As a major regulator of gene transcription, NFκB is involved in immune, inflammatory, and stress responses. In response to cell stimulatory factors such as TNFα and other cytokines, NFκB undergoes rapid phosphorylation and translocation into the nucleus to activate target genes [9, 10]. Findings show that NFκB promotes the expression of diverse target genes involved in cell proliferation, cell adhesion, and inflammatory responses [11]. However, it is unclear whether surgical stress activates NFκB in malignant cells or not.

Macrophage migration inhibitory factor (MIF) is one of the molecules upregulated by activated NFκB. MIF is well known as an important factor in the control of cell proliferation, differentiation, angiogenesis, and tumor progression [12–15]. Recently, overexpression of MIF has been shown to induce angiogenesis and a deteriorating prognosis after radical hepatic resection for hepatoma [16]. There is a possibility that surgical stress induces TNFα, which leads to increased MIF expression via NFκB. This may result in enhanced growth and invasiveness of cancer cells [17].

This study therefore aimed to evaluate the expression of TNFα and MIF mRNA as well as the activation of NFκB in tumors after CO_2 pneumoperitoneum and laparotomy in nude mice with peritoneally disseminated human gastric carcinoma.

Materials and methods

Cell preparation

All cell culture reagents were purchased from Gibco BRL (Life Technologies, Rockville, MD, USA). The human gastric cancer (MKN45) cells' poorly differentiated human gastric carcinoma cell line was grown in RPMI 1640 medium supplemented with 10% fetal bovine serum and an antibiotic/antimycotic agent containing 100 IU/ml of penicillin, 0.1 mg/ml of streptomycin, and 2.5×10^{-4} mg/ml of amphotericin B. The cells were cultured in dishes in a 5% CO_2 atmosphere at 37°C.

To prepare tumor cells for inoculation, cells in the exponential growth phase were harvested by 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA), then washed and resuspended in phosphate-buffered saline (PBS). Cell viability was determined by trypan blue exclusion, and only single-cell suspensions of 90% viability were used.

Animal models

Male 6-week-old BALB/c nude mice were obtained from Seac Yoshitomi (Tokyo, Japan) and maintained under specific pathogenic free laboratory conditions. All procedures were performed according to the Animal Experimentation

and Ethical Guidelines of Oita University. The animals were kept 1 week before tumor inoculation, and animal weight was 25 to 30 g at that time.

To create the murine peritoneal dissemination model, the mice were intraperitoneally injected with MKN45 cells (3×10^6 cells) were exposed to laparotomy, CO_2 pneumoperitoneum, anesthesia alone as a control condition, or no procedure. All the mice were anesthetized by diethyl ether inhalation and fixed to the operating table in the supine position using adhesive tape.

In the laparotomy group, a 3-cm laparotomy was performed in the midline. The abdominal content was exposed for 20 min. Then the incision was closed using polyglycolic acid sutures 3-0, and the animal was allowed to recover. In the CO_2 pneumoperitoneum group, CO_2 pneumoperitoneum was created and maintained for 20 min under pressure of approximately 6 cm H_2O, as previously reported [19]. Each mouse in the anesthesia group underwent only diethyl ether anesthesia for 20 min. In the no procedure group, the mice were killed immediately with overinhalation of diethyl ether.

The effect of the surgical procedure on TNFα and MIF mRNA expression and NFκB protein activity in peritoneal tumors was evaluated in experiment 1. On day 21, after peritoneal dissemination model preparation, the mice were exposed to laparotomy, CO_2 pneumoperitoneum, anesthesia alone, or immediate killing without any surgical procedure. After 1, 6, 24, and 48 h, and after 1 and 2 weeks, animals (7 mice per time point) were killed and tumor nodules collected. All nodules were frozen immediately in liquid nitrogen, then stored at –80°C.

The effect of surgical procedures on the growth of peritoneal tumor nodules was evaluated in experiment 2. On day 3 after peritoneal dissemination model induction, a laparotomy CO_2 pneumoperitoneum or anesthesia only procedure was performed. The animals were killed at 3 weeks. All macroscopic tumor nodules were collected and weighed. Tumor growth was assessed at the abdominal wall, omentum, mesentery, peritoneum, liver, and retroperitoneum.

Analysis of TNFα and MIF mRNA expression by reverse transcription-polymerase chain reaction (RT-PCR)

Briefly, total RNA from tumor tissue was isolated by an automated procedure using the tissue Mini Kit (BioRobot; Qiagen, Tokyo, Japan) according to the manufacturer's instructions, and purified total RNA was stored at –80°C. Total RNA (1 μg) was reverse transcribed at 22°C for 10 min, then at 37°C for 60 min and 80°C for 5 min. Amplification with specific primers for human TNFα, MIF, and glyceraldehyde-3 phosphate dehydrogenase (GAPDH) was

Table 1 Primer sequences used for reverse transcription-polymerase chain reaction (RT-PCR)

Primer	Sequence	Length (bp)
TNFα	F 5′-CAGAGGGAAGAGTTCCCCAG-3′	324
	R 5′-CCTTGGTCTGGTAGGAGACG-3′	
MIF	F 5′-CTCTCCGAGCTCACCCAGCAG-3′	255
	R 5′-CGCGTTCATGTCGTAATAGTT-3′	
GAPDH	F 5′- GCCAAAAGGGTCATCATCTCTG-3′	348
	R 5′-CATGCCAGTGAGCTTCCCGT-3′	

bp basepair, *TNFα* tumor necrosis factor-α, *MIF* migration inhibitory factor, *GAPDH* glyceraldehyde-3 phosphate dehydrogenase

performed (1 min at 94°C for denaturing; 1 min at 54°C [GAPDH], 55°C [TNFα], or 60°C [MIF] for annealing; and 1 min at 72°C for extension, for 33 cycles). The primer sequences used are in Table 1 [20–22].

Amplified products were electrophoresed using 6x loading buffer triple dye (Wako, Nippon Gene, Tokyo, Japan) on 1.8% agarose gel, with GAPDH used as an internal control and to estimate mRNA relative expression. Bands were analyzed using scanning densitometry by image J analysis software (U.S. National Institutes of Health, Bethesda, MD, USA).

Analysis of NFκB activity

Lysates from frozen tumor tissue at the sixth hour after the procedure were homogenized. Cytoplasmic and nuclear extracts were prepared using NE-PER nuclear and cytoplasmic extraction reagents (Pierce Biotechnology, Rockford, USA) according to the manufacturer's instructions. The amount of NFκB protein in each extract was measured by the Western blot method. Cytoplasmic and nuclear extracts were electrophoresed on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels. Protein then was electroblotted onto Sequi-Blot polyvinylidene difluoride membranes (Bio-Rad Laboratories, Hercules, CA, USA) as previously described [23].

Next, the membranes were incubated in 5% nonfat milk in TBST-20 (Tris-buffered saline with 1% Tween 20) overnight. The membranes were first incubated with a specific mouse monoclonal antibody that can detect NFκB P65 at 1:200 dilution, then incubated with horseradish peroxidase-conjugated immunoglobulin-G (IgG) antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

Antibody-labeled proteins were detected using protocols and reagents contained in the enhanced chemiluminescence (ECL) detection reagent and analysis system (Amersham Biosciences UK Ltd., Buckinghamshire, UK) on hyperfilm ECL (Amersham Biosciences Corp., Piscataway, NJ, USA). Bands were analyzed using scanning densitometry by image J analysis software.

Statistical analysis

Both TNFα and MIF mRNA, expressed as a ratio in relation to GAPDH mRNA and tumor weight, were shown as mean ± standard deviation. Associations between variables were tested using a nonrepeated analysis of variance (ANOVA) test. Then groups were compared post hoc by Student's *t*-test. All *p* values less than 0.05 were considered statistically significant.

Results

Effect of surgical procedures on tumor growth

Total tumor weight was significantly higher after laparotomy than after CO_2 pneumoperitoneum ($p < 0.05$) or anesthesia ($p < 0.0001$) (Fig. 1). Interestingly, only tumors located in the anterior abdominal wall showed a significant difference in tumor weight ($p < 0.01$ for laparotomy vs CO_2 pneumoperitoneum, Table 2).

Expression of TNFα mRNA after the surgical procedure

Using RT-PCR, we examined TNFα mRNA expression in peritoneal tumors after laparotomy versus CO_2
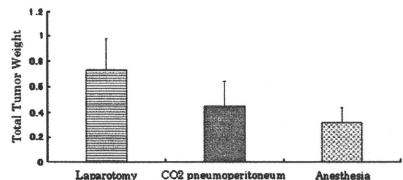

Fig. 1 Total tumor weight for peritoneal dissemination in a murine model. Tumor growth was greater in the laparotomy group than in the anesthesia ($p < 0.01$) and carbon dioxide pneumoperitoneal ($p < 0.05$) groups

Table 2 Mean tumor weight in mice 3 weeks after carbon dioxide (CO_2) pneumoperitoneum and laparotomy

Variable	Laparotomy	CO_2 Pneumo	*p* Value
Subcutis	0.39 ± 0.12	0.21 ± 0.09	0.01
Omentum	0.13 ± 0.05	0.13 ± 0.06	NS
Mesentery	0.10 ± 0.06	0.05 ± 0.06	NS
Liver	0.10 ± 0.07	0.02 ± 0.02	NS
Retroperitoneum	0.02 ± 0.04	0.02 ± 0.04	NS
Total	0.74 ± 0.02	0.41 ± 0.19	<0.05

CO_2 Pneumo carbon dioxide pneumoperitoneum, *NS* not significant

A

B
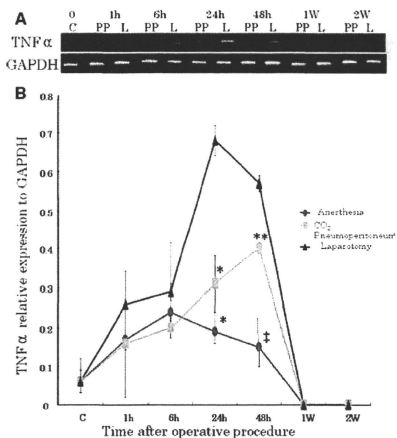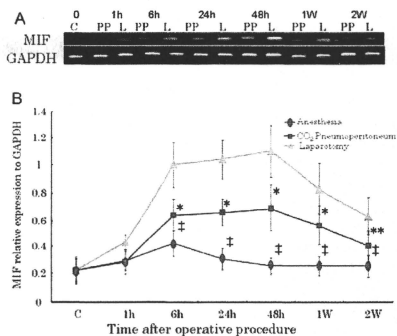

Fig. 2 Tumor necrosis factor-α (TNFα) mRNA relative expression in gastric tumors after carbon dioxide pneumoperitoneum and laparotomy in a tumor-bearing mouse model. **A** Reverse transcription-polymerase chain reaction (RT-PCR) representation. **B** Time course after experiment. A significant difference in TNFα expression was continued for up to 48 h of the experiment's time course. * $p < 0.05$; ** $p < 0.01$; ‡ $p < 0.001$. *L* laparotomy, *PP* carbon dioxide pneumoperitoneum

pneumoperitoneum. The TNFα mRNA expression started to increase in all groups 1 h after the experiment, then continued to increase progressively until 24 h in the laparotomy group and 48 h in the CO₂ pneumoperitoneal group. After that, the TNFα mRNA expression in both groups started to decrease from its peak, soon reaching the control level. Expression of TNFα mRNA was significantly higher 24 h and 48 h after laparotomy than after CO_2 pneumoperitoneum ($p < 0.05$ and $p < 0.01$ respectively). In the anesthesia-only group, TNFα mRNA expression started to decrease toward the control level soon after 6 h (Fig. 2A, B).

Expression of MIF mRNA after the surgical procedure

Because TNFα upregulates MIF expression, we examined MIF mRNA expression by RT-PCR. Expression of MIF mRNA started to increase significantly in all groups 6 h after the experiment, then continued to increase significantly and progressively until 48 h in the laparotomy and CO_2 pneumoperitoneal groups. Thereafter, MIF mRNA expression in both groups started to decrease. However, in

A

B

Fig. 3 Migration inhibitory factor (MIF) mRNA relative expression in gastric tumors after carbon dioxide pnemoperitoneum and laparotomy in a tumor-bearing mouse model. **A** Reverse transcription-polymerase chain reaction (RT-PCR) representation. **B** Time course after the experiment. A significant difference in MIF expression continued until the end of the experiment time course. * $p < 0.05$; ** $p < 0.01$; ‡ $p < 0.001$. *L* laparotomy, *PP* carbon dioxide pneumoperitoneum

contrast to TNFα, they did not reach the control level until the end of the observation period (i.e., 2 weeks).

Expression of MIF mRNA was significantly higher after laparotomy than after CO_2 pneumoperitoneum at all the examined time points ($p < 0.05$ until 1 week or $p < 0.01$ at 2 weeks). Additionally, the level in the anesthesia group started to fall toward the control level soon after 6 h (Fig. 3A, B).

Nuclear shift of NFκB protein after the surgical procedure

Using Western blotting, the NFκB protein shift into the nucleus was examined. The shift in NFκB protein from the cytoplasm into the nucleus was higher and more progressive with laparotomy than with CO_2 pneumoperitoneum 6 h after the experiment, as shown in Fig. 4.
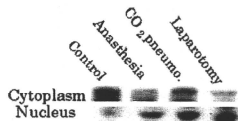
Fig. 4 Western blot for nuclear factor kappa B (NFκB) protein that shows shifting of the NFκB protein from the cytoplasm into the nucleus 6 h after the experiment, which was higher after laparotomy than after carbon dioxide pneumoperitoneum or anesthesia

Discussion

Surgical resection is the most important method for treating malignant solid tumors [24]. However, several reports show that surgical stress possibly enhances the growth of the dormant malignant cells [3, 25, 26] . Also, the mechanisms by which surgical trauma might enhance tumor growth and metastasis still are not totally understood [3, 4, 27].

Recently, minimally invasive surgeries such as laparoscopic procedures have become popular worldwide [6, 7]. The feasibility of laparoscopic surgery with minimal invasiveness for malignant tumors has been examined in the experimental and clinical settings, but it still is controversial [1–3, 5]. Furthermore, few studies have investigated the effect of surgical stress on the expression of cytokines and growth factors, which affect tumor growth and progression [1–4, 7, 25].

In this study, we showed that CO_2 pneumoperitoneum with minimal invasiveness induces less tumor growth and lower TNFα and MIF mRNA expression in peritoneal tumors than laparotomy. Moreover, we showed less activation of the DNA-binding protein; NFκB, after CO_2 pneumoperitoneum than after laparotomy.

In our study, we used nude mice with malignant peritoneal inoculation. Using this model, we showed that tumor weight was higher after laparotomy than after CO_2 pneumoperitoneum. Interestingly, the weight was higher after laparotomy than after CO_2 pneumoperitoneum only for tumors located in the anterior abdominal wall (Table 2). This finding may be associated with some growth factors induced at injury sites after laparotomy that induce not only wound healing but also tumor attachment and growth at wound sites [3, 11, 28]. These data suggest that minimally invasive surgery may be more feasible for the treatment of cancer in clinical settings.

This model also is convenient and useful for clarifying the association of surgical stress modes such as CO_2 pneumoperitoneum and laparotomy with oncologic microbiology. We could analyze the gene expression of TNFα and MIF and activation of NFκB protein after surgical procedures by using peritoneal tumor nodules in this model. The cytokine TNFα is known to promote several inflammatory events associated with tumor growth and progression [29]. It is recognized as an essential mediator of the stress response that influences immune cell function, proliferation, differentiation, and apoptosis [30]. Recently, TNFα has been reported to activate NFκB, a DNA-binding protein in epithelial cells that is essential for malignant progression [31].

In our study, TNFα mRNA was less expressed sooner after CO_2 pneumoperitoneum than after laparotomy. These data suggest that TNFα mRNA is expressed early after surgery and that less invasive surgery may cause a lower level of TNFα expression.

To our knowledge, this is the first study to investigate the association of NFκB activation in tumors with surgical stress. In our study, NFκB protein was activated less after CO_2 pneumoperitoneum than after laparotomy.

Ravi et al. [11] found that activation of NFκB causes the expression of many important genes involved in cell cycle progression, cell survival, cell adhesion/angiogenesis, and immune/inflammatory responses. The activation of NFκB not only enables malignant transformation and tumor progression but also provides a mechanism by which tumor cells escape immune surveillance and resist therapy. Hagemann showed that the activation of NFκB, caused by TNFα, induces MIF expression [17]. In the current study, lower expression of MIF in the CO_2 pneumoperitoneum group seemed to be associated with lower activation of NFκB after CO_2 pneumoperitoneum than after laparotomy.

Findings have shown MIF to be an important factor in the control of cell proliferation, differentiation, angiogenesis, and tumor progression [13–15]. Recent studies have shown that MIF may play a critical role in the development of cancers as a link between inflammation and tumorigenesis [14]. It has been shown that overexpression of MIF induces angiogenesis and deteriorates prognosis after surgery [16]. Lower expression of MIF after CO_2 pneumoperitoneum may be related to the lower tumor weight in the CO_2 pneumoperitoneum group.

In clinical settings, tumor recurrence may be explained by the residual microscopic cancer cells after surgical resection [25, 26]. Even after complete resection of primary and secondary hepatic malignancies, recurrent disease develops in nearly two-thirds of patients, and it is believed to arise from residual microscopic cancer undetected at surgery [32]. Also, microscopic viable gastric cancer cells repeatedly have been identified within the peritoneal cavity after gastrectomy [33]. Our results show that overexpression of TNFα and MIF induced by surgical stress may have a relation to the growth and progression of microscopic cancer after surgery and that less invasiveness of surgical procedures may be important from the oncologic point of view.

In conclusion, CO_2 pneumoperitoneum with minimal invasiveness induced lower levels of TNFα and MIF mRNA expression and less NFκB activation than laparotomy. This correlation needs further study. Less invasive surgical procedures may be important from the oncologic point of view. More animal experiments and clinical research to clarify the relation of surgical stress to tumor growth and progression on a microbiologic basis are necessary. Consideration of the perioperative period as oncologically risky may lead to a search for new strategies or drugs such as anti-NFκB and anti-TNFα to minimize the effect of surgical stress on tumor growth and progression.

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Cardiopulmonary and immunologic effects of transvaginal natural-orifice transluminal endoscopic surgery cholecystectomy compared with laparoscopic cholecystectomy in a porcine survival model

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Background: A few studies have addressed the physiology related to a basic natural-orifice transluminal endoscopy surgery (NOTES) procedure, such as transgastric peritoneoscopy, but the physiologic impact of more complex NOTES procedures has not been previously examined.

Objective: To evaluate the cardiopulmonary and immunologic effects of transvaginal NOTES cholecystectomy compared with laparoscopic cholecystectomy.

Setting: Survival experiments in 10 40-kg female pigs assigned to transvaginal cholecystectomy and laparoscopic cholecystectomy groups.

Interventions: Transvaginal cholecystectomy was performed with the assistance of a needlescopic device, and laparoscopic cholecystectomy was performed in the standard manner.

Main Outcome Measurements: Cardiopulmonary and immunologic parameters in the transvaginal cholecystectomy group were compared with those in the laparoscopic cholecystectomy group. Cardiopulmonary parameters included heart rate, blood pressure, saturation pulse oximetry, intratracheal pressure, and arterial blood gases. Immunologic parameters included white blood cell count, tumor necrosis factor-α, interleukin-1β, and interleukin-6.

Results: All procedures were performed successfully without complications. Although operation times were longer for transvaginal cholecystectomy than for laparoscopic cholecystectomy, cardiopulmonary changes were similar and stable in both groups. White blood cell count, interleukin-1β, and interleukin-6 did not differ between the 2 groups, and the increase in tumor necrosis factor α after transvaginal cholecystectomy was significantly smaller on postoperative day 1 than after laparoscopic cholecystectomy (133.4 pg/mL vs 200.4 pg/mL; $P < .05$).

Limitations: Animal model and small sample size.

Conclusions: Transvaginal cholecystectomy resulted in cardiopulmonary stability and well preserved immune function similar to those of laparoscopic cholecystectomy, suggesting that NOTES may be less invasive than laparoscopic surgery. (Gastrointest Endosc 2010;72:1241-8.)

Because of the development of techniques and devices for endoscopy, such as endoscopic submucosal dissection, the clinical application of therapeutic endoscopy has become widespread and has led to a new era of less-invasive therapy. Laparoscopic surgery also has become popular in the past 20 years. It has been accepted as a

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less-invasive surgery, because several studies showed that laparoscopic surgery induces less surgical injury and is therefore associated with a reduced immune response compared with open surgery.[1-4] Moreover, in some diseases, such as cholecystolithiasis, laparoscopic surgery has replaced traditional open surgery as the standard operation of choice.

Natural-orifice transluminal endoscopic surgery (NOTES) is a new evolving concept of minimally invasive surgery that may have benefits over laparoscopic surgery by eliminating abdominal wounds altogether.[5-7] Many experimental studies have shown the technical feasibility of various surgical procedures by using NOTES,[8-10] and the clinical application of NOTES has already begun.[11-14] However, one of the most significant concerns is to clarify the physiologic impact of the NOTES procedure on the patient. The physiologic effects of pneumoperitoneum during laparoscopic surgery have been well established.[1-3] However, the physiologic impact during NOTES may be different from that during laparoscopy, because it is as yet not known how best to maintain pneumoperitoneum and intra-abdominal pressure and what kind of gases should be used in NOTES procedures. Another concern with NOTES is intra-abdominal infection, because the endoscope gains access through a site such as the digestive tract or vagina, which is more contaminated than the skin. It also remains unclear whether minimal gastrotomy or colpotomy for access to the intra-abdominal cavity is less invasive than a skin wound.

Inflammatory cytokines tumor necrosis factor α (TNF-α), interleukin (IL) 1β, and IL-6 are good guides in the assessment of the activation of the systemic immune system. These cytokines were measured to clarify the surgical stress of laparoscopic cholecystectomy.[1]

To clarify the physiologic effects of the transvaginal NOTES procedure, we compared the perioperative cardiopulmonary and immunologic measurements made during transvaginal cholecystectomy with those made during laparoscopic cholecystectomy.

MATERIAL AND METHODS

Animal preparation

This study was approved by the Animal Care and Use Committee at the Oita University Faculty of Medicine. A total of 10 female pigs weighing 35 to 40 kg were randomized to receive transvaginal NOTES cholecystectomy (n = 5; NOTES group) or laparoscopic cholecystectomy (n = 5; laparoscopic group). All pigs were fed until 24 hours before the operation. After sedation with intramuscular midazolam at 30 mg per pig, the pigs were given general anesthesia. A 22-gauge venous catheter was placed in the marginal ear vein, and thiopental sodium (80 mg per pig) was administered. The pigs were orotracheally intubated and mechanically ventilated at 12 respirations per minute with a tidal volume of 15 mL/kg and FiO_2 of 100%. Main-

Take-home Message

• Transvaginal natural-orifice transluminal endoscopic surgery cholecystectomy showed intraoperative cardiopulmonary stability and well preserved postoperative immune function similar to laparoscopic cholecystectomy in a porcine survival model.

tenance of anesthesia was provided by 1% to 2% sevoflurane inhalation. Cardiopulmonary status was monitored via electrocardiogram, and a catheter was placed in the femoral artery.

The pig was placed supine in the reverse Trendelenburg position. Perioperative antibiotic (cefazolin sodium 1 g) was intravenously administered at the start and end of the operation. The surgical field, including the vaginal cavity, was prepared with povidone iodine. Each pig was covered with sterile drapes to expose only the abdominal wall or vagina. The endoscope, accessories, and all laparoscopic instruments were cleaned by high-level disinfection and were gas-sterilized.

Procedures and postoperative care

For transvaginal cholecystectomy, a flexible gastroscope (GIF-XQ200; Olympus Medical Systems, Tokyo, Japan) with a transparent hood (D-201-11804; Olympus Medical Systems) was introduced into the vaginal cavity. A 2-cm incision of the posterior vaginal sac was made with a Flex-knife (KD-630L; Olympus Medical Systems) and an insulation-tipped (IT) knife (KD-610L; Olympus). The endoscope was then carefully advanced into the abdominal cavity through the incision in the vagina. A Veress needle was placed through the abdominal wall under endoscopic guidance and was connected to a laparoscopic CO_2 insufflator to maintain an intraperitoneal pressure of 10 mm Hg. A needlescopic forceps (HA0002; Covidien Co, Tokyo, Japan) was placed in the right upper abdomen and was used to retract the gallbladder (Fig. 1A). The peritoneum covering the cystic structures was incised and dissected by using a combination of the Flex-knife and IT knife. After isolation of the cystic duct and artery, both were ligated together with endoclips and divided with an IT knife (Fig. 1B). The gallbladder was then dissected away from the liver bed with the Flex-knife and IT knife as in endoscopic submucosal dissection (Fig. 1C and D). Normal saline solution was used to inject the dissection plane with a raising solution. The resected gallbladder was removed through the vagina. The pneumoperitoneum was evacuated by endoscopic aspiration, after which the endoscope was removed. The vaginal incision was not closed at the end of the procedure.

For laparoscopic cholecystectomy, a 15-mm trocar was placed below the umbilicus by using the Hasson technique, and pneumoperitoneum was created and con-

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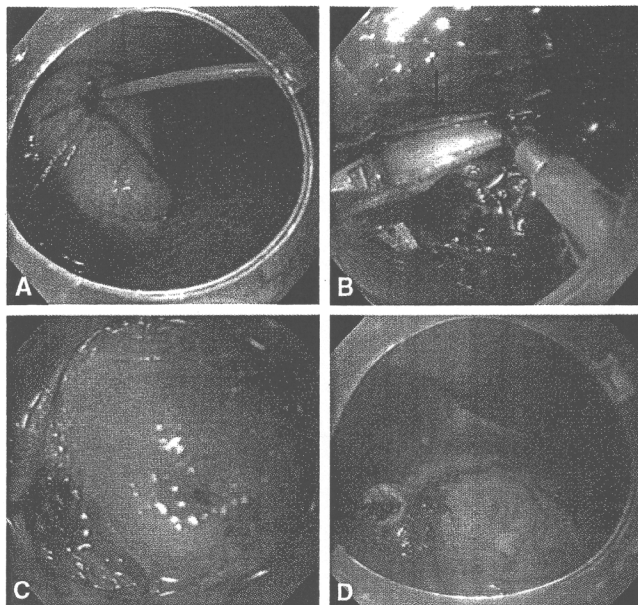
Figure 1. Endoscopic images from transvaginal cholecystectomy. **A,** Retraction of the gallbladder with needlescopic forceps. **B,** Clipping of the cystic duct and artery (*arrow*). **C, D,** Dissection of the gallbladder from the liver fossa with an IT knife.

trolled with a laparoscopic CO_2 insufflator. The intraperitoneal pressure was maintained at 10 mm Hg. Under laparoscopic visualization, a 10-mm trocar was placed in the subxiphoid area, and two 5-mm trocars were placed in the right subcostal region just as in standard laparoscopic cholecystectomy in humans. The fundus of the gallbladder was retracted with 5-mm grasping forceps. The cystic duct and artery were ligated together with a 10-mm clip applier, and dissection of the gallbladder was performed with electronic coagulating forceps. The resected gallbladder was extracted through the umbilical incision. Skin incisions were closed with absorbable sutures.

A liquid diet was offered when the pigs recovered from general anesthesia, and a regular diet was resumed the following morning. The physical activity and eating habits of the pigs were carefully monitored every day.

Data collection and analysis

All of the pigs survived for 7 days, during which time behavior, food intake, and body temperature were checked every day, after which the animals were killed and necropsy was performed. The peritoneal cavity and intraperitoneal organs were examined for signs of organ injury, peritonitis, and peritoneal adhesion. The scoring system used to evaluate adhesions in this study was adapted from the More Comprehensive Adhesion Scoring Method.[15] The severity of adhesions was classified macroscopically as follows: 0, no adhesion; 1, filmy and avascular; 2, dense or vascular; and 3, cohesive. The extent of adhesions was scored as follows: 0, no adhesion; 1, 1% to 25% involvement of total area; 2, 26% to 50%; and 3, more than 50%. The sum of the 2 scores was used as the total adhesion score. The operation time and the amount of blood loss were recorded for every operation.

Cardiopulmonary parameters were recorded at the following time points: before the operation (T1), achieving pneumoperitoneum (T2), after dividing the cystic duct and artery (T3), after dissection of the gallbladder (T4), and immediately after the operation (T5). Cardiopulmonary parameters measured included heart rate, arterial blood pressure, saturation pulse oximetry (Spo_2) (BSM-2391L; Nihon Koden, Tokyo, Japan), and arterial O_2 and CO_2 partial pressures. Blood drawings for cytokines and white blood cell (WBC) counts were performed 6 times as follows: before and immediately after the operation and 1 hour and 1, 3, and 7 days postoperatively. Measured cy-

TABLE 1. Operative outcomes

	NOTES cholecystectomy (n = 5)	Laparoscopic cholecystectomy (n = 5)	P value
Operation time, min (range)	154.0 (100-225)	64.0 (55-70)	<.01
Complications			
Intraoperative	None	None	NS
Postoperative	None	None	NS
Blood loss	Minimal	Minimal	NS
Necropsy findings			
Peritonitis	None	None	NS
Adhesion score	2.4	2.2	NS

NS, Not significant.

tokines included TNF-α, IL-1β, and IL-6. WBC counts were measured with a disposable hemocytometer (DHC-N01; NanoEnTek, Seoul, South Korea). Blood serum was separated out by centrifugation at 2000 rpm for 20 minutes. Plasma concentrations of cytokines were determined by enzyme-linked immunosorbent assay with a commercially available kit (R&D Systems, Minneapolis, Minn, USA).

All data were analyzed with StatView (version 5.0 for Windows; SAS institute, Cary, NC, USA). Differences in measurements between the NOTES group and laparoscopic groups were analyzed for statistical significance by unpaired χ^2 test or the Student t test. $P < .05$ was considered to be significant.

RESULTS

All procedures were successfully completed without intraoperative complications (Table 1). Although the operation times in the NOTES group were longer than those in the laparoscopic group (154 vs 64 minutes, respectively; $P < .05$), cardiopulmonary parameters, including heart rate, mean blood pressure, Spo$_2$, and arterial O$_2$ and CO$_2$ partial pressures were stable during the operations, and no significant differences were noted between the 2 groups (Fig. 2). Intratracheal pressure in the NOTES group remained relatively stable compared with the laparoscopic group.

All pigs gained weight and survived well after surgery for 7 days without adverse consequences. At necropsy, there was no evidence of complications such as damage to surrounding organs, bleeding, abscess, and peritonitis. The mean adhesion score in the NOTES group was similar to that in the laparoscopic group (2.4 vs 2.2, respectively). There was no adhesion around the pelvic cavity, and adhesions were observed in the right upper quadrant in all pigs in the NOTES group.

The number of WBCs increased on postoperative day 1 and returned to preoperative levels by postoperative day 7

in both groups (Fig. 3). The peak WBC count was similar between the groups. The TNF-α level was increased after surgery in both groups, but the TNF-α level on postoperative day 1 in the NOTES group was significantly lower than in the laparoscopic group (133.4 $\pm$ 56.8 pg/mL vs 200.4 $\pm$ 16.0 pg/mL, respectively; $P < .05$). A markedly low TNF-α level (45.0 pg/mL) was measured in 1 pig in the NOTES group. Even if we omit the lowest data, the TNF-α level on postoperative day 1 in the NOTES group is significantly lower than in the laparoscopic group (155.5 $\pm$ 32.4 pg/mL vs 200.4 $\pm$ 16.0 pg/mL, respectively; $P < .05$). The levels of IL-1β and IL-6 increased on postoperative day 1 and then gradually returned to preoperative levels by postoperative day 7 in both groups. There were no significant differences between the groups.

DISCUSSION

This study showed that the NOTES group required longer operation times than did the laparoscopic group, but cardiopulmonary changes were minimal and stable in both groups. Although WBC, IL-1β, and IL-6 levels did not differ between the 2 groups, the increase in TNF-α level on postoperative day 1 after transvaginal cholecystectomy was significantly smaller than that after laparoscopic cholecystectomy. There were no intraoperative or postoperative complications, and no intra-abdominal abscesses were noted at necropsy in either group. There are only a few studies on the cardiopulmonary and immunologic effects of a basic NOTES procedure (transgastric NOTES peritoneoscopy vs laparoscopy), and little is known about the physiologic changes that may occur during a more complex surgical procedure using NOTES.[16-20] To our knowledge, this is the first report to evaluate the physiologic impact of transvaginal cholecystectomy compared with laparoscopic cholecystectomy.

Laparoscopic surgery has been widely accepted in the field of GI surgery because of its minimal invasiveness.

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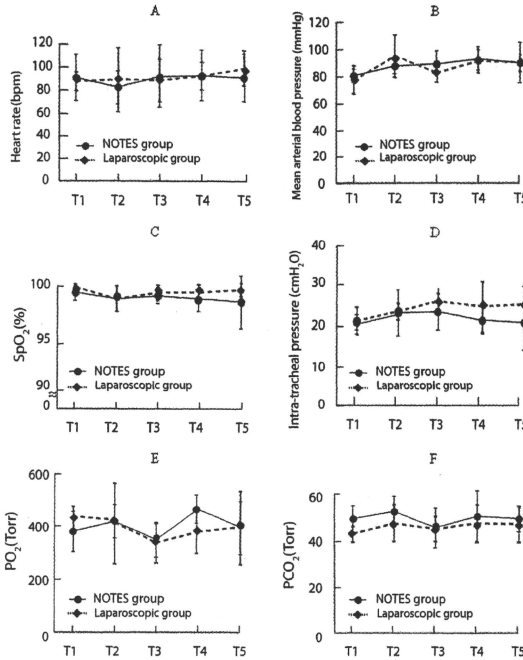
Figure 2. Comparison between NOTES and laparoscopic groups of intraoperative monitoring of (**A**) heart rate, (**B**) mean blood pressure, (**C**) saturation pulse oximetry (Spo_2), (**D**) intratracheal pressure, (**E**) arterial O_2 partial pressure, and (**F**) arterial CO_2 partial pressure at the following times: before the operation (T1), achieving pneumoperitoneum (T2), after dividing the cystic duct and artery (T3), after dissection of the gallbladder (T4), and after the operation (T5). All parameters were stable during the operating period in both the NOTES group and the laparoscopic group, with no significant differences occurring between the 2 groups. Values are shown as mean ± SD.

Experimental and clinical studies have shown that laparoscopic surgery induces less surgical trauma, such as in the size of the abdominal wound, and is therefore associated with reduced immune response compared with open surgery.[1-4] NOTES is considered to be the next logical evolutionary step in minimally invasive surgery by eliminating all abdominal wounds. Investigation of the physiologic impact of NOTES procedures is a crucial step in the further development of NOTES.

NOTES reduces the number of abdominal wounds because it requires a minimal incision in the stomach, rectum, or vagina. However, the transdigestive or transvaginal route is regarded to be a more contaminated site than the skin, and some studies have shown postoperative complications, such as intra-abdominal infection and leakage from access site, after transrectal NOTES procedures.[21,22] On the other hand, few studies have shown postoperative complications after NOTES procedures performed via transgastric, transvaginal, or transbladder

access.[21-24] The transvaginal cholecystectomies performed in the present study also showed no intra-abdominal abscess or problems associated with the access site. These findings suggest that some problems still remain with transrectal access, whereas procedures performed via transgastric or transvaginal access appear to be relatively safe.

To minimize surgical trauma to the abdominal wall, we used a needlescopic instrument, not trocars, to assist with the transgastric cholecystectomy. We consider this model of minimizing abdominal wounds to be suitable for investigating the physiologic impact of the NOTES procedure beyond the absence of abdominal incisions.

Only 2 studies have compared cardiopulmonary changes between transgastric NOTES peritoneoscopy and laparoscopy.[16,19] Von Delius et al[16] investigated the effect of pneumoperitoneum on hemodynamics and inspiratory pressure during transgastric NOTES peritoneoscopy. Although neither NOTES peritoneoscopy nor laparoscopy

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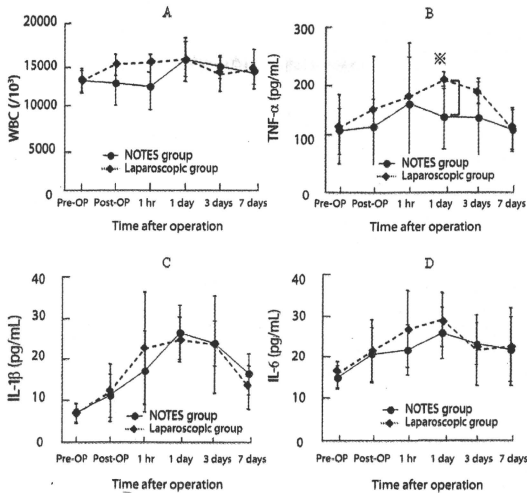
Figure 3. Comparison between NOTES and laparoscopic groups of changes in (**A**) the WBC count and (**B**) TNF-α, (**C**) IL-1β, and (**D**) IL-6 levels after NOTES and laparoscopic cholecystectomy. TNF-α was significantly elevated on postoperative day 1 in the laparoscopic group comparison with the NOTES group ($P < .05$). Values are shown as mean ± SD.

showed hemodynamic instability or a decrease in oxygen saturation, on-demand air insufflation with a standard endoscopic insufflator in the NOTES group produced wide variation in intra-abdominal pressure and significantly high peak inspiratory pressure compared with the controlled insufflation through a Veress needle in the laparoscopy group. Bingener et al[19] compared perioperative cardiopulmonary parameters during transgastric NOTES peritoneoscopy by using air insufflation with standard CO_2 laparoscopy in a porcine survival model. Although no clinically significant hemodynamic compromise was noted in either group, transgastric NOTES peritoneoscopy resulted in a decrease in pulse rate with an increase in pulse pressure. These findings suggest that on-demand air insufflation with an endoscope may have an influence on cardiopulmonary changes during NOTES. In the present study, pneumoperitoneum in the transvaginal cholecystectomy group was also controlled by using a laparoscopic CO_2 insufflator via a Veress needle, and the changes in perioperative cardiopulmonary parameters were similar and stable in both the NOTES group and the laparoscopic cholecystectomy group. Development of a system for monitoring intra-abdominal pressures with a standard endoscope is desirable to avoid the potentially adverse effects of pneumoperitoneum during NOTES.[25,26]

The cytokines IL-1β, IL-6, and TNF-α play a major role in the acute-phase response against surgical stress.[1,27] Although cytokine levels do not indicate immune status

directly, they are an objective and quantitative parameter in the assessment of immune system activation in response to operative trauma. McGee et al[17] compared the immunologic impact of transgastric NOTES peritoneoscopy with that of standard laparoscopy and laparotomy in a porcine survival model. The laparotomy group showed higher TNF-α levels than did the other groups, and the levels of TNF-α in the transgastric NOTES peritoneoscopy group were significantly reduced compared with the other groups on postoperative days 7 and 14. However, another group of investigators reported on a similar study design that was randomized and showed opposite findings[20]: The TNF-α level increased from baseline in the transgastric NOTES peritoneoscopy group on postoperative days 1, 2, and 7 and decreased from baseline in the laparoscopy group. In those 2 studies, on-demand air insufflation through the endoscope was used in the transgastric NOTES peritoneoscopy group. Insufflation pressure and the choice of insufflation gas are known to influence the immunologic reaction. In the present study, a controlled CO_2 pneumoperitoneum was used in both groups, and the NOTES group showed reduced levels of TNF-α compared with the laparoscopic cholecystectomy group. This suggests that NOTES may be associated with better preservation of postoperative systemic immune function than is standard laparoscopic surgery.

In this study, NOTES and laparoscopic cholecystectomy were done under the same intra-abdominal pressure of 10

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mm Hg. So far, there have been several reports about the intra-abdominal pressure during laparoscopic surgery, and the optimum intra-abdominal pressure was indicated to be approximately 10 mm Hg in laparoscopic surgery.[3,28] Negative effects, such as a decrease in pulmonary compliance and a decrease in renal blood flow, were observed under high intra-abdominal pressure.[3] For less invasiveness, low intra-abdominal pressure is desirable in laparoscopic surgery, but there was not good visualization under low intra-abdominal pressure in laparoscopic surgery.[28] However, wide visualization and high intra-abdominal pressure are not always necessary in NOTES, because most procedures are done with close visualization. In the present study, to clarify the surgical stress in the difference of incision length and incision site, we controlled the intra-abdominal pressure at 10 mm Hg with a laparoscopic CO_2 insufflator via Veress needle in the NOTES group and via the Hasson trocar in laparoscopic group. There was a possibility that NOTES would be a less invasive surgery if it were performed with lower intra-abdominal pressure than laparoscopic surgery.

Clinical application of transgastric and transvaginal cholecystectomy has already started. This study showed no particular advantage of transvaginal cholecystectomy over that of laparoscopic cholecystectomy. Transvaginal cholecystectomy with an ordinary endoscope and existing devices required operating times 2.5 times longer than those for laparoscopic cholecystectomy. It is well known that the operating time is associated with surgical stress.[29,30] Thus, there is still room for improvement of the NOTES procedure and its associated devices. To further determine the physiologic impact of the NOTES procedure, development of new devices and refinement of techniques are desirable, and additional studies are necessary.

CONCLUSION

Transvaginal cholecystectomy showed cardiopulmonary stability and well preserved postoperative systemic immune function similar to laparoscopic cholecystectomy. The data of this study suggest the possibility of enhanced minimal invasiveness of NOTES equal to that of laparoscopic surgery. Moreover, regarding cosmetics and postoperative wound pain, NOTES appears to be a promising new surgical technique that is less invasive than laparoscopic surgery. Additional studies, including human trials, are needed to further clarify the physiologic impact of NOTES.

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ORIGINAL ARTICLE

Risk Factors for Anastomotic Leakage Following Intersphincteric Resection for Very Low Rectal Adenocarcinoma

**Takayuki Akasu · Masashi Takawa ·
Seiichiro Yamamoto · Tomohiro Yamaguchi ·
Shin Fujita · Yoshihiro Moriya**

Abstract

Background The aim of this study was to perform a retrospective analysis of the risk factors for anastomotic leakage following intersphincteric resection (ISR) for very low rectal cancer.

Methods Between 1993 and 2007, 120 patients with T1–T3 rectal adenocarcinomas located 1 to 5 cm (median 3 cm) from the anal verge underwent ISR without radiotherapy. Univariate and multivariate analyses of 47 prospectively recorded parameters were conducted.

Results All patients had total mesorectal excision after complete bowel preparation. Of them, 103 underwent partial resection, and 17 underwent complete resection of the internal sphincter. Some 108 patients had a defunctioning stoma. Morbidity and mortality rates were 33% and 0.8%, respectively. Fifteen patients (13%) developed clinical leakage, and six (5%) had severe leakage causing relaparotomy, permanent stoma, or death. Univariate analysis of risk factors for clinical leakage revealed tumor annularity, intraoperative blood transfusion, and pulmonary disease to be significant. Multivariate analysis showed transfusion (hazard ratio, 6.5 [95% confidence interval, 1.4 to 30]; $p=0.018$) and pulmonary disease (6.3 [1.6 to 26]; $p=0.009$) to be independently significant. Moreover, transfusion (71 [3.0 to 1000]; $p=0.008$), colonic J-pouch (32 [1.8 to 500]; $p=0.018$), and pulmonary disease (32 [1.1 to 1000]; $p=0.044$) were independently associated with severe leakage.

Conclusions This study suggests intraoperative blood transfusion and pulmonary disease as independent risk factors for clinical and severe leakage following ISR and colonic J-pouch as that for severe leakage. By considering these factors, we may be able to stratify high-risk patients and prepare countermeasures.

Keywords Rectal cancer · Surgery · Intersphincteric resection · Anastomotic leakage · Risk factor

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Introduction

Although abdominoperineal resection is standard surgery for patients with massively invasive rectal adenocarcinomas located within 5 cm from the anal verge,[1] intersphincteric resection (ISR) has recently been considered as an alternative option to avoid permanent colostomy for selected patients.[2-4] ISR is defined as a procedure obtaining sufficient margins by removing part or whole of the internal sphincter and restoring bowel continuity for patients with rectal cancers involving or neighboring the anal canal.

Careful performance of ISR has been reported to allow satisfactory results both in the short and long term.[4-11] Furthermore, reported rates of anastomotic leakage follow-

ing ISR have been as comparatively low as 5% to 16% in experienced hands.[7–11] However, anastomotic leakage after rectal cancer surgery can result in reoperation, morbidity, mortality, permanent stoma, prolonged hospitalization, anal stenosis, and anal dysfunction and may be associated with a higher local recurrence rate.[12,13] To reduce such complications, clarification of the risk factors for anastomotic leakage should help in identifying high-risk patients and planning countermeasures. The aim of this study was, therefore, to perform a retrospective exploratory analysis of risk factors for anastomotic leakage following ISR for very low rectal adenocarcinomas.

Patients and Methods

Between October 1993 and February 2007, 122 patients with T1 to T3 rectal adenocarcinomas located within 5 cm from the anal verge underwent ISR at the National Cancer Center Hospital, Tokyo. All of the T1 tumors were accompanied by massive submucosal invasion. Selection criteria for ISR were as follows: (1) sufficient medical fitness; (2) normal sphincter function; (3) distance between the tumor and the anorectal junction (upper edge of the surgical anal canal) less than 2 cm; (4) no involvement of the external sphincter; and (5) no signs of disseminated disease. Preoperatively, the patients were assessed with chest and abdominal computed tomography (CT), digital anorectal examination, and radiological studies, including endorectal ultrasonography, thin-section helical CT, or high-resolution magnetic resonance imaging.

Univariate and multivariate analyses of 47 prospectively recorded clinicopathologic variables were conducted for the 120 consecutive patients who did not receive neoadjuvant radiotherapy. Data from the remaining two given radiotherapy were excluded from the present analysis. Approval by the institutional review board was not required for the observational study. All patients gave informed consent for usage of their data for analysis.

Surgical Procedures

The day before surgery, bowel lavage with 2 L of polyethylene glycol was carried out, and all patients received parenteral antibiotic prophylaxis no more than 30 min before skin incision. The surgical procedures were as described previously[11] and basically similar to those originally documented by Schiessel et al.[4,7] The intersphincteric plane between the puborectalis and the internal sphincter was dissected cautiously as caudad as possible under direct vision, using long right-angle retractors and electrocautery. When the lower edge of the tumor was

reached, the anal canal was closed just below the tumor and then irrigated with povidone iodine followed by saline. After retractors were applied to the anal canal, the anal canal mucosa and internal sphincter were circumferentially incised, and the intersphincteric plane was dissected cephalad. A resection margin of at least 1 cm was always attempted. If the rectum was not closed in the abdominal phase, it was closed using sutures during per-anal dissection. After removal of the rectum, the pelvic cavity and anal canal were washed, and then a coloanal anastomosis was made using 3-0 absorbable vertical mattress sutures. A pelvic drain was placed, and a defunctioning stoma was made.

Definition of Anastomotic Leakage

Clinical anastomotic leakage was defined as clinically apparent leakage including gas, pus, or fecal discharge from the pelvic drain or peritonitis. All anastomotic leakages were confirmed as extravasation of endoluminally administered water-soluble contrast material on radiography or computed tomography. An abscess around the anastomosis or a rectovaginal fistula was also considered as leakage. Radiological examination was performed by the surgeon and only when there was clinical suspicion of anastomotic leakage. Pouch fistula, pouch necrosis, and necrosis of neorectum were also regarded as evidence of a leakage. Severe leakage was defined as causing emergency relaparotomy, permanent stoma, or death.

Statistical Analysis

The chi-square test was used to compare proportions. The influence of each variable on the risk of clinical anastomotic leakage or severe leakage was calculated using the chi-square test. All variables associated with clinical leakage or severe leakage at $p<0.05$ were entered in a multivariate analysis using the multiple logistic regression model with the forward stepwise method (likelihood ratio). All statistical analyses were performed using SPSS for Windows, version 11.0J (SPSS-Japan Inc., Japan). A two-sided p value of less than 0.05 was considered significant.

Results

Of 39 patients (33%) who suffered complications, 30 were treated conservatively and nine received reoperations. Fifteen patients (13%) had clinical anastomotic leakage, and six underwent an emergency relaparotomy (Table 1). Five of those six had permanent stoma and one dying of

Table 1 Details of the Patients with Anastomotic Leakage

Severity	Reconstruction	Site of leakage	Treatment
Severe[a]	Colonic J-pouch (5)[a]	Pouch necrosis (2)[a] Anterior wall of pouch (1)	Pouch resection, colostomy and drainage (3)[a]
		Pouch anal anastomosis (1)	Ileostomy and drainage (1)
		Pouch-vaginal fistula (1)	Drainage and fistulectomy (1)
	Straight end to end (1)	Anovesical fistula (1)	Drain irrigation and fistulectomy (1)
Minor	Straight end to end (6)	Anastomosis (6)	Transanal drainage (3), Observation (2), Drain irrigation (1),
	Transverse coloplasty (3)	Anastomosis (3)	Drain irrigation (1), Transanal drainage (1), Observation (1)

Numbers in parentheses are numbers of patients

[a] One patient died

anastomotic leakage and sepsis (30-day mortality rate= 0.8%). Seven patients had permanent stoma due to complications (six patients) or local recurrence (one). Other complications included wound infection (nine patients), bowel obstruction (six), urinary tract infection (four), anal pain (two), cholecystitis (two), anastomotic stenosis (one), anal prolapse (one), peristomal hernia (one), and thrombocytopenia (one).

Of the 47 variables analyzed, 28 are summarized in Table 2. The remaining 19 variables were tumor size, pT, pN, pM, lateral pelvic lymph node metastasis, preoperative vital capacity, serum carcinoembryonic antigen, CA19-9, C-reactive protein, hemoglobin A1c levels, white blood cell count, hamatocrit, lymphocyte count, arterial blood oxygen tension, carbon dioxide tension, bicarbonate, base excess, liver disease, and drinking habit.

There were 92 male and 28 female patients with a median age of 57 years (range 26 to 75 years). Thirteen had pulmonary disease including chronic obstructive pulmonary disease in eight patients and restrictive respiratory disease in five. The median distance from the anal verge to the tumor was 3 cm (range 1 to 5 cm).

All patients underwent total mesorectal excision. In addition, 46 patients received extended lateral pelvic lymph node dissection. Sixty-seven patients underwent high ligation of the inferior mesenteric artery. A total of 103 patients underwent partial resection of the internal sphincter, and 17 underwent complete resection. A small part of the external sphincter was resected in six patients to obtain sufficient surgical margins. Combined resection of adjacent organs was performed for 12 patients. Two patients with solitary liver metastases and one with a solitary lung metastasis underwent complete resection of their metastases. Mobilization of the splenic flexure was performed for 35 patients. A colonic J-pouch was constructed for 24 patients, a transverse-coloplasty pouch for 38, and a straight anastomosis for 58. Some 108 patients had a defunctioning stoma which was closed 3 months after ISR. Median operating time was

339 min (range 200 to 590 min). Median blood loss was 462 mL (range 45 to 3,644 mL), and nine patients received intraoperative blood transfusions (Table 3).

The median tumor diameter was 3.7 cm (range 1 to 12 cm). Pathologic findings are shown in Table 2. Resection margins were macroscopically negative in all patients but microscopically positive in four. The median number of lymph nodes removed at surgery was 29 (range 4 to 88), and 108 patients (90%) underwent dissection of 12 or more.

Univariate Analysis

Clinical anastomotic leakage was statistically significantly associated with tumor annularity, intraoperative blood transfusion, and pulmonary disease (Table 2). Severe leakage was significantly associated with tumor annularity, extended lateral pelvic lymph node dissection, a colonic J-pouch, intraoperative transfusion, preoperative serum total protein and albumin levels, the preoperative platelet count, and pulmonary disease (Table 2). Neither overall clinical leakage nor severe leakage showed significant association with the 19 variables not shown in Table 2.

Multivariate Analysis

In a multivariate analysis for clinical leakage, the significant variables in the univariate analysis were entered. Pulmonary disease (hazard ratio, 6.3 [95% confidence interval, 1.6 to 26]; $p=0.009$) and intraoperative transfusion (6.5 [1.4 to 30]; $p=0.018$) were found to be independently significant. The incidences of clinical leakage for patients with 0, 1, and 2 positive risk factors were estimated to be 8%, 28%, and 100%, respectively.

In a multivariate analysis for severe leakage, the eight significant variables in the univariate analysis were used.

Table 2 Univariate Analyses of 28 Clinicopathologic Variables Related to Clinical Anastomotic Leakage and Severe Leakage

	Number of patients	Clinical leak (%)	p Value	Severe leak (%)	p Value
Gender					
Male	92	12 (13)	1	5 (5)	1
Female	28	3 (11)		1 (4)	
Age					
<60 years	71	6 (8)	0.16	2 (3)	0.22
≥60 years	49	9 (18)		4 (8)	
Distance of the tumor from the anal verge					
<2.5 cm	21	1 (5)	0.47	0 (0)	0.59
≥2.5 cm	99	14 (14)		6 (6)	
Tumor annularity					
<3/4	101	10 (10)	0.033	3 (3)	0.033
≥3/4	16	5 (31)		3 (19)	
Unknown	3				
Histopathologic grade					
Well-differentiated	59	9 (15)	0.62	3 (5)	1
Moderately differentiated	53	6 (11)		3 (6)	
Poorly differentiated	8	0 (0)		0 (0)	
Pathological UICC TNM stage					
Stage I	50	7 (14)	0.91	1 (2)	0.23
Stage II	21	3 (14)		3 (14)	
Stage III	46	5 (11)		2 (4)	
Stage VI	3	0 (0)		0 (0)	
Microscopic resection margins					
Negative	116	15 (13)	1	6 (5)	1
Positive	4	0 (0)		0 (0)	
Internal sphincter resection					
Partial	103	15 (15)	0.13	6 (6)	0.59
Complete	17	0 (0)		0 (0)	
Combined resection					
No	108	15 (14)	0.36	6 (6)	1
Yes	12	0 (0)		0 (0)	
Extended lateral pelvic lymph node dissection					
No	74	8 (11)	0.57	1 (1)	0.03
Yes	46	7 (15)		5 (11)	
High ligation of the inferior mesenteric artery					
No	50	6 (12)	1	3 (3)	1
Yes	67	9 (13)		3 (4)	
Mobilization of the splenic flexure					
No	63	8 (13)	1	1 (2)	0.129
Yes	35	5 (14)		3 (9)	
Reconstruction					
Straight anastomosis	58	7 (12)	0.18	1 (2)	0.001
Transverse coloplasty	38	3 (8)		0 (0)	
Colonic J-pouch	24	5 (21)		5 (21)	
Defunctioning stoma					
No	14	1 (7)	1	0 (0)	1
Yes	106	14 (13)		6 (6)	
Anastomosis height from the anal verge					
<2.0 cm	57	5 (9)	0.28	1 (2)	0.21
≥2.0 cm	63	10 (16)		5 (8)	

Table 2 (continued)

	Number of patients	Clinical leak (%)	p Value	Severe leak (%)	p Value
Operating time					
<6 h	68	8 (12)	0.79	1 (1)	0.084
≥6 h	52	7 (13)		5 (10)	
Blood loss					
<500 mL	64	6 (9)	0.29	2 (3)	0.42
≥500 mL	56	9 (16)		4 (7)	
Intraoperative blood transfusion					
No	111	11 (10)	0.014	2 (2)	<0.001
Yes	9	4 (44)		4 (44)	
Preoperative body mass index					
<25	89	10 (11)	0.53	4 (4)	0.65
≥25	31	5 (16)		2 (6)	
Preoperative FEV$_1$ (%)					
<70%	8	3 (38)	0.061	2 (25)	0.051
≥70%	112	12 (11)		4 (4)	
Preoperative serum total protein level					
Normal (6.3–8.3 g/dL)	113	13 (12)	0.21	4 (4)	0.039
Abnormal	7	2 (29)		2 (29)	
Preoperative serum albumin level					
Normal (3.7–5.2 g/dL)	110	12 (11)	0.11	3 (3)	0.007
Abnormal	10	3 (30)		3 (30)	
Preoperative blood hemoglobin level					
Normal (11.3–14.9 g/dL)	85	8 (9)	0.13	2 (2)	0.059
Abnormal	35	7 (29)		4 (11)	
Preoperative platelet count					
Normal (125,000–375,000/µL)	115	13 (11)	0.12	4 (3)	0.02
Abnormal	5	2 (40)		2 (40)	
Diabetes mellitus					
No	106	12 (11)	0.38	6 (7)	1
Yes	14	3 (21)		0 (0)	
Cardiovascular disease					
No	98	10 (10)	0.15	4 (4)	0.30
Yes	22	5 (23)		2 (9)	
Pulmonary disease					
No	107	10 (9)	0.011	3 (3)	0.017
Yes	13	5 (38)		3 (23)	
Smoking habit					
No	79	11 (14)	0.58	6 (8)	0.094
Yes	41	4 (10)		0 (0)	

The remaining 19 variables not shown here did not demonstrate any significant association

FEV_1 forced expiratory volume in the first second of expiration

Intraoperative transfusion (hazard ratio, 71 [95% confidence interval, 3.0 to 1,000]; $p=0.008$), a colonic J-pouch (32 [1.8 to 500]; $p=0.018$), and pulmonary disease (32 [1.1 to 1,000]; $p=0.044$) were independently associated with adverse outcomes. The incidences of severe leakage for patients with 0, 1, 2, and 3 positive risk factors were estimated to be 0%, 6%, 67%, and 100%, respectively.

Discussion

In this study, the incidences of clinical anastomotic leakage and mortality after ISR were 13% and 0.8%, respectively. These are comparable to the respective incidences of 5% to 16% and 0 to 0.8% in recent ISR series.[7–11] Since these figures are even comparable to the 2.8% to 19.2% and 0%

to 2.5% observed with anterior resection,[14–26] appropriately administered ISR can be regarded as safe in terms of leakage and mortality. However, such figures should be interpreted cautiously because incidences of anastomotic leakage depend on the definition, patient selection, and treatment details. Patient factors like gender,[15,16,18,22,25] age,[25] American Society of Anesthesiology score,[25] heart disease,[26] malnutrition,[17] weight loss,[17] obesity,[15] smoking habit,[26] and alcohol abuse[17] have been reported to independently influence the incidences of leakage after anterior resection, and so have treatment factors such as neoadjuvant chemoradiotherapy,[18,22] bowel preparation,[19] timing of surgery,[25] surgeon caseload,[25] anastomotic level,[14,15,18,19,22] intraoperative contamination,[17,18] pelvic drainage,[21] defunctioning stoma,[16,20,21,24] operation time,[17] and blood transfusion.[17,19]

To our knowledge, there have only been few studies addressing risk factors for anastomotic leakage following ISR. Rullier et al.[15] investigated 272 anterior resections for rectal cancer, in which 131 anastomoses were situated 5 cm or less from the anal verge. Multivariate analysis of their overall population showed that male sex and the level of anastomosis were independent factors for leakage. In a second analysis of 131 very low anastomoses, obesity was an independent factor. The authors concluded that a protective stoma is suitable after anastomoses situated at or less than 5 cm from the anal verge, particularly for men and obese patients.

In the present study, all of the patients had undergone complete bowel preparation, elective surgery by high-volume colorectal specialists, and pelvic drainage, all of which have been reported to be independently beneficial for reducing leakage.[19,21,25] Most had a defunctioning stoma as well.[16,20,21,24] None had received neoadjuvant chemoradiotherapy which is reported to be an independent risk factor for leakage.[18,22] Therefore, these already known significant factors could not be evaluated in this study. Our multivariate analysis revealed intraoperative blood transfusion and pulmonary disease to be independently associated with overall clinical leakage and severe leakage, and a colonic J-pouch was associated with severe leakage. These results suggest that under the circumstances prevailing in our institution, we can stratify high-risk patients by using these factors and prepare countermeasures against them.

Although the exact mechanism whereby anastomotic leakage may be related to blood transfusion is unclear, it is known that allogeneic blood transfusion induces immunosuppression and predisposes to postoperative infection.[27] Allogeneic leukocytes have a critical role in the induction of transfusion-induced immunosuppression.[27] Tang et al.[27] reported that intra- or postoperative blood transfusion was an independent risk factor for overall surgical site infection, incisional infection, and organ/space infection with and without clinical anastomotic leakage in a prospective study

of 2,809 consecutive patients undergoing elective colorectal resection. Therefore, susceptibility to infection induced by transfusion may promote development of anastomotic leakage.

To avoid intraoperative transfusion, it is preferable to treat anemia before surgery using oral and parenteral iron therapy. Transfusion should be reserved for patients with cardiovascular instability and continued and excessive blood loss. Furthermore, it should be given before the operation because deleterious effects appear to be more likely with intra- or postoperative transfusion.[27] Operative blood loss should be minimized by cautious procedures. If excessive blood loss is expected, autologous blood transfusion should be considered, especially in the presence of other risk factors.

In line with previous reports on intestinal anastomotic leakage, we found an independent association with pulmonary disease. Jonsson et al.[28] measured oxygen tension and collagen deposition in subcutaneous wounds in 33 postoperative patients and found that this and the resultant tensile strength are limited by perfusion and tissue oxygen tension. Hopf et al.[29] measured subcutaneous wound oxygen tension in 130 surgical patients and observed that this factor is a strong predictor of infection. Millan et al.[23] determined intramucosal pH at colorectal anastomoses, which reflects blood supply and oxygenation of the mucosa, and found that it can accurately predict the risk of anastomotic leakage. Smoking is a major cause of chronic obstructive pulmonary disease and is known as an independent risk factor for anastomotic leakage after anterior resection.[26] Therefore, although the exact pathophysiology remains to be clarified, it is reasonable to speculate that pulmonary disease predisposes to anastomotic hypoxia which in turn hinders wound healing, aggravates infection, and promotes anastomotic dehiscence.

Because of their chronic and irreversible nature, the chronic obstructive pulmonary disease and restrictive respiratory diseases seen in our series are difficult to treat. However, intensive respiratory management including continuous pulse oximetry monitoring, supplemental oxygen, appropriate analgesia, bronchoscopy when needed, and early mobilization, similar to the management applied after esophageal cancer surgery,[30] may prevent the respiratory complications and hypoxemia which can lead to anastomotic leakage.

Although the incidence of leakage with a colonic J-pouch was reported to be significantly lower than with straight coloanal anastomosis[31] and transverse coloplasty[32] in anterior resection, we paradoxically found a J-pouch to be an independent risk factor for severe leakage in our ISR series. Of the five patients who underwent J-pouch construction and suffered severe leakage, four were male, four received an intraoperative transfusion, and two had

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pulmonary disease. Therefore, it appears that a colonic J-pouch reconstruction after ISR may confer extra risk on males with intraoperative transfusion and/or pulmonary disease. Since males have a longer anal canal than females, the presence of a bulky J-pouch and anastomosis may increase the sphincteric squeeze pressure and worsen anastomotic blood and oxygen supply, thereby predisposing to leakage. Thus, in the presence of other risk factors, countermeasures including a switch to other reconstruction methods may need to be considered.

There are limitations to the present study. First, the study design is retrospective, and this may cause biases. Especially, because all or nearly all patients had complete bowel preparation, elective surgery by high-volume colorectal specialists, pelvic drainage, and defunctioning stoma and did not have neoadjuvant chemoradiotherapy, the significance of these factors could not be evaluated in this study. Second, because the numbers of events were limited particularly for severe leakage, many other risk factors which were significant in the previous studies on leakage after anterior resection were not significant in this study. Thus, further confirmation with a larger number of patients would be preferable.

In conclusion, the present retrospective exploratory study suggests that intraoperative blood transfusion and pulmonary disease are independently significant risk factors for overall and severe anastomotic leakage after ISR, and a colonic J-pouch was associated with severe leakage. By taking account of these factors, we may be able to stratify high-risk patients and prepare countermeasures. However, because numbers of patients and events in this study were limited, further investigation and validation are warranted with larger datasets or in future prospective trials.

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