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ORIGINAL ARTICLE

Early Detection of Anastomotic Leakage After Elective Low Anterior Resection

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Abstract
Background Colorectal anastomotic leakage is a serious complication leading to major postoperative morbidity and mortality. In the present study, we investigated the early detection of anastomotic leakage before its clinical presentation.
Method Fifty-six patients with rectal cancer were included prospectively in this study. All patients underwent elective low anterior resection. Peritoneal samples were collected from the abdominal drains at the first, third, and fifth days postoperatively for bacteriological study (quantitative cultures for both aerobes and anaerobes) and cytokines (IL-6, IL-10, TNF) measurement. Patients were divided into two groups: those without symptomatic or clinical evidence of anastomotic leakage (AL; group 1) and those with clinical evidence of AL (group 2). Study variables included hospital stay, wound infection, operative time, blood loss, height of anastomosis, *intraperitoneal* cytokines, and microbiological study of peritoneal fluid.
Result Clinically evident AL occurred in eight patients (14.3%) and diagnosed postoperatively on median day 6. Intraperitoneal bacterial colonization and cytokine levels were significantly higher in patients with clinical evidence of AL. Wound infection was significantly higher in anastomotic leakage group. The hospital stay for the patients with anastomotic leakage was significantly longer than those without AL (14±1.41 vs. 5.43±0.89 days). A significant difference among two groups was observed regarding operative time, blood loss, blood transfusion, and height of the anastomosis.
Conclusion The peritoneal cytokines levels and intraperitoneal bacterial colonization might be an additional diagnostic tool that can support the decision making of surgeons for early detection of anastomotic leak in colorectal surgery.

Keywords Anastomotic leakage · Cytokines · Bacterial colonization

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Introduction

Anastomotic leakage after large bowel resections remains one of the most serious and important complications, despite recent advances in colorectal surgery. Recently, with an increasing number of sphincter-preserving procedures, there are more patients at risk for possible leakage. The leakage rate varies from 0.5% to 30%.[1–5]

Clinical anastomotic leakage is associated with multiple morbidities, poor functional outcome,[6] increased mortality rate ranged from 10% to 15% and increased overall and local recurrence in patients who underwent resection for rectal cancer.[7,8]

Clinically, anastomotic leakage diagnosis has been reported to be on median postoperative days 7 to 11;[9,10]

some studies reported an even longer interval, up to 45 days postoperatively.[11,12] Such long intervals are associated with increased morbidity and mortality therefore exclusion or confirmation of the diagnosis of anastomotic leakage (AL) have to take priority in patients with any suspicion of AL after colorectal surgery.[13]

Several studies investigated early prediction of anastomotic leakage after colorectal surgery, to allow the treatment to be instituted before the patient develops serious complications such as organ failure and death.[9,14]

After abdominal surgery, proinflammatory cytokines such as TNF-α and IL-6 A are released into the peritoneal cavity and generate an inflammatory reaction, which is inhibited by other cytokines such as IL-10.[14–16] The increase in peritoneal cytokines can predict anastomotic complications, thus uncomplicated postoperative course is associated with decreasing peritoneal cytokine levels, whereas increasing levels indicate an unfavorable postoperative course.[14,17]

The clinical approach of bacterial quantitation to treatment has been emphasized during the last decades.[18] There is a correlation between the wound microbial load and the likelihood of infection as evidenced by delayed wound healing. [19–21] There are not many current researches that correlate the quantitative microbial load of specific bacteria to the clinical significance of specific isolates in causing anastomotic leakage.

So, this study aimed to investigate intraperitoneal bacterial colonization and cytokine (IL-6, IL-10, TNF-α) level in the early postoperative period that might possibly serve as an indicator of early prediction of anastomotic leakage in patients undergoing elective low anterior resection.

Patients and Methods

Patients

Consecutive patients who were treated for rectal cancer with low anterior resection at the Colorectal Surgery Unit of Mansoura University Hospital, Mansoura, Egypt, during the period from March 2007 to December 2009 were eligible for the study.

Informed consent was obtained from all patients to be included in the study, after explanation of the nature of the disease and possible treatment. The study was approved by the local ethics committee.

Pre-operatively, all patients underwent mechanical bowel preparation and were given cefuroxim and metronidazole for antibiotic prophylaxis. Low molecular weight heparin was used for deep vein thrombosis prophylaxis.

Low anterior rectal resections were performed for patients with rectal cancer. Anastomoses were handsewn or EEA stapler of appropriate size. After removal of the stapler, both doughnuts were inspected for integrity. The integrity of the anastomosis can be tested by transanal air insufflation after the true pelvis was filled up with saline solution. A leakage that was revealed during this procedure was treated by oversewing (small leakage) and performance of a new anastomosis (leakage over 25% of the circumference). A temporary loop ileostomy was constructed in cases where the presence of risk factors were thought to increase the risk of anastomotic leakage: comorbidity factors or adverse effect during operation. At the end of the operation, intraoperative irrigation was done and an 18-French drain was placed in the pelvis or abdomen. Postoperative complications were recorded in all patients.

Definition of Anastomotic Leakage

AL was defined clinically as gas, pus, or fecal discharge from the drain, fecal discharge from the operative wound, pelvic abscess, peritonitis, and rectovaginal fistula. All symptomatic anastomotic leakages were confirmed by one or more of the following methods: radiological contrast study, CT scan, digital rectal palpation.[13,14,17]

Patients were divided into two groups: those without symptomatic or clinical evidence of AL (group 1) and those with clinical evidence of AL (group 2).

Sampling

Peritoneal samples were collected from the abdominal drains at the first, third, and fifth days postoperatively for peritoneal microbiological study and cytokines (IL-6, IL-10, TNF) level measurement. Samples were centrifuged at 3,000*g* for 10 min at 4°C and stored at 20°C, until cytokines analysis.

Assays for TNF-α, IL-6, and IL-10

Tumor necrosis factor TNF-α, IL-6, and IL-10 were measured by enzyme-linked immunosorbent assay (ELISA; Ray Bio^{R} Human IL6, IL10, and TNF ELISA Kit protocol). All kits were used according to the instruction of the manufacturers.

Microbiological Study

Sample Collection and Transport Specimens submitted to microbiology laboratory were obtained from the abdominal drain at first, third, and fifth days postoperatively by aspiration of the drained fluid by a syringe after evacuation of the air and capping of the needle. The samples were transported to the medical diagnostic and infection control unit as soon as possible without delay. The time between

collection of the material and inoculation of the specimens ranged from 30 min to 2 h at the most.[22]

Sample Processing Quantitative culture was done according to Collee et al.[23] A measure of 0.1 ml was obtained to make tenfold serial dilutions of bacterial suspension. A 0.1-ml measure from each dilution was pipetted and distributed widely with sterile glass spreader on sheep blood agar 5%, chocolate, and MacConkey *agar* plates for aerobes and facultative anaerobic organisms. An additional neomycin blood agar plate was inoculated for anaerobic cultivation. The plates were incubated aerobically at 37°C and examined at 24 and 48 h. While for anaerobic organism isolation, the plated media were incubated in GasPak Jars and examined at 48, 96 and 120 h.[22] The viable count was calculated from the plates with average colony count (30 to 300 *colonies*).

Microbial Identification Isolates were identified by Gram-stain and colonial morphology. Further identification of the isolates was done using API 20 E and API 20NE systems (bioMerieux, Marcy l'Etoile, France) for facultative anaerobic and aerobic organisms, respectively. With regard to organisms isolated anaerobically, identification was based on the cultural, microscopical, and antimicrobial sensitivity characteristics of the organism (sensitivity to metronidazole and resistance to penicillin and aminoglycosides).

Statistical Analysis

Statistical analysis of the data in this study was performed using SPSS software, version 10. For continuous variables, descriptive statistics were calculated and were reported as mean±standard deviation. Categorical variables were described using frequency distributions. The Student's t test for paired samples was used to detect differences in the means of numerical variables; Chi-square test was used for nominal variables, and Fisher's exact test was used in cases with low expected frequencies. P values<0.05 were considered to be significant.

Results

Of the 56 patients, with rectal cancer who underwent elective low anterior resection during the period from March 2007 to December 2009, clinically evident AL occurred in eight patients (14.3%; five men and three women). The mean age was 53.37±8.41 (range, 42–66) years; see Table 1.

Anastomotic leakage was diagnosed on median day 6 (range, 2–8) and all occurred before discharge from hospital. Of the eight patients who developed symptomatic leakage, one patient died of sepsis secondary to anastomotic leakage, one patient already had covering ileostomy passed conservatively, and the other six patients were urgently re-operated on and had loop ileostomy.

Wound infection was significantly higher in anastomotic group 7 (87.5%) versus seven (14.6%) in patients without anastomotic leakage. The hospital stay for the patients with anastomotic leakage was 14±1.41 days[12–16], which took significantly longer than those without AL at 5.43+ 0.89 days[4–8] (Table 1).

The operative details of the two groups are presented in Table 2. A significant difference among two groups was

Table 1 Demographic data of the patients operated on with anterior resection of the rectum

Variables	No anastomotic leakage	With anastomotic leakage	*P* value	95% confidence interval of the difference	
				Upper	Lower
Age	51.22±8.55 (35–67)	53.37±8.41 (42–66)	0.52	−8.6827	4.3910
Sex					
Male	35 (72.1%)	5 (62.5%)	0.55		
Female	13 (27.9%)	3 (37.5%)			
BMI	23.62±4.62 (19–35)	25.87±4.48 (21–33)	0.20	−5.7742	1.2742
Smoking	20 (41.7%)	3 (37.5%)	0.82		
Staging					
Stage A	9 (18.8%)	2 (25%)	0.59		
Stage B	23 (47.9%)	4 (50%)			
Stage C	16 (33.3%)	2 (25%)			
Level of tumor	11.06±2.85 (6–15)	11.62±2.19 (9–15)	0.59	−2.6893	1.5643
Pre-operative radiotherapy	12 (25%)	1 (12.5%)	0.44		
Hospital stay	5.43±0.89 (4–8)	14±1.41 (12–16)	0.0001	.3741	−9.3125
Wound infection	7 (14.6%)	7 (87.5%)	0.0001		

Table 2 Intraoperative data

Variables	No anastomotic leakage	With anastomotic leakage	*P* value	95% confidence interval of the difference	
				Upper	Lower
Operative time	149.89±20.35 (110–195)	175.25±34.37 (130–290)	0.0001	−66.3103	−24.3980
Blood loss	331.25±189.23 (100–750)	593.75±187.91 (250–800)	0.001	−407.2550	−117.7450
Blood transfusion	9 (18.8%)	4 (50%)	0.04		
Height of anastomosis	6.43±2.21 (2–10)	5±1.06 (4–7)	0.009	0.4024	2.4726
Stapled used	14 (29.2%)	3 (37.5%)	0.63		
Defunction stoma	16 (33.3%)	1 (12.5%)	0.24		

observed regarding operative time, blood loss, blood transfusion, and height of the anastomosis.

The causes of the anastomotic leak were anastomotic ischemia in two patients, staple failure in one patient, the lower level of anastomosis below 6 cm from anal verge in three patients, and AL also occurred in two patients with no obvious risk factors.

TNF-α, IL-6, and IL-10 Levels

The peritoneal cytokine response in both groups is shown in Table 3. Intraperitoneal IL-6 was increased postoperatively on first, third, and fifth day (P=0.0001; P=0.0001, P=0.0001, respectively) and IL-10 was increased on postoperative first, third, and fifth day (P=0.0001; P=0.04, P=0.0001, respectively). TNF-α was increased in AL group on third and fifth postoperative day (P=0.0001; P=0.0001, respectively) but not on the first day.

There was no a significant difference in the level of peritoneal cytokine response in patient with AL and already had a diverting ileostomy (one patient) from the rest of the patients with AL (seven patients).

Although peritoneal cytokine levels were significantly higher beginning on day 1, clinically evident anastomotic leakage occurred on day 6. All patients developed temperature above 38 C and absence of bowel movement. One patient died of sepsis secondary to AL; one patient already had covering ileostomy passed conservatively, and other six patients with proven AL were re-operated and defunctioning ileostomy was done.

Microbiological Result

Escherichia coli, *Klebsiella*, *Pseudomonas* species, and bacteriod micro-organism were significantly more in AL group in first, third, fifth days postoperatively as shown in Table 4.

E. coli was the most common micro-organism detected in patients with AL. In AL group starting from the first, third, and fifth postoperative day showed that CFU/

Table 3 Intraperitoneal cytokines level in both groups

Variables	No anastomotic leakage	With anastomotic leakage	*P* value	95% confidence interval of the difference	
				Lower	Upper
IL6					
First day	35482.50±9455.35 (22,100–50,700)	52482.50±14364.42 (32,100–75,900)	0.0001	−24828.9	−9171.06
Third day	28159.17±4839.05 (21,000–35,200)	115,450±34974.81 (35,200–145,400)	0.0001	−97532.9	−77048.9
Fifth day	22219.58±1825.13 (20,100–25,900)	148,125±50753.85 (23,600–175500.00)	0.0001	−139,957	−111,854
IL10					
First day	23,800±9687.80 (11,300–50,700)	41982.50±10974.88 (21,700–55,900)	0.0001	−25734.7	−10630.3
Third day	22209.17±6079.59 (11,200–35,200)	33,355±36141.13 (15,200–122,500)	0.04	−22013.7	−277.9923
Fifth day	19708.33±3919.36 (11,200–25,900)	77842.50±62181.47 (19,700–151,400)	0.0001	−75502.0	−40766.3
TNF					
First day	161.94±16.10 (142–200)	169.65±8.63 (154–182)	0.19	−19.4527	4.3060
Third day	141.31±7.39 (130+155)	511.25±54.61 (427–555)	0.0001	−385.8895	−353.9855
Fifth day	78.10±19.88 (47+121)	824.50±113.94 (617–945)	0.0001	−780.8636	−711.9281

Table 4 Intraperitoneal bacteriological study in both groups

Variables	No anastomotic leakage	With anastomotic leakage	*P* value
E. coli			
First day	2 (4.2%)	3 (37.5%)	0.002
Third day	5 (10.4%)	6 (75%)	0.0001
Fifth day	3 (6.3%)	8 (100%)	0.0001
Bacteroides			
First day	2 (4.2%)	3 (37.5%)	0.002
Third day	2 (4.2%)	5 (62.5%)	0.0001
Fifth day	2 (4.2%)	6 (75%)	0.0001
Pseudomonas			
First day	3 (6.3%)	3 (37.5%)	0.009
Third day	3 (6.3%)	3 (37.5%)	0.009
Fifth day	2 (4.2%)	4 (50%)	0.0001
Klebsiella			
First day	4 (8.3%)	2 (25%)	0.16
Third day	4 (8.3%)	3 (37.5%)	0.02
Fifth day	5 (10.4%)	4 (50%)	0.005

milliliters was $\geq 10^6$ for *E. coli*, *Pseudomonas* species, and bacteriod micro-organism.

In the patient who died of sepsis, in the fifth postoperative day, secondary to anastomotic leakage, we found that CFU/ml was $\geq 10^6$ for *E. coli*, *Klebsiella*, *Pseudomonas* species, and bacteriod micro-organism in all samples except the sample which taken after abdominal closure where CFU/ml was $\leq 10^5$ for *E. coli* with no detected other micro-organism

In patients without AL, 41 patients showed no growth all over the duration of the study, and seven patients showed CFU/ml$\leq 10_5$ for the *E. coli*, *Klebsiella*, *Pseudomonas* species, and bacteriod micro-organism.

Discussion

Anastomotic leak following colorectal surgery is a significant complication that can result in severe sepsis, is a requirement for further surgery and a stoma, and is associated with prolonged hospital stay, considerable cost, multiple morbidities, and poor functional results.[24–26] The mortality rate associated with symptomatic leaks is 6% to 22%. The highest mortality rate was reported by the West of Scotland and Highland Anastomosis Study Group.[27] Also, Anastomotic leak has been associated with a higher local recurrence rate after curative treatment of colorectal malignancies.[28,29]

The most important risk factor for leakage is height of anastomosis form the anal verge; the lower the anastomosis, particularly below 6 cm, the higher the risk.[11,30] Other risk factors that have been attributed to anastomotic leakage are patient-specific risk factors, such as chronic obstructive pulmonary disease, ischemic heart disease, and diabetes mellitus; ASA score≥3; systemic hypertension; tobacco and alcohol use; prolonged use of high-dose steroids; under-nutrition; obesity; and male sex[2,11,31], and operative risk factors as poor colonic preparation, presence of peritonitis, adverse effect during operation, intraoperative blood loss/transfusion, anastomotic ischemia or tension, presacral hematoma, or fluid collection with subsequent infection and pelvic drainage.[1,11,30–33] In addition, cancer itself has been reported as a risk factor of anastomotic leakage.[5,23] Nevertheless, anastomotic leakage also occurs in patients with no obvious risk factors.[9,34]

The role of a temporary diverting stoma has been a matter of controversy, whether it could reduce the incidence of anastomotic leakage or not. Some studies have demonstrated a reduction in leakage rates in patients with covering stoma;[5,35] however, others showed no clear benefit of using a diverting stoma.[3,10] Moreover, a protective stoma is also associated with increased hospital stay and cost. Also, stoma reversal can cause morbidity and even mortality.[1,3,10] The role of mechanical bowel preparation and prophylactic antibiotic therapy in preventing AL is unclear, despite some studies that describe a low incidence of AL.[14]

The early detection of these complications within the first postoperative day by clinical examination is difficult, and the exact laboratory biomarkers of early prediction of these complications are unknown. Dulk et al.[13] and Peel AL[36] described the clinical signs of AL which included fever, increased leukocyte count, and increased C-reactive protein (CRP) level Furthermore; Systemic Inflammatory Response Syndrome (SIRS) was indicated to be a sign of AL. The following signs could also occur with SIRS: changed mental status, oliguria, increased levels of serum

creatinine, and ileus. But, neither clinical signs and symptoms nor systemic analysis of parameters such as CRP and leukocytosis are specific for anastomotic leakage diagnosis.[37,38]

Patients with an anastomotic intramucosal pH<7.28 in the first 24 h postoperatively have 22 times more risk of anastomotic leak. Therefore, by measuring intramucosal pH using Tonometry in the early postoperative period, the risk of anastomotic leak can be more accurately predicted.[30] Also, oxygen-tension measurements can be an additional diagnostic tool that can support the early prediction of AL in colorectal surgery. So, adequate tissue oxygenation pre- and postoperatively (continuously for 7 days) showed clear benefit towards prevention of anastomotic leakage. Reduced blood flow induces a switch from aerobic to anaerobic metabolism; the level of lactate will rise and pyruvate will decrease, resulting in an increased lactate/pyruvate ratio and decreased glucose levels which may be early signs of symptomatic anastomotic leakage before clinical symptoms are evident.[9]

A number of recent studies have investigated the cytokine response within the peritoneal cavity after abdominal surgery.[14,15] Polymorphonuclear leukocytes, macrophages, and peritoneal mesothelial cells are all probably production sites for this local cytokine as a part of the peritoneal response to surgical and infectious injury.[39,40]

Some studies have shown increased levels of the peritoneal cytokines including TNF-α, IL-1, IL-6, and IL-10 in patients with postoperative complications, and its concentrations reflect the severity of stress caused by abdominal operations[14], whereas others have failed to demonstrate this.[9] More specifically, peritoneal IL-6, IL-10, and TNF-α levels were significantly higher in patients with AL compared with patients without AL.[14,15]

Burak Ugrasx et al. [14] investigated the early prediction of peritoneal IL-6, IL-10, and TNF-α levels in developing AL after colorectal surgery and found that peritoneal cytokine response was suitable for the early identification of AL. Peritoneal cytokine levels in the patients with AL were significantly higher than in the patients without AL. Postoperatively, peritoneal cytokine levels in patients without AL were decreased; however, peritoneal cytokine levels in patients with AL were increased.

In our study, peritoneal IL-6, IL-10, and TNF-α levels were significantly higher in patients with AL than in patients without AL. On the first postoperative day, cytokine level indicated severe local inflammation, which occurred days before the clinical signs of AL.

It has been shown that during the first postoperative day, the peritoneal concentrations of cytokines reflect the severity of stress caused by abdominal operations. It is suggested that decreasing peritoneal cytokine levels occurred in a normal postoperative course, whereas increasing levels indicate an abnormal postoperative course. The overexpression of peritoneal cytokines, as a local inflammatory response, in response to microbial invasion might be a very early event in the development of AL.[14,17,41–43]

Ruiter et al. [44] reported that the composition of the microbial flora present in the abdominal fluid of patients critically ill with abdominal sepsis varies depending on location of the perforation. In lower gastrointestinal perforation, the most frequently isolated aerobic organisms were *E. coli*, *Klebsiella*, and *Pseudomonas* species. The predominant anaerobes were *Bacteroides*.[22,44]

The first stage of microbial infection is colonization which is defined as the presence of a micro-organism in an internal organ that is normally sterile; failure to clear colonizing micro-organisms invariably leads to high concentrations of potentially pathogenic micro-organisms (PPMs).[45]

Infection is a microbiologically proven clinical diagnosis of inflammation, local and/or generalized. This includes not only clinical signs, but also the presence micro-organisms of $\geq 10^5$ colony forming units (CFU)/milliliters in diagnostic samples obtained from an internal organ or the isolation of a micro-organism from peritoneal fluid.[35,44–46]

Secretions from internal organs of healthy individuals are normally sterile. The main mechanism by which micro-organisms cause endogenous colonization/infection is migration which is the movement of live PPMs from one place, e.g., gut where they are present in overgrowth, to other sites, in particular, normally sterile internal organs.[47–49]

The vast majority of postoperative infectious complications after colorectal surgery are caused by colonic flora. The predominant bacteria are the anaerobic *Bacteroides* accompanied by a smaller amount of aerobic coliforms.[22,50]

The organisms that predominate in peritonitis are the endotoxin-generating facultative anaerobes such as *E. coli* and the obligate anaerobes such as *Bacteroides fragilis* which are involved in the later phases of the infection while *E. coli* is responsible for the acute peritonitis phase of infection.[35,44–46]

The bacterial flora in the human colon is normally a stable ecologic environment. After perforation or spillage of the colon, more than 400 different species of bacteria will contaminate the peritoneal cavity.[44,51] But, of the vast number of species of bacteria that invade the peritoneum, only few will survive outside their native intraluminal environment. If an infection results, it will be polymicrobial in nature.[22]

It has long been thought that cleaning the bowel preoperatively reduced the bacterial load. However, while reducing fecal mass, pre-operative bowel preparation does not alter the concentration of fecal organisms intraluminally.[52] Previous studies have shown that a vigorous 72-h mechanical

cleansing regimen only produced a significant reduction in coliforms, while the residual colonic microflora remained unchanged.[52–54] In unprepared colon, besides the possibility of wound infections, the bacteria can also lead to local infection of the anastomosis, causing leakage.[55,56]

In our study, we found higher frequency of *E. coli*, *Klebsiella*, *Pseudomonas* species, and *Bacteroides* in patients with anastomotic leakage after low anterior resection. The more types of bacteria isolated from the patients, the higher the postoperative morbidity.

We suggest that the estimation of the peritoneal cytokine levels might be an additional diagnostic tool that can support the decision making of surgeons for early detection of anastomotic leak in colorectal surgery. Our data will need confirmation to define precise cut-off values for the identification the patients with ongoing anastomotic leak after low anterior resection.

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