Frequent occurrence of fever in patients who have undergone endoscopic submucosal dissection for colorectal tumor but bacteremia is not a significant cause

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Running head: Post-ESD fever and bacteremia

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Abstract

**Background:** We examined the incidence of and factors associated with fever, as well as the frequency of bacteremia, in patients who had undergone endoscopic submucosal dissection (ESD) for colorectal tumor.

**Method:** A total of 199 patients (120 male and 79 female) were included. The patients were classified into 2 groups based on the body temperature on the day after ESD treatment: group A, body temperature <37°C; and group B, body temperature ≥37°C. The following factors were analyzed to determine their potential association with post-ESD fever: gender; age; tumor size, form, location; and presence or absence of intraoperative perforation. In addition, blood samples from 50 patients were obtained for blood culture and 16S rRNA gene analysis by polymerase chain reaction.

**Results:** Group A included 106 patients (70 male and 36 female), with a median age of 63 y. Group B included 93 patients (50 male and 43 female), with a median age of 70 y. The incidence of post-ESD fever in the entire cohort was 46.7%. Univariate analysis based on comparison between groups A and B showed that the following factors were significantly associated with post-ESD fever: age (mean±SD), 64.5±9.2 vs. 68.5±10.8 (y), p=0.006; and tumor size (mean±SD) 30.6±10.8 vs. 39.1±16.6 (mm), p<0.001. Logistic regression analysis for post-ESD fever also found that age (odds ratio 1.04
(95% CI [1.01-1.07], P=0.009) and lesion size (odds ratio 1.05 (95% CI [1.03-1.08], P=0.0002) were closely associated with post-ESD fever. Of the 50 patients who had blood samples cultured and 16S rRNA gene analyzed, bacteria in blood culture and the 16S rRNA gene were not detectable in any of the samples from the 50 patients.

Conclusions: This study indicated that older patients and patients with large tumors were more likely to develop post-ESD fever, but there was a low probability that bacteremia was the cause of fever.

Key words:

Post-ESD fever, 16S rRNA, Polymerase chain reaction (PCR), Tumor size, Age.
INTRODUCTION

Endoscopic submucosal dissection (ESD) is indicated for the treatment of large superficial colorectal tumors (1-4). During the ESD treatment, physical stimuli (e.g., injection and heat coagulation to submucosal and muscle layers) are thought to influence ESD lesions, and some enterobacteria may infect the body from the denuded area made by ESD. The human body is inhabited by a vast number of microorganisms including bacteria. It is estimated that about 4,000 bacterial species reside in the gastrointestinal tract; the number of bacteria in the colon is $10^{12}$ per gram of contents, and bacteria account for 60% of the dry weight of feces (5). As a result of ESD, large mucosal defect areas are made in the large intestine and are influenced by exposure to stool and some enterobacteria rather than by exposure to contents of the stomach and esophagus.

Fever is sometimes observed in patients who have undergone complete ESD for colorectal tumor. Fever secondary to colorectal ESD is thought to arise from enhanced susceptibility to bacterial infection due to exposure to stool and residues from the epithelial denuded area of the large intestine. Blood culture is widely used for the diagnosis of bacteremia. Min et al. (6) reported that bacteremia associated with EMR or ESD for colorectal tumors was not found in blood cultures obtained immediately before
and those obtained 5 minutes after the procedure in 40 patients who did not develop infectious symptoms. However, in many clinical situations the yield from blood culture is low; positive cultures are obtained from fewer than 30% (7), and only 5–20% of the intestinal microflora can be cultured and culture can reliably distinguish among bacterial phylogenetic groups, but not down to the species- or strain-level (8-9). In recent years, polymerase chain reaction (PCR) of the sequence of the 16S rRNA gene has been used to detect and identify bacterial infection in clinical practice (10-11). This method is able to detect microbes that are captured by white blood cells and leads to accurate diagnosis of post-ESD bacteremia.

The aim of this study was to evaluate the incidence of and factors correlated with fever, as well as the frequency of bacteremia using blood culture and PCR for bacterial 16S rRNA analysis, in patients who had undergone colorectal ESD.
METHODS

Patients and methods

Patients who were admitted to our hospital for ESD of colorectal tumor from April 2006 to March 2011 were enrolled. A total of 199 patients (120 male and 79 female) were included. The inclusion criteria of this study were patients who had a laterally spreading tumor (LST) which is defined as a superficial spreading-type tumor of at least 10mm in size, tumor size greater than 20 mm, tumor in any location in the large intestine, age over 20 years old, gave informed consent, and body temperature under 37 ºC on the day before and just before ESD. The exclusion criterion was tumors suspected of being submucosal massive invasive cancers. Patients were not given prophylactic antibiotics. We retrospectively analyzed the frequency of fever (body temperature ≥37ºC) after colorectal ESD until recovery of body temperature to under 37 ºC. Post-ESD fever was defined as body temperature ≥37ºC on the day after ESD treatment. Gender, age, tumor size, tumor form, tumor location, and presence or absence of intraoperative perforation were analyzed to determine their potential association with post-ESD fever.

In addition, between August 2010 and March 2011, blood samples from 50 patients who were among the 199 patients were obtained just before ESD and 16 hours
after ESD for blood culture and 16S rRNA gene analysis using polymerase chain reaction (PCR) to determine whether bacteremia after ESD was present. This study was approved by the ethics committee of Juntendo University Hospital. Written informed consent was obtained from the 199 subjects before they were included in the study.

Endoscopic procedure

The ESD procedure was performed as follows. Bowel preparation was performed using polyethylene glycol electrolyte lavage solution (Niflec Ajinomoto Pharma, Tokyo, Japan), which was administered at a volume ranging from 1 to 2 L, starting at 4 h before the examination until the stools became colorless and watery. ESD was performed in all patients using an electric endoscope (PCF Q260J or PCFQ260AZI; Olympus Optical Co., Ltd., Tokyo, Japan). During ESD, the patient was sedated with an intravenous injection of midazolam (2 mg) and pethidine hydrochloride (35 mg) and, if necessary, conscious sedation was maintained with an additional injection of midazolam. We performed all ESD procedures with carbon dioxide (CO2) insufflation. The standard colorectal ESD procedure was started with a circumferential incision. First, after injection of glyceol and 0.4% sodium hyaluronate with diluted epinephrine and indigocarmine, a circumferential incision in the submucosal layer was made. Then, the mucosa at the periphery was circumferentially cut with a Flush knife (Fujifilm Co., Ltd.,
Tokyo, Japan) by using a 40 W effect 2 Endocut (VIO300D). After an additional submucosal injection of glyceol and 0.4% sodium hyaluronate, the submucosal layer below the lesion was dissected by the Flush knife. If it was difficult to perform safe resection using the Flush Knife, we switched to another knife, Mantisfook (PENTAX Co, Ltd, Tokyo, Japan). The colonoscopist was accustomed to using both of these knives. All procedures were mainly performed two expert endoscopists (N.S and T.O. specialists of Japan Gastroenterological Endoscopy Society). If perforation or microperforation were recognized or suspected during ESD procedure, the perforated sites were closed using endoscopic clips (EZ Clip; Olympus Medical Systems Co., Tokyo, Japan) and the degree of air leakage was checked using abdominal CT after ESD.

**Blood culture**

Blood was obtained from a vein in the antecubital fossa. Prior to each sampling, the site was wiped with 10% povidone-iodine solution to minimize the number of skin contaminants. Forty milliliters of blood was drawn into a 50-ml syringe. For culture, blood samples were inoculated into two blood culture bottles (BACTEC Plus Aerobic ® and Plus Anaerobic ®, Nippon Becton Dickinson Company, Ltd., Tokyo, Japan). All inoculated culture media were incubated at 37°C in an incubator with shaking.
These culture bottles were assessed every day for 5 days. The contents of bottles with positive bacterial growth were inoculated onto a non-selective agar plate. The grown bacterial plaques were Gram stained, and identified using commercial bacteria identification kits.

**DNA purification from blood**

Two 3ml whole-blood samples drawn into sterile tubes were obtained from each participant. All samples were coded to conceal the patient’s identity and diagnosis. The samples were processed in a class II biosafety cabinet. We used a QIA amp® DNA Mini Kit (QIAGEN K.K, Tokyo, Japan) for purification of DNA from bacteria in whole blood.

**PCR amplification of 16S rRNA gene**

Polymerase chain reaction (PCR) amplification of 16S rRNA gene was performed in a class II biosafety cabinet used only for PCR. The primers used for PCR amplification of 16S rRNA gene sequences were 27F (5’-AGAGTTTGATCCTGGCTCAG-3’) or 350F (5’-TACGGGAGGCAGCAG-3’) and 1492R (5’-GGCTACCTTGTTACGACTT-3’) or 920R (5’-GTCAATTCMTTTGAGTTT-3’). Amplification reactions were performed in a total volume of 50μl containing 5μ dissolved DNA (100ng), 1.25U Ampli Taq Gold LD® or Prime STAR GXL® (TAKARA BIO INC, Shiga, Japan), 5 μl 10x Ex taq
buffer, 4μl dNTP mixture (2.5 mM each), and 10 pmol of each primer. 16SrDNAs were amplified in a thermal cycler using the following program: 95°C for 3 min, followed by 30 cycles consisting of 95°C for 30s, 52°C for 30s, and 72°C for 1.5 min, with a final extension period at 72°C for 10 min. Amplified DNA was verified by electrophoresis of aliquots of PCR mixtures (2μl) on 1.5% agarose gels in 1x TAE buffer.

**Statistical analysis**

Age and tumor size were expressed as mean ± SD (standard deviation) in cases with fever and cases with no fever after colorectal ESD. Comparison of age and tumor size between patients with or without fever was performed using Student’s t-test (unpaired), and comparison of gender, tumor form, tumor location and perforation was performed using Chi-square test or Fisher's exact test. Multivariate analysis of risk factors associated with fever after colorectal ESD was performed using logistic regression analysis. Differences with P <0.05 were considered to be statistically significant.
RESULTS

A total of 199 lesions were resected by ESD in 199 patients. The characteristics of the patients are summarized in Table 1. The patients comprised 120 males and 79 females, with a mean age of 66.4±10.2 y (range, 35-90 y). One hundred twenty-seven lesions were LST-G and 72 lesions were LST-NG. Seventy-eight lesions (39.2%) were located in the rectum-sigmoid colon and 121 lesions (60.8%) were located in the descending colon-cecum. The mean size of the resected specimen (the length of the major axis) was 34.6±14.4 mm (range, 20-110 mm). All tumors were completely resected. Eleven patients had micro-perforation of the large intestine. However, these 11 patients avoided laparotomy by undergoing endoscopic clipping of the perforated site.

Post ESD bleeding was recognized in only two patients who did not develop post-ESD fever. No other serious complications were recognized. Post-ESD fever was observed in 93 patients (46.7%); body temperature ≥37ºC on the day after ESD treatment was not recognized in the other 106 patients.

The patients were classified into the following 2 groups based on the body temperature on the day after ESD treatment: group A, body temperature <37ºC; and group B, body temperature ≥37ºC. Group A included 106 patients (70 male and 36 female), with a median age of 63 y (range 35-87 y). Group B included 93 patients (50
male and 43 female), with a median age of 70 y (range 39-90 y). Univariate analysis based on comparison between groups A and B showed that the following factors were significantly associated with post-ESD fever: age [(mean±SD), 64.5±9.2 vs. 68.5±10.8 (y), p= 0.006] and tumor size [30.6±10.8 vs. 39.1±16.6 (mm), p<0.0001]. Other factors including gender, tumor form, tumor location, and presence or absence of intraoperative perforation did not show a significant association with post-ESD fever (Table 2).

Multivariate analysis for post-ESD fever showed the following odds ratios: gender (male/female) 1.75 (95% CI [0.93-3.28], P=0.08); age 1.04 (95% CI [1.01-1.07], P=0.009); tumor form (LST-G/LST-NG) 0.87 (95% CI [0.44-1.70], P=0.67), tumor location (rectosigmoid/proximal colon) 1.42 (95% CI [0.75-2.73], P=0.28), tumor size 1.05 (95% CI [1.03-1.08], P=0.0002) and intraoperative perforation 3.28 (95% CI [0.76-14.1], P=0.11) (logistic regression analysis). Therefore, age and lesion size were closely associated with post-ESD fever, while gender, tumor form, tumor location and intraoperative perforation were not. Especially, post-ESD fever in the patients over or under 80 years old was recognized in 70.6% (12 out of 17 patients) and 44.5% (81 out of 182 patients), respectively (Fisher's exact test P=0.035) (Figure 1), and post-ESD fever in patients with size of lesion over or under 60mm was observed in 71.4% (10 out of 14 patients) and 44.9% (83 out of 185 patients), respectively (Fisher's exact test
P=0.049) (Figure 2). The mean duration of post-ESD fever in the 93 group B patients was 3.3 days, with a maximum of 13 days.

Of the 50 patients who had blood samples cultured and 16S rRNA gene analyzed, post-ESD fever was observed in 22 patients (44.0 %). Bacteria in blood cultures and the 16S rRNA gene were undetectable in all samples obtained just before ESD and 16 hours after ESD from the 50 patients.
DISCUSSION

Some complications such as bleeding and perforation are often associated with endoscopic resections, especially when the lesions removed are large (13-14). Post-ESD complications were reported in a large multicenter trial; the delayed bleeding rate in colorectal ESD cases was 1.5% (17/1111), whereas the delayed perforation rate was 0.4% (4/1111) (3). However, the incidence of post-ESD fever and factors associated with fever, as well as the frequency of bacteremia using molecular biological assay, in patients who had undergone ESD had not been reported. Numerous studies have shown that the rate of demonstrable bacteremia is maximal during and shortly after endoscopic examination, and diminishes rapidly within the following 30–240 min (15). The highest rates of bacteremia in endoscopic procedures have been reported for esophageal dilatation and sclerotherapy (16). The rate of bacteremia after esophageal bougienage has been demonstrated to be 12–22% (17-19). The rate of bacteremia after variceal sclerotherapy was 0–52%, with a mean of 14.6% (20). The cultured microorganisms are usually oral commensals such as Streptococcus viridians (21). Min et al. reported the frequency of bacteremia after colonic ESD or EMR in 40 patients (6). Bacteremia was not recognized in any blood cultures obtained at 5 min and was recognized in 1 sample obtained at 30 min after colonic ESD. However, inflammatory symptoms or signs after
Colonic ESD are observed more often than has been supposed from the low rate of ESD-related bacteremia. The rate of post-ESD fever was 46.7% among all patients in our study. The reason that bacteria were not detectable in the current study may be that the detection sensitivity of blood culture is low; positive cultures are obtained from fewer than 30% of bacteria-positive samples (7).

The evaluation of bacteremia after ESD or EMR has been performed using aerobic and anaerobic blood culture bottles only; we evaluated the presence of bacteremia after ESD using a molecular biological technique, i.e., detection of a sequence of the 16S rRNA gene of offending bacteria. This assay is able to detect microbes in a short period of time that are captured by white blood cells; therefore, it has high sensitivity and specificity regardless of whether the patient is using antibiotics (10-11). However, the results of the 16S rRNA assay and blood culture in our 50 patients were negative regardless of whether they developed a fever or not. Conclusively, colorectal ESD results in a low or very low frequency of bacteremia.

Post-ESD fever was closely associated with the patient’s age and tumor size. This indicates that the frequency of post-ESD fever increases with advancing age and with increasing tumor size. Especially, the frequency of post-ESD fever among those over 80 years old or those with size of lesion over 60mm was greater than 70%.
However, almost cases of post-ESD fever resolved within 3 days after ESD and were low-grade fever. Itaba et al. (22) reported that post-ESD fever (≥37°C) after ESD for gastric tumor was recognized in 41.3% of patients; however, almost all patients had low-grade fever (<38°C) and the source of fever was unlikely to be bacteremia using blood culture. In our study, bacteremia was not recognized as a major cause of post-ESD fever using a molecular biological diagnostic method which was a more accurate method for detecting bacteremia. Post-ESD fever was more likely to develop in aged patients and patients with larger lesions; bacteremia was not the cause and the fever resolved within several days. Moreover, post-ESD fever developed not only after colorectal ESD but also after gastric ESD, and the frequency was over 40%.

Our study indicated a low probability of bacteremia after colorectal ESD. The reason for the development of post-ESD fever is not fully understood yet. A larger tumor requires longer treatment time and contact time of electric knives to the submucosal layer. Many physical stimuli (e.g., injection and heat coagulation to submucosal and muscle layers) also influence the epithelial denuded area apart from intestinal bacteria. Physical stimuli during the ESD treatment may influence the development of fever secondary to colorectal ESD. Older patients might be readily influenced by these physical stimuli due to their low body resistance.
This study had several limitations. We drew blood samples for culture and PCR 16 h after ESD. It has been reported that transient bacteremia was observed using blood cultures obtained from 5 to 240 min after ESD (23-25). However, we evaluated the presence of bacteremia 16 hr after ESD. This timing made it possible to detect sustained bacteremia which would manifest as a clinical condition. When considering this point, the results of this study seem to be sufficiently meaningful for clinical purpose. We detected bacteremia using the PCR method which is highly-sensitive method of detecting pathogens. However, this method has a limit of detection. Its sensitivity ranged from 3 to 50 CFU (26). Bacteremia was not completely ruled out, but no patient had symptoms of sepsis in our study.

In conclusion, age and tumor size were significantly associated with post-ESD fever. This study indicated that older patients and patients with large tumors were more likely to develop Post-ESD fever, but there was a low probability that bacteremia was the cause of fever.

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Technology of Japan.
References


Figure 1: Patients having Fever after Colorectal ESD according to Age, Under 80 vs. over 80 years old.

Post-ESD fever was observed in 70.6% (12/17) of patients over 80 years old and in 44.5% (81/182) of patients under 80 years old. Patients over 80 years old were significantly more likely to develop fever after colorectal ESD (P=0.035, Fisher's exact test).

Figure 2: Patients having Fever after Colorectal ESD according to Tumor Size, Under 60 vs. over 60 mm

Post-ESD fever was observed in 71.4% (10/14) of patients with tumor size over 60 mm and 44.9% (83/185) of patients with tumor size under 60 mm. Patients with tumors over 60 mm were significantly more likely to develop fever after colorectal ESD (Fisher's exact test P=0.049).
Table 1: Characteristics of Study Cases

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tr>
<td>Gender (male/female)</td>
<td>120 / 79</td>
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<tr>
<td>Age (years)</td>
<td>66.4±10.2 (35-90)</td>
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<tr>
<td>Form (LST-G/LST-NG)</td>
<td>127 / 72</td>
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<tr>
<td>Location (rectum-sigmoid /descending-cecum)</td>
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<tr>
<td>Tumor size (mm)</td>
<td>34.6±14.4 (20-110)</td>
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<tr>
<td>Perforation (cases)</td>
<td>11</td>
</tr>
<tr>
<td>Post-ESD fever / no fever (cases)</td>
<td>93 / 106</td>
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</table>

Table 2: Comparison of Cases with Fever and no Fever after Colorectal ESD.

<table>
<thead>
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<th>Group B</th>
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</tr>
<tr>
<td>Age (years)</td>
<td>64.5±9.2</td>
<td>68.5±10.8</td>
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</tr>
<tr>
<td>Form (LST-G/LST-NG)</td>
<td>62 / 44</td>
<td>65 / 28</td>
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</tr>
<tr>
<td>Location (rectum-sigmoid /descending-cecum)</td>
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<td>37 / 56</td>
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<tr>
<td>Tumor size (mm)</td>
<td>30.6±10.8</td>
<td>39.1±16.6</td>
<td>&lt;0.001*</td>
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<tr>
<td>Perforation (cases)</td>
<td>3</td>
<td>8</td>
<td>0.11</td>
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</table>

Table 3: Multivariate Analysis of Risk Factors Associated with Fever after Colorectal ESD.

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<th>95% CI</th>
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<td>1.01 -1.07</td>
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<tr>
<td>Form</td>
<td>0.87</td>
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<td>Location</td>
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<td>0.75 -2.73</td>
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