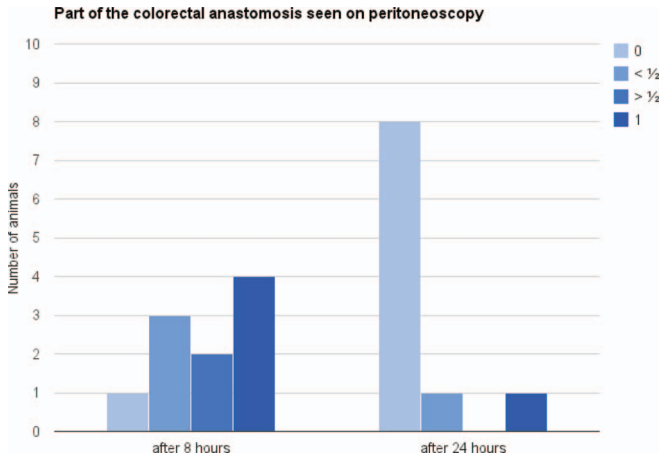

Figure 2. Part of the colorectal anastomosis seen on peritoneoscopy 8 and 24 hours after surgery.

Table 1.
Distribution of Peritoneal Adhesions on Peritoneoscopy

	Endoscopy 1 (8 h)	Endoscopy 2 (24 h)
Localized adhesions	8 (80)	5 (50)
Diffuse adhesions	1 (10)	4 (40)
Adhesions in all	9 (90)	9 (90)

N = 10. Data are expressed as the number (percentage) of the total sample.

hours after the operation. Adhesions were otherwise distributed equally in the animals with and without AL (**Figure 3**).

Eight hours after the operation, endoscopy revealed the whole anastomosis in 4 subjects: 3 with AL and 1 without. In all 3 animals with AL, the examiner did not see the defect and in 1 without leakage, an anastomotic defect was observed on peritoneoscopy (**Videos 1–3**; videos are available on request).

Overall, the sensitivity and specificity of peritoneal flexible endoscopy in detecting AL 8 hours after the operation was low. The sensitivity was 0% and the specificity 40%.

DISCUSSION

Three reviews gave us insight into the diagnostic workup and timing of AL diagnosis in colorectal surgery over the past 2 decades.[3,13,14] The diagnostic workup for colorectal anastomotic dehiscence has partially changed from routine contrast radiography (from postoperative day 4 to 14) or as-needed after surgery, to clinical diagnosis followed by confirmation with radiologic,[5,6,8,15] endoscopic, or surgical examination. There is evidence that early diagnosis and treatment of the patients with lower gastrointestinal (GI) AL reduces mortality.[16,17] At present, we do not have a reliable method for early diagnosis of colorectal anastomosis insufficiency.

In an attempt to improve early diagnosis of GI AL, different diagnostic methods have been tested on animals and humans.[18] The tests are based on detection of the mediators of inflammation, ischemia, or tissue repair parameters in peritoneal fluid.[3,18–23] Although these methods have shown promising results, they are still not used in clinical practice, perhaps because of the lack of defined cutoff values, the complexity of the method, and the necessity for postoperative peritoneal drainage, which is currently not recommended.[24]

We argue that if we can provide safe and easy access to the peritoneal cavity in the postoperative course, we can inspect the intestinal anastomosis with a flexible endoscope on an as-needed basis. Such a capability would have the benefit of direct visualization of the anastomosis and surrounding peritoneal cavity and would provide a fast and reliable diagnosis. In the absence of a device developed for this purpose, we used a BGFT as an access port in our study. This type of device has been in use for many years, with a record of safe outcomes. The BGFT is inserted under direct visualization, and the risk of complications related to its placement is therefore minimal. In laparoscopic surgery, peritoneal cavity access points can be used for this purpose. The BGFT can be removed when it is no longer needed for observation of the abdominopelvic cavity, at discharge from the hospital, or at any point in the postoperative course. Device removal does not require instrumentation. We used the BGFT as an access port in our study because of its small size, and the risk of incisional hernias after removal of the BGFT was estimated to be insignificant. Incidental removal of the tube is not expected to add additional risk in comparison with planned removal.

Safety

Despite an attempt to protect the BGFT from contamination by covering it with transparent adhesive film, gross fecal contamination was observed around the external part of the BGFT in all animals on the day 2 after tube placement, from their lying in the pen. Laparotomies 48 hours after tube insertion did not exhibit signs of peritoneal contamination. In one swine, a moderate amount of cloudy ascites was observed, but without signs of peritonitis on peritoneoscopy 8 hours later. There were no complications related to the BGFT placement. Additional animal studies are needed to examine whether the prolonged presence of the button port in the abdominal wall is safe and does not cause clinically significant peritoneal contamination.

Feasibility

We used PDS and prolene sutures to mark the anastomosis. These marking sutures not only enabled identification of the anastomosis, but also facilitated manipulation and visualization of the large bowel containing the anastomosis. This unexpected additional benefit of the marking sutures helped us to overcome some of the restrictions in

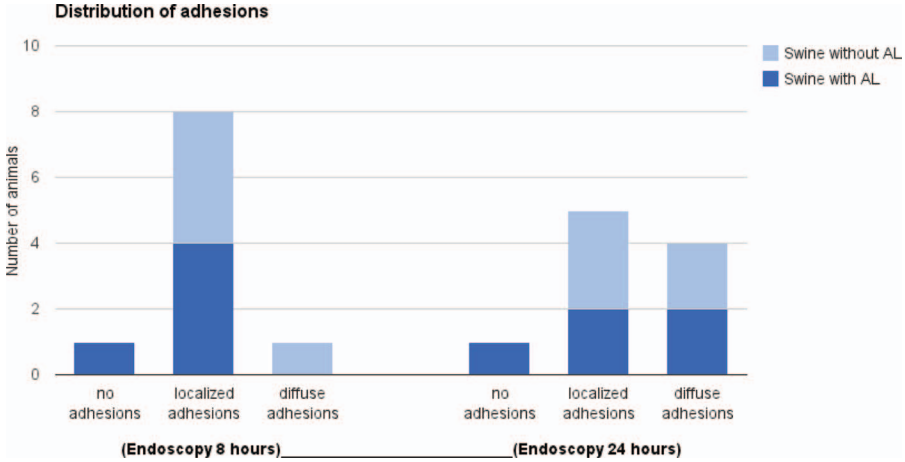

Figure 3. Distribution of adhesions in animals with and without AL, 8 and 24 hours after surgery.

examining the peritoneal cavity, caused by the percutaneous tube's fixed position in the abdominal wall (**Video 1**). Some may argue that manipulating the bowel containing the anastomosis could have a detrimental effect on AL that could result in a sealed perforation or formation of an abscess. We believe that detecting AL early in the postoperative course may have a positive impact on treatment of the dehiscence, making manipulation a worthwhile risk.

All peritoneoscopies were performed by a consultant surgeon experienced in endoscopy. Even though it was the first time he had examined the peritoneal cavity with an endoscope, the examination time remained stable throughout the study, indicating a very short learning curve for physicians skilled in endoscopy. We performed 12 peritoneoscopies on 6 animals as a pilot trial before the start of the study.

We found that visibility was substantially limited 24 hours after the operation, irrespective of the presence or absence of AL, mostly due to adhesions that had developed in most of the swine in the early postoperative course. Adhesions developed in 90% of the swine 8 hours after the operation, irrespective of anastomotic dehiscence (**Video 4**). The incidence of diffuse adhesions rose from 10% at 8 hours after the procedure to 40% within 24 hours. Distribution of the adhesions was even in the animals with and without leakage (**Figure 3**). In their study, 2 weeks after surgery, Dubcenco et al[25] found adhesions in 100% of swine that had undergone laparotomy. They demonstrated that even minor surgical intervention, such as liver biopsy, performed in the setting of laparotomy, could provoke adhesion formation in swine. We found similar results in our current study. We conclude that peritoneal adhesions after open abdominal surgery in a porcine model cannot be used as an indicator of peritonitis.

Regardless of excessive adhesion formation, we could inspect more than half of the anastomosis line in 60% of the swine 8 hours after surgery. In 4 of the 10 animals, the full circumference of the anastomosis was inspected, and video was recorded during endoscopy 8 hours after the operation. Three of the animals with fully visualized anastomoses had AL that was not recognized during endoscopy. This unexpected result can be explained by species-related differences in the inflammatory response to surgical stress and peritoneal contamination, which is more extensive in swine than in humans. This profuse inflammatory reaction prevented leakage of large bowel content into the peritoneal cavity, despite the defect's involving a quarter of the bowel's circumference. As Hoeppner et al[26] found in their study, "distinctive formation of adhesions prevented development of peritonitis or intraabdominal abscess" in swine with leakage of the colonic anastomosis. In our study, we could not confirm the results of our Danish colleagues that a 21-mm-diameter defect in the anastomotic line is sufficient to provoke diffuse peritonitis,[27] at least not during the first 24 hours after AL occurs.

In 1 swine without AL, the whole anastomosis was inspected, and a leak was identified on endoscopy. Postmortem examination revealed that the anastomosis site was intact (**Figure 4**; **Video 3**). Additional video analysis explained this false-positive result as discoloration of the mesocolonic defect that was formed during creation of the anastomosis and was misinterpreted as an anastomotic insufficiency. On closer examination, the anastomotic de-

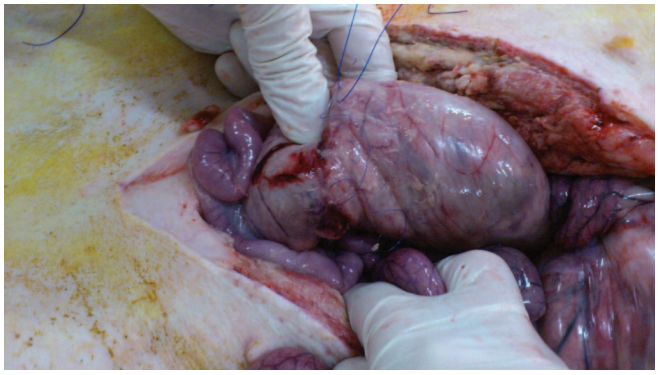

Figure 4. Postmortem examination of a sufficient anastomosis, misinterpreted on peritoneoscopy as an AL.

fect was seen on the other side of the PDS suture, marking the mesenteric side of the large bowel, with a sufficient anastomosis line between the prolene suture and PDS sutures on both sides.

Accuracy of the Method

In our study, we found unimpressive sensitivity and low specificity of the proposed method for the detection of both peritonitis and AL. When viewed in the context of excessive adhesion formation after laparotomy in swine, the results do not reflect the true accuracy of the method, but rather the limitations of the porcine model's transferability to humans.

Limitations and Perspectives

There are 2 main limitations of this study. First, we had a small sample size that might have been inadequate to determine the actual accuracy of the tested method. Second, the use of the swine as an animal model for colonic AL and testing of the visualization of peritoneal diagnostic method was limited by significant adhesion formation. Our results imply that the excessive adhesion formation after laparotomy prevents reliable reproducibility of an anastomotic defect to provoke diffuse peritonitis, at least early after leakage.

Peritoneal flexible endoscopy could be an alternative to diagnostic laparoscopy offering the advantage of repeated examination, possibly as a bedside procedure. It can be easily learned, and, in the hands of a physician with previous experience in endoscopy, it does not require additional training. The method is not limited to diagnosis of AL in lower GI surgery and may also be used as an instrument for visualization of the peritoneal cavity after upper GI surgery. As an additional benefit, it can facilitate sampling of peritoneal fluid for measuring biomarkers of inflammation, ischemia, and bacterial contamination.

CONCLUSION

Peritoneal flexible endoscopy is a safe and simple procedure that probably can be performed bedside in unsedated patients. The results of our feasibility study in a swine model, however, do not answer the question of whether this method provides satisfactory visualization of the anastomosis in the early postoperative course in humans after colorectal surgery. We believe that extensive adhesion formation in the swine model prevents extrapolation of the data to humans and is the reason that we could not determine the method's accuracy. Additional studies are needed to address these methodological concerns.

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References:

1. Hallböök O, Sjödhal R. Anastomotic leakage and functional outcome after anterior resection of the rectum. *Br J Surg*. 1996; 83:60–62.

2. Mirnezami A, Mirnezami R, Chandrakumaran K, Sasapu K, Sagar P, Finan P. Increased local recurrence and reduced survival from colorectal cancer following anastomotic leak: systematic review and meta-analysis. *Ann Surg*. 2011;253:890–899.

3. Hirst NA, Tiernan JP, Millner PA, Jayne DG. Systematic review of methods to predict and detect anastomotic leakage in colorectal surgery. *Colorectal Dis*. 2014;16:95–109.

4. Hyman N, Manchester TL, Osler T, Burns B, Cataldo PA. Anastomotic leaks after intestinal anastomosis it's later than you think. *Ann Surg*. 2007;245:254–258.

5. Nicksa GA, Dring RV, Johnson KH, Sardella WV, Vignati PV, Cohen JL. Anastomotic leaks: what is the best diagnostic imaging study? *Dis Colon Rectum*. 2007;50:197–203.

6. Khoury W, Ben-Yehuda A, Ben-Haim M, Klausner JM, Szold O. Abdominal computed tomography for diagnosing postoperative lower gastrointestinal tract leaks. *J Gastrointest Surg*. 2009; 13:1454–1458.

7. Nesbakken A, Nygaard K, Lunde OC, Blücher J, Gjertsen Ø, Dullerud R. Anastomotic leak following mesorectal excision for rectal cancer: true incidence and diagnostic challenges. *Colorectal Dis*. 2005;7:576–581.

8. Kornmann VN, Treskes N, Hoonhout LH, Bollen TL, van Ramshorst B, Boerma D. Systemic review on the value of CT scanning in the diagnosis of anastomotic leakage after colorectal surgery. *Int J Colorectal Dis*. 2013;28:437–445.

9. Platell C, Barwood N, Dorfmann G, Makin G. The incidence of anastomotic leaks in patients undergoing colorectal surgery. *Colorectal Dis*. 2007;9:71–79.

10. DuBrow RA, David CL, Curley SA. Anastomotic leaks after low anterior resection for rectal carcinoma: evaluation with CT and barium enema. *AJR*. 1995;165:567–571.

11. Matthiessen P, Henriksson M, Hallböök O, Grunditz E, Norén B, Arbman G. Increase of serum C-reactive protein is an early indicator of subsequent symptomatic anastomotic leakage after anterior resection. *Colorectal Dis*. 2008;10:75–80.

12. Power N, Atri M, Ryan S, Haddad R, Smith A. CT assessment of anastomotic bowel leak. *Clin Radiol*. 2007;62:37–42.

13. Rahbari NN, Weitz J, Hohenberger W, et al. Definition and grading of anastomotic leakage following anterior resection of the rectum: a proposal by the International Study Group of Rectal Cancer. *Surgery*. 2010;147:339–351.

14. Bruce J, Krukowski ZH, Al-Khairy G, Rusell EM, Park KGM. Systematic review of the definition and measurement of anastomotic leak after gastrointestinal surgery. *Br J Surg*. 2001;88:1157–1168.

15. Eckmann C, Kujath P, Schiedeck TH, Shekarriz H, Bruch HP. Anastomotic leakage following low anterior resection: results of a standardized diagnostic and therapeutic approach. *Int J Colorectal Dis*. 2004;19:128–133.

16. Alves A, Panis Y, Pocard M, Regimbeau JM, Valleur P. Management of anastomotic leakage after nondiverted large bowel resection. *J Am Coll Surg*. 1999;189:554–559.

17. den Dulk M, Noter SL, Hendriks ER, et al. Improved diagnosis and treatment of anastomotic leakage after colorectal surgery. *EJSO*. 2009;35:420–426.

18. Komen N, de Bruin WF, Kleinrensink GJ, Jeekel J, Lange JF. Anastomotic leakage, the search for a reliable biomarker: a review of the literature. *Colorectal Dis*. 2008;10:109–115.

19. Yamamoto T, Umegae S, Matsumoto K, Saniabadi AR. Peritoneal cytokines as early markers of peritonitis following surgery for colorectal carcinoma: a prospective study. *Cytokine*. 2011;53:239–242.

20. Fouda E, El Nakeeb A, Magdy A, Hammad EA, Othman G, Farid M. Early detection of anastomotic leakage after elective low anterior resection. *J Gastrointest Surg*. 2011;15:137–144.

21. Matthiessen P, Strand I, Jansson K, et al. Is early detection of anastomotic leakage possible by intraperitoneal microdialysis and intraperitoneal cytokines after anterior resection of the rectum for cancer? *Dis Colon Rectum*. 2007;50:1918–1927.

22. Ellebæk Pedersen M, Qvist N, Bisgaard C, Kelly U, Bernhard A, Møller Pedersen S. Peritoneal microdialysis: early diagnosis of anastomotic leakage after low anterior resection for rectosigmoid cancer. *Scand J Surg*. 2009;98:148–154.

23. Pasternak B, Matthiessen P, Jansson K, Andersson M, Aspenberg P. Elevated intraperitoneal matrix metalloproteinases-8 and -9 in patients who develop anastomotic leakage after rectal cancer surgery: a pilot study. *Colorectal Dis*. 2010;12:e93–e98.

24. Karliczek A, Jesus EC, Matos D, Castro AA, Attalah AN, Wiggers T. Drainage or nondrainage in elective colorectal anastomosis: a systemic review and meta-analysis. *Colorectal Dis*. 2006;8:259–265.

25. Dubcenco E, Assumpcao L, Dray X, et al. Adhesion formation after peritoneoscopy with liver biopsy in a survival porcine model: comparison of laparotomy, laparoscopy, and transgastric natural orifice transluminal endoscopic surgery (NOTES). *Endoscopy*. 2009;41:971–978.

26. Hoeppner J, Crnogorac V, Hopt UT, Weiser HF. The pig as an experimental model for colonic healing study of leakage and ischemia in colonic anastomosis. *J Invest Surg*. 2009;22:281–285.

27. Nordentoft T, Sørensen M. Leakage of colon anastomoses: development of an experimental model in pigs. *Eur Surg Res*. 2007;39:14–16.