EXPERIMENTAL STUDY

# The fate of suboptimal anastomosis after colon resection: An experimental study

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## ABSTRACT

**BACKGROUND:** The fate of suboptimal anastomosis is unknown and early detection of anastomotic leakage after colon resection is crucial for the proper management of patients.

**METHODS:** Twenty-six rats were assigned to "Control", "Leakage" and "Suboptimal anastomosis" groups where they underwent either sham laparotomy, cecal ligation, and puncture or anastomosis with four sutures following colon resection, respectively. At the fifth hour and on the third and ninth days; peripheral blood and peritoneal washing samples through relaparotomy were obtained. The abdomen was inspected macroscopically for anastomotic healing. Polymerase chain reaction (PCR) with 16s rRNA and *E.coli-specific* primers were run on all samples along with aerobic and anaerobic cultures.

**RESULTS:** The sensitivity and specificity of PCR on different bodily fluids with 16s rRNA and *E.coli-specific* primers were 100% and 78%, respectively. All samples of peritoneal washing fluids on the third and ninth days showed presence of bacteria in both PCR and culture. The inspection of the abdomen revealed signs of anastomotic leakage in eight rats (80%), whereas mortality related with anastomosis was detected in two (20%).

**CONCLUSION:** Anastomotic leakage with suboptimal anastomosis after colon resection is high and the early detection is possible by running PCR on peritoneal samples as early as 72 hours.

**Key words:** Anastomotic leakage; colorectal anastomosis; early detection; polymerase chain reaction; suboptimal anastomosis.

## INTRODUCTION

Anastomotic leakage is one of the most feared complications of colorectal surgery. Although reported rates of anastomotic leakage vary between 1% and 23%, 3% to 6% rate is considered acceptable for modern surgery.[1] Besides many negative impacts on patient's morbidity, mortality and life quality, it is also associated with higher tumor recurrence rate and poor survival.[2,3]

Wide variations in reported incidences of colorectal anastomotic leakage are partly due to lack of consensus on the definition. There have been several reports using highly variable definitions of anastomotic leakage in colorectal surgery.[4] A recent consensus definition of anastomotic leakage as "a communication between intra-and extraluminal compartments owing to a defect of the integrity of the intestinal wall at the anastomosis between colon and rectum or the colon and anus" has been proposed by International Study Group of Rectal Cancer.[4] Although more general, UK Surgical Infection Study Group has also defined anastomotic leakage as the "leak of luminal contents from a surgical join between two hollow viscera".[5] While many studies used clinical signs

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of peritonitis such as fever, tachycardia, leukocytosis, and etc.; some used radiological findings detected by computed tomography (CT) or other modalities. Lack of consensus in detection methods could partly explain the reason of great variations in the incidence and outcome of the leakage.

Although clinical anastomotic leakage has received great interest in surgery, the fate of suboptimal anastomosis is largely unknown. Since the presenting clinical symptoms are often vague and confused with benign postoperative complications, the true rate of anastomotic leakage is difficult to estimate. It is important to distinguish leakage from such benign conditions to act as early as possible in order to avoid severe consequences. The luminal contents leaked into peritoneum are principally cleared by local defense mechanisms. However, bacteria in the peritoneum can easily find access to the bloodstream in a very short time.[6] The early detection of microorganism in the peritoneum and blood could alter the management of the patient. Since conventional methods of bacterial detection such as culture is sometimes insufficient, molecular techniques like polymerase chain reaction (PCR) could be of assistance. PCR has been shown to detect small amount of bacteria in different bodily fluids with great success.[7] However, the role of PCR in the early detection of anastomosis is not clearly depicted.

This experimental study was designed to understand the fate of suboptimal anastomosis and explore the possibility of early diagnosis of leakage in colon anastomosis by means of detecting bacterial DNA in different bodily fluids by polymerase chain reaction. It was also aimed to evaluate the efficacy of PCR in anastomotic leaks.

## MATERIALS AND METHODS

### Animals

The local Animal Ethics Committee of Istanbul University, Faculty of Medicine approved the experiment (Protocol number: 28/2005, Protocol Date: 20.09.2005). Twenty-six male Wistar rats weighing 200-250 grams were used. National Research Council guidelines were followed for the use and care of the animals. Briefly, the animals were put two per cage, fed on standard chow, and let free access to water. They were observed for two weeks before the experiment started. They were allowed to eat and drink before and after the operation. The animals were followed for two months after the operation.

### Experimental Design

The animals were randomly assigned to three groups: Group I was the "control group" consisted of eight animals which underwent sham laparotomy. Five hours after the operation, peripheral blood sample was obtained to evaluate any bacterial contamination by PCR and culture. Group II was the "leakage group" and consisted of eight animals. After laparotomy, the cecal ligation and puncture were performed and the abdomen was closed. Five hours later, peripheral blood was withdrawn and both PCR and culture were run on blood samples to detect bacterial presence. The rats were then sacrificed. Group III was the "suboptimal anastomosis group" and consisted of ten animals. After laparotomy, resection of the left colon and anastomosis were performed. Five hours, three and nine days after the operation, peripheral blood samples were taken to run PCR and culture. On the third and ninth days, the rats underwent relaparatomy. Peritoneal fluid collections were obtained for both PCR and culture. The abdomen was inspected and anastomosis was evaluated macroscopically for healing. Experimental design was summarized in Figure 1.

### Procedures and the Operation

All interventional procedures and operations were performed under strict sterility and dissociative anesthesia. Intramuscular 90 mg/kg ketamine HCl (Ketalar, Parke-Davis, Eczacibasi, Turkey) was used for anesthesia. Peripheral blood to study the bacteremia with PCR and culture was withdrawn from the femoral region. In order to prevent contamination from skin bacteria, the femoral region was cleaned with polyvinyl pirolidon iodine (Batticon, Adeka, Turkey). Groin region was covered with sterile drapes and an incision of 2 cm was made. Under sterile conditions, femoral artery was found and 1 to 2 ml of blood was withdrawn into 2 Na2EDTA containing sterile tubes. One blood sample was used for DNA isolation and placed on ice immediately and transferred to -20°C freezers until analysis. The blood in the second tube was inoculated into cultural medium. For each time point, opposite site of the femoral region from the previous attempt was used to minimize the contamination risk. The femoral region was sutured after the procedure under sterile condition.

Laparotomies were performed via 3-cm midline incisions after cleaning the surgical area with povidone iodine and covering with sterile drapes. The area was covered to prevent spillage. A segment of left colon was isolated and resected without compromising the vasculature network. Anastomosis of the resected colon was performed with four sutures using 4/0 vicryl suture. The use of less than five sutures in anastomosis has been defined as a model for suboptimal anastomosis.[8] Then, the abdominal wall and skin were closed with 3/0 silk sutures.

Seventy-two hours later, the animals were prepared for peripheral blood withdrawal under anesthesia with strict adherence to asepsis, as described. After the closure of the femoral incision, the abdomen was incised and subcutaneous tissue was inspected for abscesses. In case of abscess, discharge sample was obtained for both PCR and culture. Abdominal wall was opened through previous sutures. Without touching the abdominal organs, abdominal cavity was washed with 2 ml of sterile saline and the fluid was collected with a syringe. Afterwards, inspection of the abdomen and anastomosis was

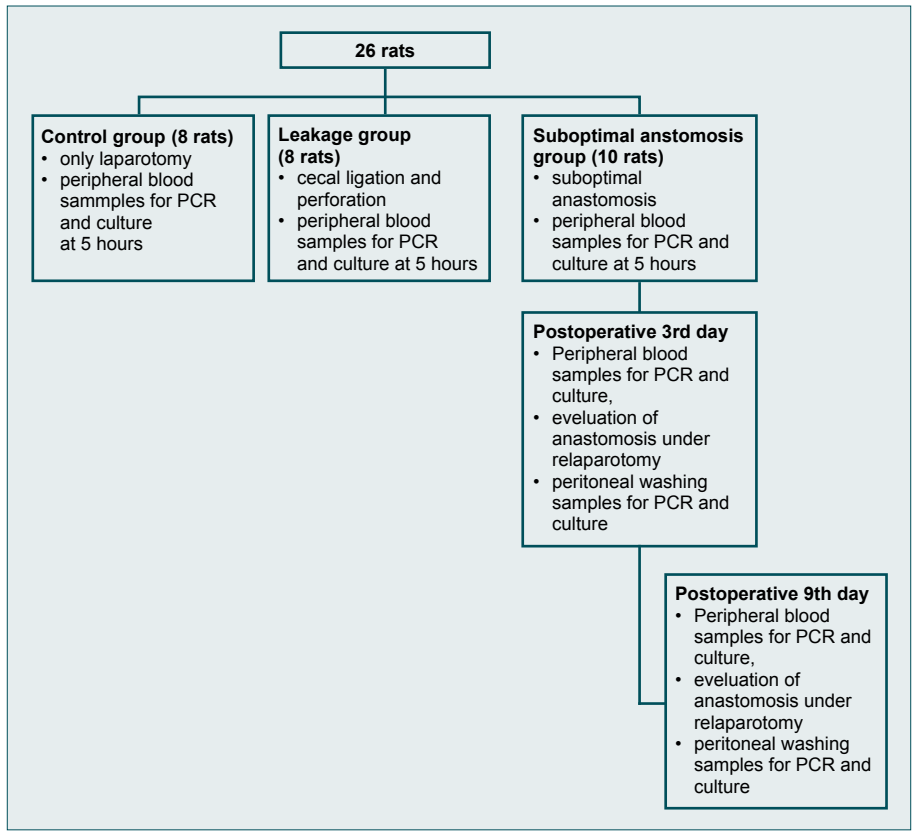


**Figure 1.** An overview of the experimental design.

performed. Any collections in the abdominal cavity were noted and anastomosis was evaluated. The leak was considered in the case of gross contamination with luminal content, the presence of healing defect in anastomosis, the presence of small abscess around anastomosis, and the omental attachment covering the healing defect. The abdomen was closed in an orderly fashion. After nine days, the same procedures with peripheral blood withdrawal and anastomosis evaluation were repeated. The animals were followed for a two-month-period for observation. Any animal lost during study period underwent autopsy and the abdominal cavity and anastomosis were evaluated.

## The Detection of Bacterial DNA in the Blood and Peritoneal Fluid

### DNA Isolation

All samples collected from each animal were stored at -20°C. For the extraction of DNA, 200-400 uL blood, whole blood or peritoneal wash fluid were used. DNA was extracted from whole blood or peritoneal fluid using the DNA extraction kit (Roche Diagnostics GmbH, Mannheim, Germany,) according to the manufacturer's protocol. DNA samples were stored at -20°C.

### Polymerase Chain Reaction

Two primer pairs were used for the detection of bacteria. First set of primers (540 bp) were used to amplify prokaryotic 16S rRNA for the detection of any bacterial contamination regardless of origin: 355F (5'-CCTACGGGAGGCAGCAG-3'), and 910R (5'-CCCGTCAATTCCTTTGAGTT -3').[9] The second set of primers (486 bp) were used to amplify β- glucuronidase of *Escherichia coli* to detect *E. coli* specifically: P1 (5'-ATCACCGTGGTGACGCATGTCGC-3') and P2 (5'-CACCACGATGCCATGTTCATCTGC-3').[10] 40 ul of reaction mixture was formed from 1x PCR buffer, 3.5 mmol/L $MgCl_2$, 2 U Taq DNA polymerase, 800 µmol/L dNTP mix, 1 µmol/L primer 1, and 1 µmol/L primer 2, and 10 ul extracted DNA was used for the 50 ul of total volume of PCR mixture. The amplification reaction consisted of one cycle of 5 min at 94°C and 35 cycles of 45 s of denaturation at 94°C, 45 s of annealing at 55°C, and 60 s of extension at 72°C, with a final extension cycle of 10 min at 72°C. At the end of the program, 10 ul of the amplification product was detected by electrophoresis on 1.5% agarose gel with ethidium bromide staining, and the products were then photographed under UV light (304 nm). The amplified DNA products (16s rRNA, 540 bp; and *E. coli*-specific, 486 bp) were compared with control DNA and molecular weight standards.

### Cultures

One ml of blood and peritoneal samples collected from rats were inoculated into BACTEC aerobic and anaerobic medium bottles (Becton Dickinson, Sparks, Md.) in order to detect

bacterial growth. After incubation for seven days at 37°C, the bottles were punctured under sterile conditions, and 100 µl was subcultured onto sheep blood (5%) agar and MacConcey agar medium. A subculture was incubated in anaerobic conditions at the same time. The subcultures were incubated for 72 hours at 37°C. If bacterial growth was detected, the bacteria were identified according to standard microbiological methods.

### Statistical Analysis

The sensitivity, specificity, negative and positive predictive value of PCR compared to culture was calculated. Presence of bacterial DNA in either *E. coli* or 16s rRNA, PCR was accepted as positive. Being negative in PCR meant both PCR samples (*E. coli* and 16s rRNA) didn't detect any bacterial DNA.

## RESULTS

### General

Two mortalities were observed in the suboptimal anastomosis group, while no mortality was detected in the control group. One of the rats died at the forty-eighth hour. Blood samples for both PCR and culture were positive at the fifth hour of anastomosis and *E. coli, Klebsiella sp.* and *Bacteroides fragilis* were identified from culture. Exploration of the abdomen revealed complete dehiscence of anastomosis with gross fecal contamination. The PCR and cultures run on blood sample at the forty-eighth hour and peritoneal washing cultures were positive for *E. coli* and *B. fragilis*. The second rat died on the sixteenth day of anastomosis. PCR and cultures from blood and peritoneal samples until postoperative ninth day were all negative for any bacterial presence. However, on ninth day, blood and peritoneal samples showed positivity for PCR. Culture identified *E. coli*, *K. pneumoniae* and *B. fragilis*. Nevertheless, inspection of the abdomen on the ninth day didn't show any gross contamination of fecal material and anastomosis healing was normal. On postmortem examination sixteenth day, complete dehiscence of anastomosis with gross contamination of abdomen was noted.

The inspection of anastomosis on postoperative third day revealed that 80% of anastomosis showed some signs of anastomotic leakage either at one time point or both (Fig. 3). Leaked anastomosis were covered by omentum and associated with microabscesses showing some signs of inflammation. On ninth day, more anastomosis appeared healed macroscopically (66%).

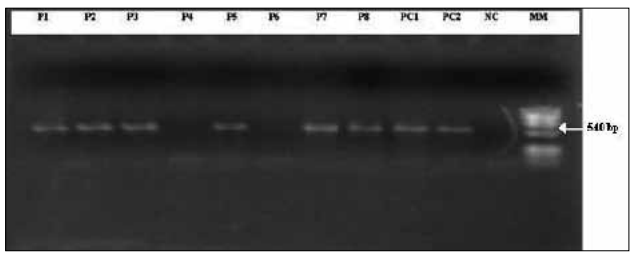


**Figure 2.** Gel electrophoresis of PCR products run on blood samples obtained at the fifth hour from the rats. (PC: Positive control, NC: Negative control; MM: Molecular marker; P1-P8: blood samples of the rats from leakage group. 16 s r RNA primers were used).

### The Sensitivity and Specificity of PCR

Sixty eight samples (blood, peritoneal washings, wound and abcesses) from 26 rats were cultured. Two PCRs, one with E. coli primers and other with 16s rRNA primers, were run on each sample ending up with a total number of 136 PCRs. The sensitivity and specificity of PCR with both *E. coli* primers and 16s rRNA primers on samples from different body fluids were found 100% and 78%, respectively (Table 1). The accuracy of PCR samples from blood, peritoneal washings, and wound abscesses changed from 84% to 100% depending on the primers and the sample type. The sensitivity of PCR was 100%, regardless of the primers and sample type, while the specificity changed between 57 and 100% depending on primers and samples. The negative predictive value of PCR was found 100%. However, the positive predictive value was between 65 to 100%. The details of sensitivity, specificity and accuracy with the use of different primers on different bodily fluids were shown in Table 2. The accuracy of PCR using *E. coli* primers on any sample (97%) was greater than the one using 16s rRNA primers (88%).

### Early Detection of Bacterial DNA in the Blood of Rats With Gross Abdominal Leak

Animals in the leakage group served as control group for the gross bacterial contamination. After five hours of cecal ligation and puncture, the animals were sick-appearing with tachypnea and lethargy. The culture performed on blood

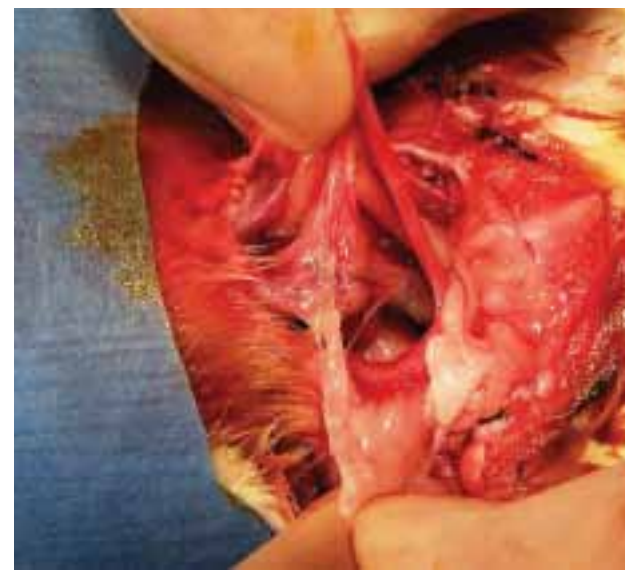

**Figure 3.** Macroscopic examination of anastomosis. Please note suture and adhesions, edema and inflammation around the anastomosis.

**Table 1.** Comparison of PCR with *E. coli* primers and 16s rRNA primers run on different body fluids with corresponding cultures

| | | Culture | | |
|---|---|---|---|---|
| | | **Positive** | **Negative** | |
| PCR | Positive | 35 (TP) | 7 (FP) | 83% PPV TP / (TP + FP) |
| | Negative | 0 (FN) | 26 (TN) | 100% NPV TN / (TN + FN) |
| | | 35 | 33 | 68 |
| | | 100% Sensitivity TP / (TP + FN) | 78% Specificity TN/(FP+TN) | |

TP: True positive; FP: False positive; PPV: Positive predictive value; FN: False negative; TN: True negative; NPV: Negative predictive value.

samples at postoperative fifth hour from 75% of rats revealed bacterial growth (n=6/8). PCR using with *E. coli* primers revealed no positivity. However, same PCR reaction with 16s rRNA primers revealed a complete overlap with culture positivity (Fig. 2). The isolated bacteria from culture were monobacterial in five rats (*B. fragilis* in four rats, *Enterococcus sp.* in one rat), and multibacterial in one. *B. fragilis* was the most frequently isolated bacteria in the leakage group (83%). In this group of animals, the accuracy, sensitivity and specificity of PCR were 100%. In the control group with only laparotomy, both PCR with *E. coli* and 16s rRNA primers and culture were negative for bacterial contamination or growth.

## The Detection of Bacteria in the Blood of Rats With Suboptimal Anastomosis

Five hours after anastomosis, PCR on the blood withdrawn from femoral vein showed two positive results. One had not been confirmed with culture. However, the other one was confirmed with culture since *E. coli*, *B. fragilis*, and *K. pneumoniae* were identified. The rat had gross anastomosis leak on second day of exploration and died. The positivity of bacterial detection at the fifth hour was 20%.

On the third day of anastomosis, three rats showed positivity of bacterial DNA in the PCR. However, the cultures of two rats didn't confirm positivity and correlate with gross anastomosis leak ending with excitus. The only positive result correlating with the culture was the blood sample obtained earlier from the heart of the rat which died at the forty-eighth hour due to anastomotic leak. Therefore, the positivity of bacterial DNA at 72 hours in blood was 30%.

On postoperative ninth day, PCR with *E. coli* primers was positive on 33% of rats, whereas PCR with 16s rRNA primers showed 88% positivity. Culture showed 60% positivity on blood. However, the identified bacteria from two cultures showed methicilline sensitive *S. aureus*, which could possibly result from contamination of skin flora. Therefore, if culture positivity with intestinal flora is taken into account, the positivity decreases to 40%. Table 3 summarizes the results.

**Table 2.** The sensitivity, specificity, NPV (negative predictive value), and PPV (positive predictive value) of PCR with different primers on different body fluids compared with corresponding cultures

| | **E. coli primers** | | | **16s rRNA primers** | | |
|---|---|---|---|---|---|---|
| | **All samples (%)** | **Blood (%)** | **Peritoneal fluid (%)** | **All samples (%)** | **Blood (%)** | **Peritoneal fluid (%)** |
| Sensitivity | 100 | 100 | 100 | 100 | 100 | 100 |
| Specificity | 93 | 100 | 57 | 75 | 78 | 0 |
| PPV | 87 | 100 | 80 | 81 | 65 | 95 |
| NPV | 100 | 100 | 100 | 100 | 100 | NC |

**Table 3.** PCR and culture results from blood samples of suboptimal anastomosis group

| Rat | 5th h | 3rd d | 9th d | Culture 3rd d / 9th d | Anastomotic leak | Mortality |
|---|---|---|---|---|---|---|
| 1 | N | N | P16sRNA | Sterile / Sterile | Yes | No |
| 2 | N | N | P16sRNA | Sterile / MSSA | No | No |
| 3 | N | P16sRNA | P16sRNA | Sterile / MSSA | Yes | No |
| 4 | N | N | P16sRNA | Sterile / Sterile | Yes | No |
| 5 | N | N | PEc+16sRNA | Sterile / *E. coli* | Yes | No |
| 6 | P16sRNA | P16sRNA | P16sRNA | Sterile / Sterile | Yes | No |
| 7 | N | N | PEc+16sRNA | Sterile / *E. coli, B. fragilis* | Yes | No |
| 8 | P16sRNA | N | N | Sterile / Sterile | Yes | No |
| 9 | P16sRNA | PEc+16sRNA* | NA | *K. pneumonia*# *E.coli**, *B. fragilis**/ NA | Yes | Yes |
| 10 | N | N | PEc+16sRNA | Sterile / *E. coli* | No | Yes |
| % | 30% | 30% | 88% | 40%* | 80% | 20% |

N: Negative; PEc: Positive PCR with *E.coli* primers; P16sRNA: Positive PCR with 16s rRNA primers; PEc+16sRNA: Positive PCR with *E.coli* and 16 s r RNA primers. *Denotes the blood sample obtained at 48 hours of anastomosis. #Shows the culture result obtained at 5 hours after anastomosis.

**Table 4.** PCR and culture results from blood samples of suboptimal anastomosis group

| Rat | 3rd day | 9th day | Culture 3rd d / 9th d | Anastomotic leak | Mortality |
|---|---|---|---|---|---|
| 1 | PEc+16sRNA | P16sRNA | *E. coli* / MSSA | Yes | No |
| 2 | PEc+16sRNA | P16sRNA | *E. coli* / *K. pneumonia* | No | No |
| 3 | PEc+16sRNA | PEc+16sRNA | *E. coli, Fusobacterium* / *E. coli* | Yes | No |
| 4 | PEc+16sRNA | P16sRNA | *E. coli* / *K. pneumonia* | Yes | No |
| 5 | PEc+16sRNA | PEc+16sRNA | *E. coli* / *E. coli* | Yes | No |
| 6 | P16sRNA | PEc+16sRNA | *B. fragilis* / *E. coli* | Yes | No |
| 7 | PEc+16sRNA | PEc+16sRNA | Sterile / *Proteus spp.* | Yes | No |
| 8 | PEc+16sRNA | PEc+16sRNA | *K. pneumonia, MSSA* / *E. coli, B. fragilis* | Yes | No |
| 9 | PEc+16sRNA* | NA# | *E. coli**/ NA | Yes | Yes |
| 10 | PEc+16sRNA | PEc+16sRNA | *E. coli* / *E. coli* | No | Yes |
|  | 100% | 100% | 100% | 80% | 20% |

PEc: Positive PCR with E.coli primers; P16sRNA: Positive PCR with 16s rRNA primers; PEc+16sRNA: Positive PCR with E.coli and 16 s r RNA primers. *Denotes the sample obtained at 48 hours of anastomosis. #The rat died at 48 hours due to anastomotic leak.

## The Detection of Bacteria in the Peritoneal Washing of Rats with Suboptimal Anastomosis

On postoperative third and ninth days, 100% PCR positivity for bacterial DNA was detected. However, on the third day, 90% of the culture was positive. On postoperative ninth day, culture positivitiy was 100%. The bacteria isolated from peritoneal washings were all from intestinal flora. In three cultures, MSSA was isolated additionally to gram negative or anaerobic bacteria, which could more likely come from contamination of the surgical procedures. *E. coli* was present in 90% of the culture. The details were shown on Table 4.

## DISCUSSION

Early diagnosis of anastomotic leakage and subsequent treatment are essential for the prognosis and prevention of devastating consequences. It has been shown that reoperation of the patients with anastomotic leakage before postoperative day five of index surgery significantly reduces the mortality compared to patients operated after day five.[11] However, presenting symptoms and timing of the leak vary greatly. Majority of the reports consider gross indicators of clinical situations like peritonitis. However, many patients present with vague or weak symptoms of neurological and respiratory ori-

gin, which could easily be confused.[12] In many studies, anastomotic leakage has appeared between postoperative seventh and twelfth days with gross signs of peritonitis and systemic sepsis. Traditional signs and symptoms of an anastomotic leak such as elevated white blood cell level (WBC), fever, and peritonitis usually develop as late as postoperative 5-7 days. The return of bowel function after colorectal resection and anastomosis does not preclude the possibility of a leak.[12] Besides clinical indicators, many biochemical and radiological tests have been studied with the expectation of timely diagnosis.[13,14] A recent study using CRP as an indicator of anastomotic leakage after colorectal resection showed that higher levels might help to detect the leage before becoming clinically apparent.[15] The detection of cytokines through intraperitoneal microdialysis has also been implicated as a tool for detection of anastomotic leakage prior to the emerging of clinical symptoms.[16] However, none of the methods have been proven effective and accepted universally.

The fate of bacteria in peritoneal cavity has been studied substantially during last decades. It has been shown that intraperitoneal elimination starts immediately and continues for approximately six hours.[17] The bacteria gain access to circulating blood through the pores located in the abdominal part of the right diaphragm to the thoracic duct and eventually to peripheral blood circulation. The process is very rapid, since the bacteria could be observed in the thoracic duct as early as 6 to 10 min after intraperitoneal injection and in the blood after 30 to 40 min.[18] The blockade of absorption from peritoneal cavity by destruction of diaphragmatic pores decreased the positive blood culture and increased the survival time in rats with double colonic perforation.[19] However, the detection of small amount of bacteria in blood by conventional methods like blood culture could be ineffective especially in postoperative period while the patient is under antibiotic regime. Recently, PCR based molecular methods have gained acceptance in many aspects of clinical application. The detection of the amount of bacteria in various bodily fluids, like blood, using PCR with specific primers designed from bacterial DNA could be accomplished, even when the patient is under antibiotic treatment.[7,20]

The fate of suboptimal anastomosis in humans is largely unknown. There are few reports on minor anastomotic leaks and their comparisons with major ones. Recently, an experimental model of suboptimal anastomosis has been introduced. The authors claim that an animal model of colorectal anastomotic leakage can be created with five interrupted sutures resulting with 44% of anastomotic leak.[8] In this study, four interrupted sutures were used to establish anastomosis and 80% of the anastomotic leak was detected with inspection. The bacteria were detected by both PCR and culture in the peritoneal washings (100% and 90%, respectively). Even as late as the ninth day after anastomosis, PCR and culture showed 100% positivity with bacteria. The identification of bacteria from culture was clearly from intestinal origin. Based upon our results, it can be suggested that most anastomosis after suboptimal suturing has leaks continuing even after the clinical healing has occurred. Although anastomotic leak continues microbiologically, the healing process becomes completed except in 20% of anastomosis, which ended up with mortality. This figure also comprised late anastomotic leaks, since the rats were followed for two months after anastomosis. It can be concluded that in suboptimal anastomosis, although anastomotic leak is present microscopically, the leak ending up with mortality only comprises 20%. Our result and clinical experience show that not all anastomotic leakages end up with devastating complications. A variety of different clinical presentations ranging from asymptomatic cases to severe peritonitis ending up with mortality could be seen. While some leaks are contained and healed without intervention, some requires surgery. The question of how a leak will progress could be influenced by many facors related with the host (immune response, genetic variations like single nucleotide polymorphism in critical genes controlling the inflammation), amount of leakage, origin of the leak like from the small or large intestine, virulence of bacteria, and etc. Therefore, the experimental model of suboptimal anastomosis can be used to titrate and investigate the variables controling the outcome of anastomotic leakages.

The use of PCR in different bodily fluids has been studied with great success.[21-24] The PCR method has been found to be more sensitive than blood cultures for detecting bacterial presence in the blood of critically ill surgical patients.[7] The detection of bacterial DNA in the blood of patients with liver cirrhosis, acute pancreatitis, and major abdominal surgery has also been reported.[25-27] The use of PCR in an experimental model of anastomotic leakage has already been shown in a study where the authors claim that the detection of microbial DNA in blood might be used in patients with dubious findings suggesting anastomotic leakage.[28] In this study, it was shown that the sensitivity of PCR was 100%, while the specificity changed between 57% and 100%. Hence, the negative predictive value of PCR was perfect as shown earlier. In other words, by negative PCR any infectious source can be ruled out. Low positive predictive level was expected since the detected DNA could come from dead organisms, which had already been phagocytosed and engulfed. The difference between the detection rates of anastomotic leakages (100% with PCR and culture, 80% by inspection) could partly be explained by the sensitivity of PCR. Furthermore, a miniscule anastomotic leakage can be missed by the naked eye, while PCR and culture are more sensitive since they detect the bacterial contamination to the peritoneum. The bacterial detection in suboptimal anastomosis either by PCR or culture at both time points from peritoneal washings was significantly higher than the blood. Using peritoneal washing solutions, PCR can detect the presence of bacteria significantly earlier and more precisely than blood. In the presence of anastomotic leakage, the management mainly depends on the patient's clinical situation and response. However, there

are some circumstances where symptoms are dubious to suggest anastomotic leakage. Although the decision will still be clinically oriented, earlier diagnosis of anastomotic leakage strongly helps clinicians to direct the treatment. Indeed, there are some clinical studies attempting to use peritoneal fluid to detect anastomotic leakages earlier than the clinical symptoms. Matthiessen et al.[16] have collected intraperitoneal cytokines, IL-6, IL-10, and TNF-alfa through a pelvic drain from patients who underwent anterior resection for rectal cancer. They have concluded that through intraperitoneal monitoring of cytokines anastomotic leakage might be detected before clinical symptoms are manifested. A recent article by Fouda et al.[29] checked the utility of intraperitoneal cytokine concentration and detection of bacteria in patients who underwent low anterior resection due to rectal cancer. Peritoneal samples were collected from the abdominal drains on the first, third, and fifth days postoperatively for peritoneal microbiological study and cytokine (IL-6, IL-10, TNF) level measurement. They found that intraperitoneal bacterial colonization and cytokine levels were significantly higher in patients with anastomotic leakages, concluding that the technique could be used as an adjunct to the decision of the surgeon for colorectal anastomotic leakages. Therefore, early detection of bacteria in peritoneal samplings by PCR and culture in patients with colorectal anastomosis could be an alarming sign of anastomotic leak.

The present study indicated that with suboptimal anastomosis, although anastomotic leakage was very high, clinically significant anastomotic leak was rather infrequent. Early detection of anastomotic leakage was possible by running PCR on peritoneal samples as early as 72 hours. The clinical significance of the use of peritoneal washing samples from colorectal anastomosis remains to be determined.

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DENEYSEL ÇALIŞMA - *ÖZET*

## Kolon rezeksiyonları sonrası suboptimal anastomoz: Deneysel çalışma

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AMAÇ: Suboptimal anastomozun nasıl sonuçlanacağı bilinmemektedir. Kolon rezeksiyonları sonrası anastomoz kaçağının erken tanısı hastanın doğru yönetiminde çok önemlidir.

GEREÇ VE YÖNTEM: Yirmi altı sıçan "kontrol", "kaçak" ve "suboptimal anastomoz" adıyla üç gruba ayrıldı. Sırasıyla gruplara sham laparotomi, çekum ligasyonu-perforasyon ve kolon rezeksiyonu sonrası dört dikişle anastomoz yapıldı. Beşinci saatte, üçüncü ve dokuzuncu günlerde periferden kan örnekleri ve relaparotomi sonrası periton yıkama örnekleri alındı. Karnın içi makroskopik anastomoz kaçağı varlığı için incelendi. Alınan yıkama örneklerinden aerobik ve anaerobik kültürlerle beraber 16 RNA ve E. Coli'ye özgü primerler kullanılarak polimeraz zincir reaksiyonu (PZR) yapıldı.

BULGULAR: Değişik vücut sıvılarında 16 s RNA ve *E. coli*'ye özgün primerler kullanılarak yapılan PZR'nin sensitivitesi ve spesifisitesi sırasıyla %100 ve %78 olarak bulundu. Üçüncü ve dokuzuncu günlerde alınan periton yıkama sıvılarında hem kültürde hem de PZR'de bakteri varlığı gösterildi. Karın içinin incelenmesinde 8 (%80) sıçanda anastomoz kaçağı işaretleri gözlenirken, anastomozla ilişkili mortalite sadece 2 (%20) sıçanda saptandı.

TARTIŞMA: Kolon rezeksiyonu sonrası suboptimal anastomoza bağlı anastomoz kaçağı oranı yüksektir ve 72 saat gibi erken bir sürede periton örneklerinden yapılan PZR ile tanı koymak olasıdır.

Anahtar sözcükler: Anastomoz kaçağı; erken tanı; kolorektal anastomoz; polimeraz zincir reaksiyonu; suboptimal anastomoz.

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