Title: Effect to Gastrointestinal Barrier Function During Laparoscopic Gastrectomy with Deep vs Moderate Neuromuscular Blockade

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Study Protocol and Statistical Analysis Plan

Study Protocol

1. Perioperative vital signs and surgical conditions

Arterial blood pressure, heart rate, pulse oxygen saturation and other perioperative vital signs in moderate neuromuscular blockade group (group M) and deep neuromuscular blockade group (group D) will be recorded. Volume of perioperative blood loss and infusion volume (including crystalloid solution, colloidal solution and blood products) will be recorded either. Surgical condition scores, intraoperative pressure of CO_2 pneumoperitoneum and duration of pneumoperitoneum will be recorded. Blood gas analysis will be performed hourly during the operation.

2. Extubation

After the operation, the extubation time of patients in the two groups and the modified Aldrete score at 1min, 5min and 15min after the extubation will be recorded.

3. Postoperative pain scores

Assessment and record of Visual analogue scale (VAS) at rest and activity 6 h, 12 h, 24 h, 48 h after extubation. (VAS pain score: 0 - completely painless, 10 - unbearable pain.)

4. Evaluations of intestinal barrier function

4.1 The concentrations of d-lactic acid and diamine oxidase

5ml peripheral venous blood will be extracted from the patients of the two groups on the first day before surgery and the first day after surgery for plasma d-lactic acid level and diamine oxidase activity detection.

4.2 Parameters of intestinal mucosal

(1) After the removal of gastric tumor, jejunum tissues of the two groups of patients will be collected. The height, width and number of villi in the intestinal mucosa will be observed and determined by microscopy. The density of mitochondria, length and width of villi, and clearance of intestinal epithelial cells will be analyzed by scanning electron microscopy to detect the damage of intestinal mucosal mechanical barrier.

(2) The expression level of sIgA+ in the submucosal lamina propria will be detected by immunohistochemistry to reflect the damage of intestinal mucosal immune barrier.

5. Intestinal microecology

The change of intestinal mucosal biological barrier function was reflected mainly through selective bacterial culture of feces. The first, second, third and one-month postoperative defecation of the patients in two groups before and after surgery will be

collected and analyzed with the following methods:

(1) Routine examination of feces;

(2) Selective culture of aerobic bacteria (such as Enterococcus, Enterobacter spp.) and anaerobic bacteria (such as Bifidobacteria, Lactobacillus), calculate the ratio of Bifidobacteria to Escherichia Coli. (B/E value), which is an important indicator to evaluate the status of intestinal flora, in the health condition, B/E value should be at least >10, otherwise it is regarded as flora imbalance;

(3) 16S rRNA sequencing of the feces to analyzed the microbiota;

(3) Determine the pH of feces;

(4) Determination of fecal ammonia.

6. The postoperative recovery of intestinal function

The postoperative recovery of intestinal function in the two groups will be evaluated, including the first postoperative exhaust time, defecation time and feeding time, time of removal of abdominal drainage tube, postoperative hospital stays the incidence of diarrhea and abdominal distension within one month after operation.

Statistical Analysis Plan

SPSS 20.0 software will be used for statistical analysis. The measurement data will be tested by t test or Mann-Whitney U test. The data will be expressed as mean ± standard deviation. Nominal variables are summarized as number of subjects (proportion, %) and will be compared with the Chi-square test or Fisher's exact probability. $P < 0.05$ will be considered statistically significant. On the basis of the primary parameters, we estimated that 83 patients will be needed with an α error of 0.05, a β error of 0.2 and a drop-out rate of about 10%.