SUPPLEMENTARY MATERIAL 1

A – Definitions

Clinical condition assessment: Defined by daily clinical evaluation performed by senior surgeons in the postoperative period, including their subjective appraisal and scoring by the Glasgow Coma Scale. Classified as stable, improved, or deteriorated.

Abdominal pain: Defined by the presence of pain localized in the abdominal region, identified during daily physical examination performed by senior surgeons, and applying a pain visual analogue scale (VAS). Classified as absent/low (VAS ≤ 3), wound pain (VAS > 4), localized pain (VAS > 4), or diffuse pain (VAS > 4).

Postoperative ileus: Defined by the combination of at least one of the following signs from postoperative day (POD) 3 through POD7, with no improvement: nausea and vomiting; inability to tolerate solid or semi-liquid diet during the preceding 24 h; no gas or stool for the preceding 24 h; abdominal distension; radiological evidence of ileus; and need for nasogastric tube insertion.

Surgical site infection (SSI): Defined by the presence of inflammatory signs or purulent discharge from the surgical wound.

Urinary tract infection: Defined by a positive urine culture associated with fever or leukocytosis.

Pneumonia: Defined by suggestive clinical signs of respiratory infection (*e.g.*, fever, cough, dyspnea) associated with radiological signs of pulmonary infiltration.

B – Study protocol

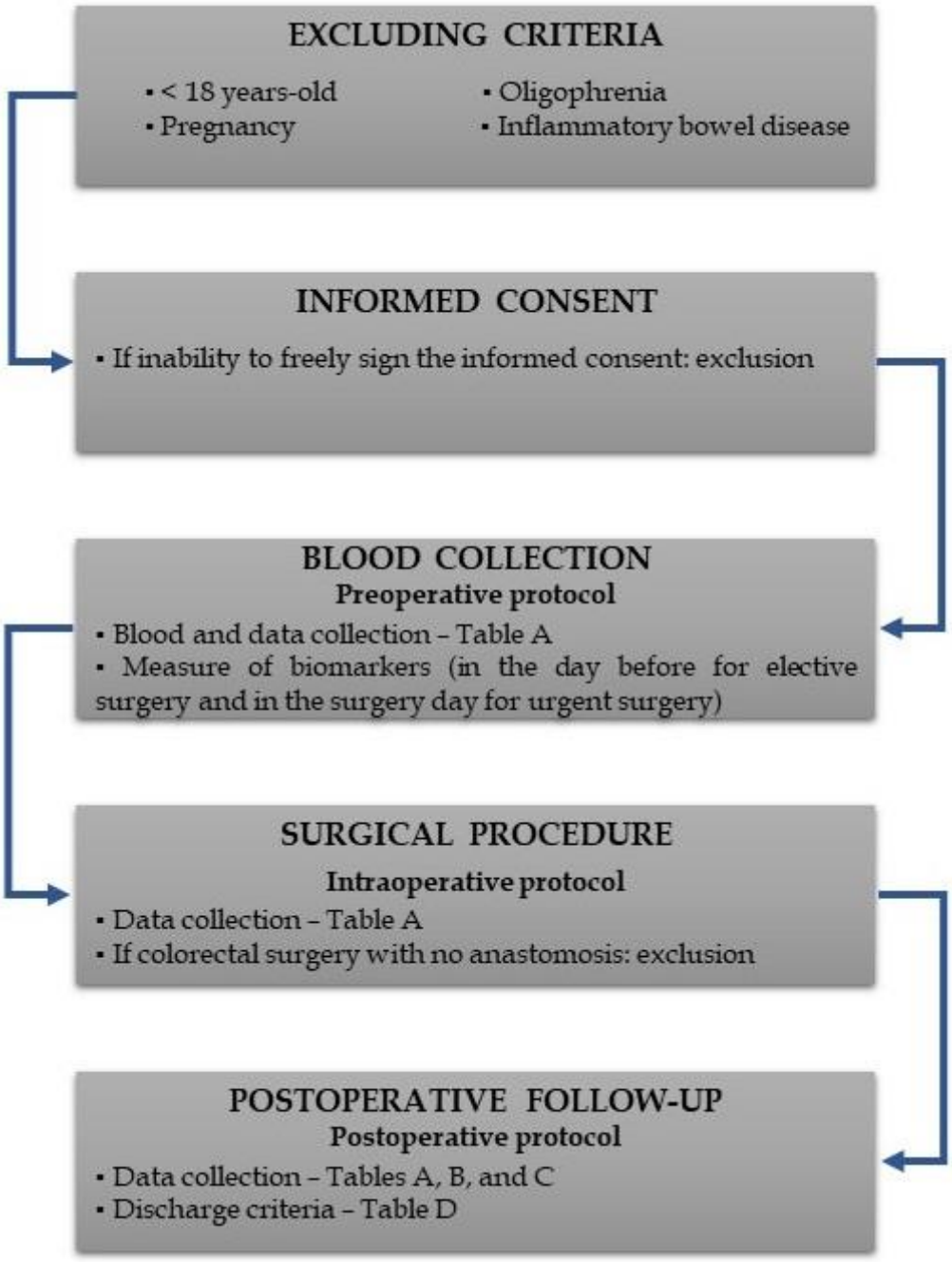

Table A Protocol variables

Variable	
Demographic	Age
	Sex
Preoperative	Health-related quality of life – EQ5D5L

	Nutritional status
	Comorbidities
	Charlson Comorbidity Index score
	Smoking and alcohol habits
	Allergies
	Previous abdominal surgery
	Steroids or immunosuppression in the last 6 mo
	Preoperative diagnosis
	Preoperative staging
	Bowel preparation
	American Society of Anesthesiologists grade
Intraoperative	Type of anesthesia
	Anastomosis technique
	Blood loss
	Blood transfusion
	Surgical complications
	Level of surgical contamination
	Duration of surgical procedure
	Surgical specimen
	Surgical approach
Postoperative	Morbidity

	Mortality
	Time of follow-up
	Intensive care unit stay

Table B Postoperative follow-up: clinical findings

Sign/Symptom	DBS	POD1	POD2	POD3	POD4	POD5
Temperature						
Heart rate						
Respiratory rate						
Urinary debit						
Mental status						
Clinical status						
Gastric emptying						
Bowel movements						
Abdominal pain						
Surgical wound infection						
Pain (VAS)						
Complications						
Intensive care unit						

DBS: Day before surgery; POD: Postoperative day; VAS: Visual analogue scale.

Table C Postoperative follow-up: laboratory findings

Biomarkers	DBS	POD1	POD2	POD3	POD4	POD5

White blood cell count
Eosinophil cell count
Urea
Creatinine
C-reactive protein
Procalcitonin
Calprotectin
Albumin

DBS: Day before surgery; POD: Postoperative day.

Table D Discharge clinical criteria

Oral tolerance
Bowel movements
Pain control with oral analgesic
No signs of sepsis
Institutional social criteria for discharge fulfilled

C – Laboratory

White blood cell count and eosinophil cell count: A complete blood count (CBC) with white blood cell (WBC) count differential is a widely-available and frequently requested blood test, given the variety of information it offers. For the diagnosis of sepsis, the presence of WBC > 12000/mm^3 or < 4000 mm^3 has been accepted as a criterion. In turn, eosinopenia has been identified as a potential sepsis biomarker, although it is not used much compared with other more commonly accepted biomarkers (C-reactive protein [CRP] and procalcitonin [PCT])[40,50].

The determination of WBC and WBC differential, in particular eosinophil cell count (ECC), is carried out in whole blood samples; therefore, a peripheral blood sample was collected by venipuncture into K_3-EDTA tubes (BD Vacutainer® K_3-EDTA; Becton Dickinson). The sample was homogenized by gentle inversion and processed on the UniCel DxH 800 automated hematology analyzer (Beckman Coulter®, Sykesville, MD, United States) in the Hematology Laboratory Department. This analyzer used the complementary data obtained by three methodologies: impedance (to obtain the cell volume); radiofrequency conductivity (to analyze the internal composition of the cell and the nucleus-to-cytoplasm ratio); and light scattering in five different angles (to obtain information about cellular granularity). The analyzer was standardized prior to sample processing by using controls provided by Beckman Coulter®.

CRP: CRP is an acute phase protein primarily produced in the liver in response to the release of inflammatory cytokines, such as interleukin-6, by macrophages, T lymphocytes, and adipocytes. It plays a role in the recognition and clearance of apoptotic or necrotic cells and foreign pathogens, through binding to the phosphocholine expressed on their surface, triggering the activation of the classical complement pathway, and promoting its phagocytosis by macrophages.

There are several causes of elevated CRP, including acute or chronic inflammatory processes of infectious or non-infectious etiology, trauma, tissue necrosis, immune or autoimmune diseases, and neoplastic processes including colorectal cancer. Due to the rapid increase in its concentration in response to injury (rise within the first 6-8 h, with a peak after 48 h), as well as the rapid return to baseline values after resolution of the condition (relatively short half-life), it is recognized as a good and sensitive indicator of systemic inflammation. Its serum levels have been useful for monitoring disease activity and response to treatment. Its role in screening and monitoring the progression or remission of neoplastic disease, however, is not well defined. Nonetheless, mild elevations can be detected in some cases, without any underlying

inflammatory pathology, for example in the elderly, smokers, obese people, and diabetics. Thus, given the non-specificity and wide inter-individual variation of CRP, interpretation of its values must always take into consideration the clinical context and previous measurements[51-54].

Laboratory analysis of CRP was performed on serum samples, which were obtained by the collection of peripheral blood by venipuncture into tubes with a separating gel and clot activator (BD Vacutainer® SST™ II Advance; Becton Dickinson, Franklin Lakes, NJ, United States) and centrifugation in a refrigerated centrifuge (3200 rpm for 10 min).

An immunoturbidimetric assay was used for the quantitative determination of CRP in human serum or plasma (CRP Latex; Beckman Coulter®) on an automated clinical chemistry analyzer (AU5800; Beckman Coulter®). During the procedure, the patient's sample was mixed with a suspension of latex particles coated with goat anti-CRP antibody; the CRP present in the sample reacted with this antibody, forming insoluble immune complexes. The turbidity produced by immune complexes, which decreases the intensity of transmitted light (due to the portion of light that is reflected, absorbed, or scattered), can be measured by a spectrophotometer; it is proportional to the concentration of CRP in the sample. The test is linear within the concentration range of 0.2–480 mg/L. Values > 5 mg/L are considered pathological.

The technique was calibrated (obtaining a six-point curve) and controlled (using two levels of control, normal and pathological) prior to the sample processing, by using specific material supplied by Beckman Coulter®.

PCT: PCT is a polypeptide prohormone of calcitonin, synthesized mainly by thyroid C cells, and to a lesser extent, in neuroendocrine tissues of other organs (*i.e.* lung, intestine). Usually undetectable in healthy individuals, its values may increase early (3-12 h) after stimulation by inflammatory cytokines and bacterial endotoxins. Currently, it is considered one of the most useful and specific biomarkers of severe systemic inflammation, infection, and sepsis of bacterial origin. Negative values virtually exclude this diagnosis, with a negative

predictive value > 95%. It is useful in the differential diagnosis against viral infections or non-infectious systemic inflammations to monitor the effectiveness of the treatment, because with a half-life of 24 h, its value rapidly decreases in the presence of an effective therapeutic response; and it has prognostic value, as its levels correlate with the severity of the infection and organ dysfunction. However, elevation in its concentration can also result from other causes, namely trauma, major surgery, extensive burns, or pancreatitis[55-58].

Until April 2018, the PCT laboratory assay was performed on serum samples obtained after the collection of peripheral blood by venipuncture into tubes with separator gel and clot activator (BD Vacutainer® SST ™ II Advance) and centrifugation in a refrigerated centrifuge (3200 rpm for 10 min).

The Elecsys BRAHMS PCT (Roche®, Basel, Switzerland) electrochemiluminescence immunoassay for quantitative determination of PCT in human serum or plasma was used on the Elecsys cobas e411 analyzer (Roche®). During the sandwich procedure, the calibrator, control, or user sample is incubated with a biotinylated monoclonal anti-PCT antibody (capture antibody) and a monoclonal anti-PCT antibody labeled with a ruthenium complex (antibody marker), forming a sandwich complex. Then, streptavidin-coated microparticles are added, which bind to the complex formed by the biotin-streptavidin interaction. The reaction mixture is aspirated into a reading cell, where the microparticles magnetically attached to the electrode surface. After a wash to remove unbound elements, an electric current is applied to the electrode, inducing a chemiluminescent reaction that is measured by a photomultiplier. The measured signal is converted to a PCT concentration in ng/mL, by using an analyzer-fitted two-point calibration curve and a lot-dependent reagent-specific curve. This immunoassay has a measuring range of 0.02-100 ng/mL.

Since May 2018, the laboratory assay of PCT has been performed on whole blood samples obtained after collection by venipuncture into tubes with K_3-EDTA anticoagulant (BD Vacutainer® K_3-EDTA). The assay is performed by using Radiometer's Procalcitonin Immunoassay in AQT90 FLex® equipment, a

point-of-care apparatus that uses temporal resolution immunoassay and fluorometry technology. This assay also uses the sandwich technique, described above. During the procedure, the calibrator, control, or user sample is incubated at 37 °C in a test well that is coated with a biotinylated mouse anti-PCT monoclonal antibody (capture antibody), immobilized on the streptavidin surface, and a mouse anti-PCT monoclonal antibody labeled with europium (marker antibody), forming a sandwich-like complex. After a wash to remove unbound elements, the time-resolved fluorescence of the europium-labeled sandwich complex is measured, after excitation with 340-nm light. The measured signal is converted to a PCT concentration in ng/mL using the lot-specific analyzer-fitted calibration curves of the reagent. The PCT concentration is directly proportional to the measured europium signal. The limit of quantification determined for this assay is 0.12 ng/mL.

PCT values can be interpreted as follows: PCT < 0.5 ng/mL represents a low risk of sepsis and/or septic shock, while PCT > 2 ng/mL represents an increased risk of sepsis and/or septic shock.

Calprotectin: Calprotectin (CLP) is a calcium-binding protein secreted predominantly by neutrophils and monocytes. The heterocomplex consists of the two proteins, S100A8 (calgranulin A) and S100A9 (calgranulin B), also designated MRP8 and MRP14, respectively. Expression of these proteins in epithelial tissues was first described in the context of squamous epithelia and with murine and human wound repair. More recently, an association of CLP expression with adenocarcinoma in humans has emerged. Elevated CLP has been found in many sites of inflammation and in the extracellular fluid of patients with many types of inflammatory conditions. The concentration of CLP in blood is increased in patients with rheumatoid arthritis, cystic fibrosis, multiple sclerosis, and human immunodeficiency virus infections, while elevated CLP has been detected in the stool of patients with Crohn's disease and colorectal cancer[59-63]. Enhanced expression of CLP is an early event in prostate tumor genesis and may contribute to the development and progression

or extension of prostate carcinoma[64]. Furthermore, the CLP level has been tested as a serum marker for prostate cancer, with researchers comparing the serum concentrations in patients with cancer with healthy controls or patients with benign prostatic hyperplasia (BPH). A significant increased CLP serum level in prostate cancer was found in patients with prostate cancer compared to patients with BPH, with the latter exhibiting values like those obtained for healthy individuals.

To quantify serum CLP, we used an enzyme-linked immunosorbent assay (ELISA) intended for the quantitative determination of CLP in serum and plasma (IDK® Calprotectin [MRP8/14]; Immundiagnostik AG, Bensheim, Germany). The assay utilizes the two-site sandwich technique with two selected monoclonal antibodies that bind to human CLP. Standards (0, 3.9, 15.6, 62.5, and 250 ng/mL), controls, and diluted patient samples were added to the wells of a microplate coated with a high-affinity anti-human CLP monoclonal antibody using the Triturus ELISA Instrument, a completely open and fully-automated ELISA analyzer for testing and processing batches of samples for infectious diseases, autoimmunity, and biological drug monitoring (Grifols, S.A., Barcelona, Spain). During the first incubation step, CLP in the samples is bound by the immobilized antibody. Then, a peroxidase-labeled conjugate is added to each well to form the following complex: capture antibody-human CLP–peroxidase conjugate. Tetramethylbenzidine is used as a substrate for peroxidase. Finally, an acidic stop solution is added to terminate the reaction and to change the solution color from blue to yellow. The intensity of the yellow color is directly proportional to the CLP concentration of the sample. A dose-response curve of the absorbance unit (optical density at 450 nm) *vs* concentration is generated, using the values obtained from standards. CLP, present in the patient samples, is determined directly from this curve. The obtained results are multiplied by the dilution factor of 30 to obtain the actual concentrations. The reference range for CLP in plasma of healthy people is < 3 µg/mL.

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SUPPLEMENTARY MATERIAL 2

A – Clinical criteria

Table E Summary of the predictive performance of the studied clinical criteria

	AUROC	SS	SP	NPV	PPV	PLR	NLR
Abdominal pain							
POD3	0.77	0.83	0.71	0.98	0.16	2.84	0.25

POD4	0.84	0.73	0.91	0.98	0.34	7.67	0.30
POD5	0.83	0.65	0.97	0.98	0.60	23.99	0.36
Clinical condition							
POD3	0.62	0.26	0.95	0.95	0.29	6.05	0.77
POD4	0.82	0.96	0.48	0.99	0.11	1.83	0.01
POD5	0.90	0.90	0.79	0.99	0.23	4.33	0.13

AUROC: Area under the receiver operating characteristic curve; NLR: Negative likelihood ratio; NPV: Negative predictive value; PLR: Positive likelihood ratio; POD: Postoperative day; PPV: Positive predictive value; SP: Specificity; SS: Sensitivity.

B – White blood cell count and eosinophil cell count

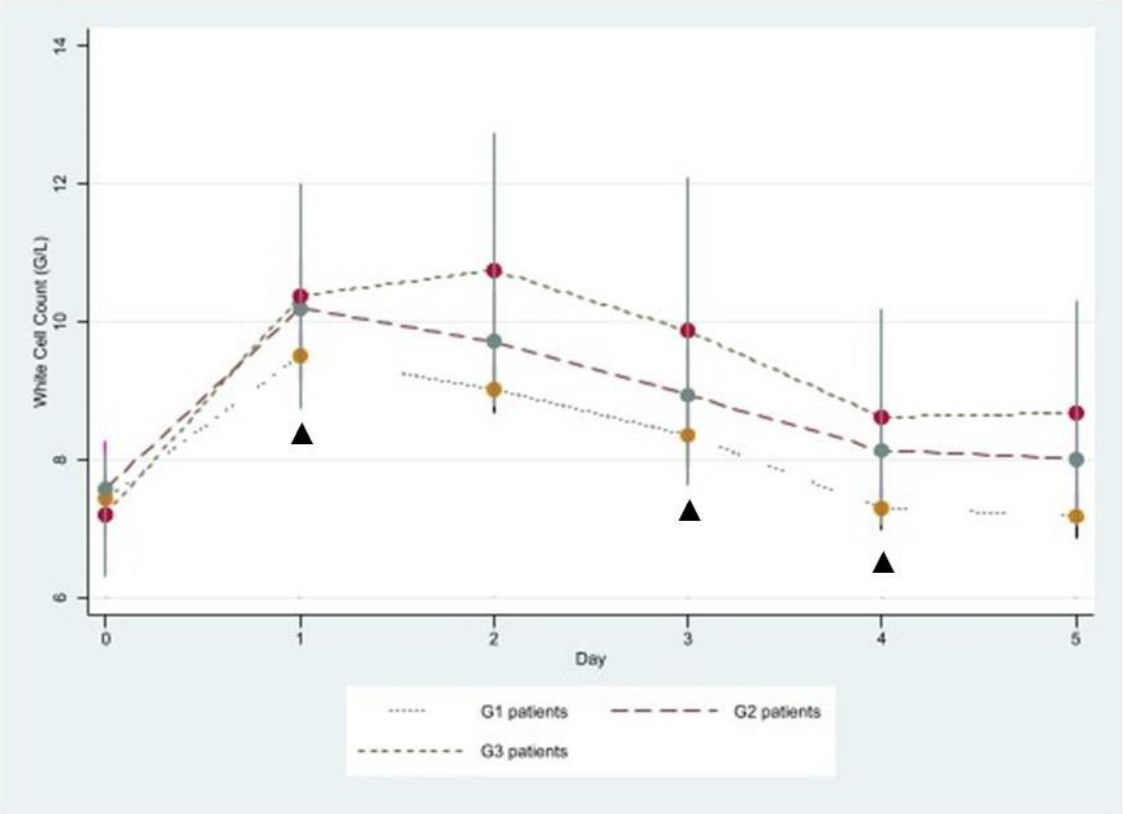

Figure 1 White blood cell count. Values are the mean ± standard error. G1: No complications; G2: Complications not related to the colorectal anastomotic leak (CAL); G3: CAL. ▲, *P* statistically significant ($P < 0.05$).

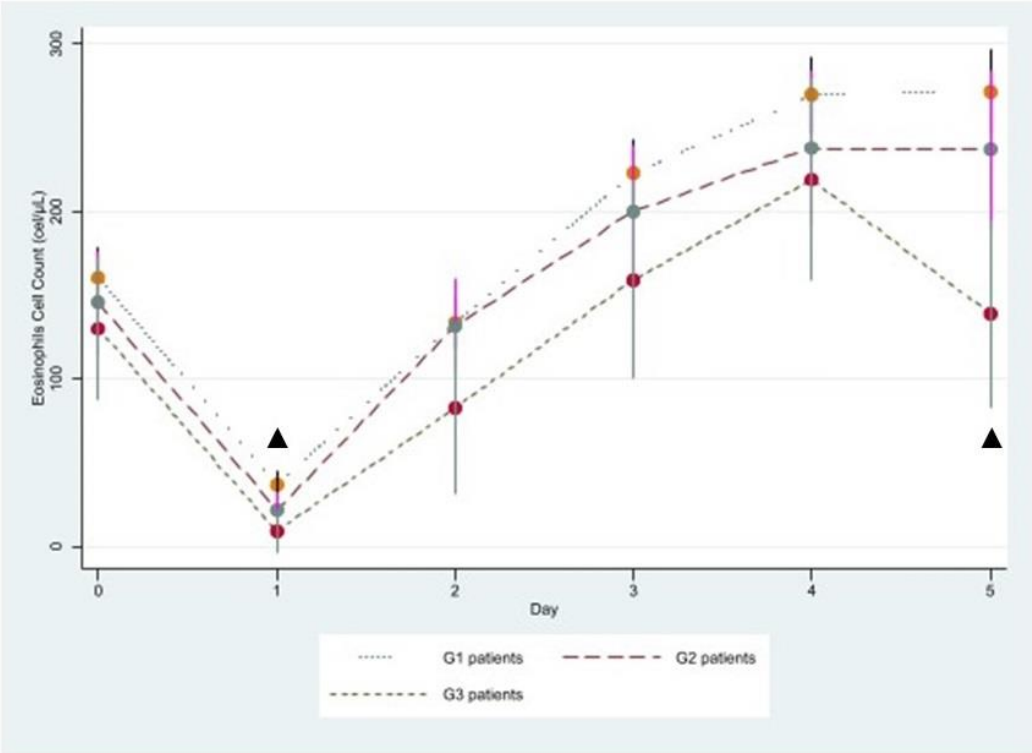

Figure 2 Eosinophil cell count. Values are the mean ± standard error. G1: No complications; G2: Complications not related to colorectal anastomotic leak (CAL); G3: CAL. ▲, *P* statistically significant ($P < 0.05$).

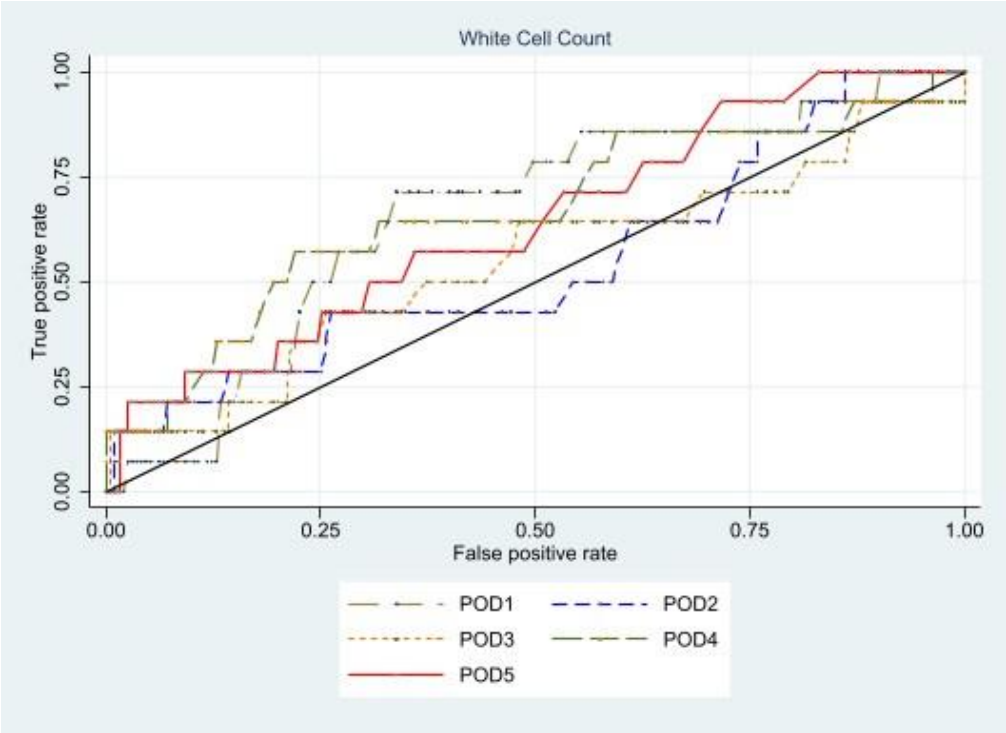

Figure 3 Area under the receiver operating characteristic curve of colorectal anastomotic leak for white blood cell count, from postoperative day (POD) 1 to POD5.

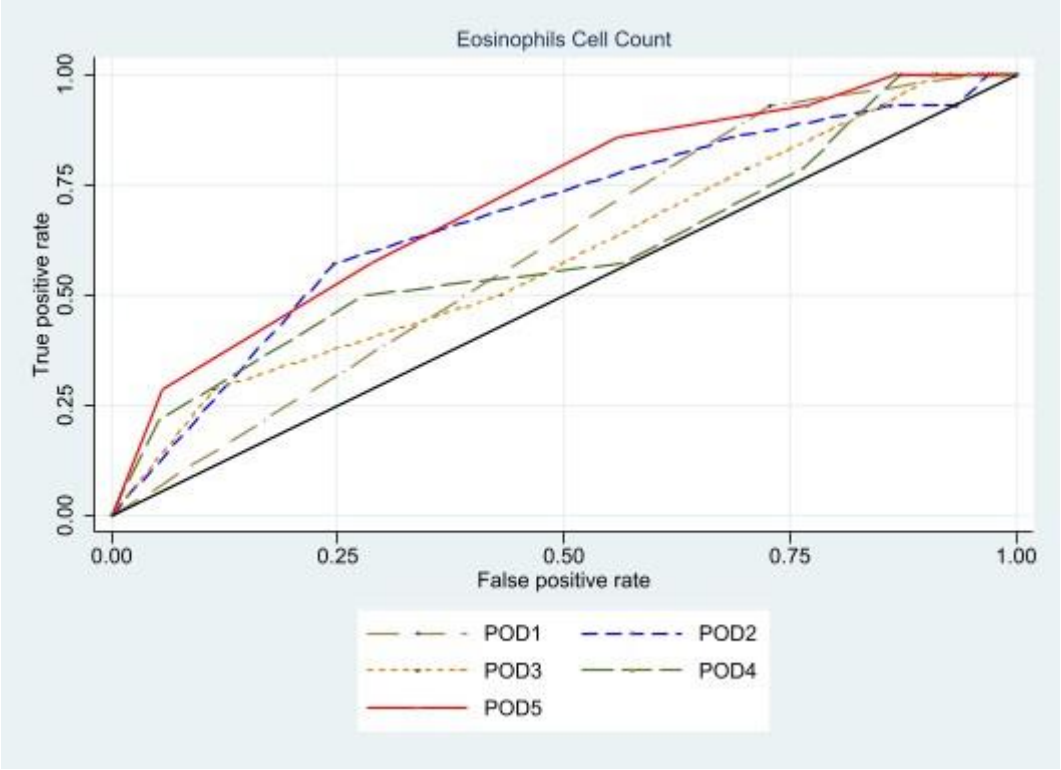

Figure 4 Area under the receiver operating characteristic curve of colorectal anastomotic leak for eosinophil cell count from postoperative day (POD) 1 to POD5.

C – Procalcitonin

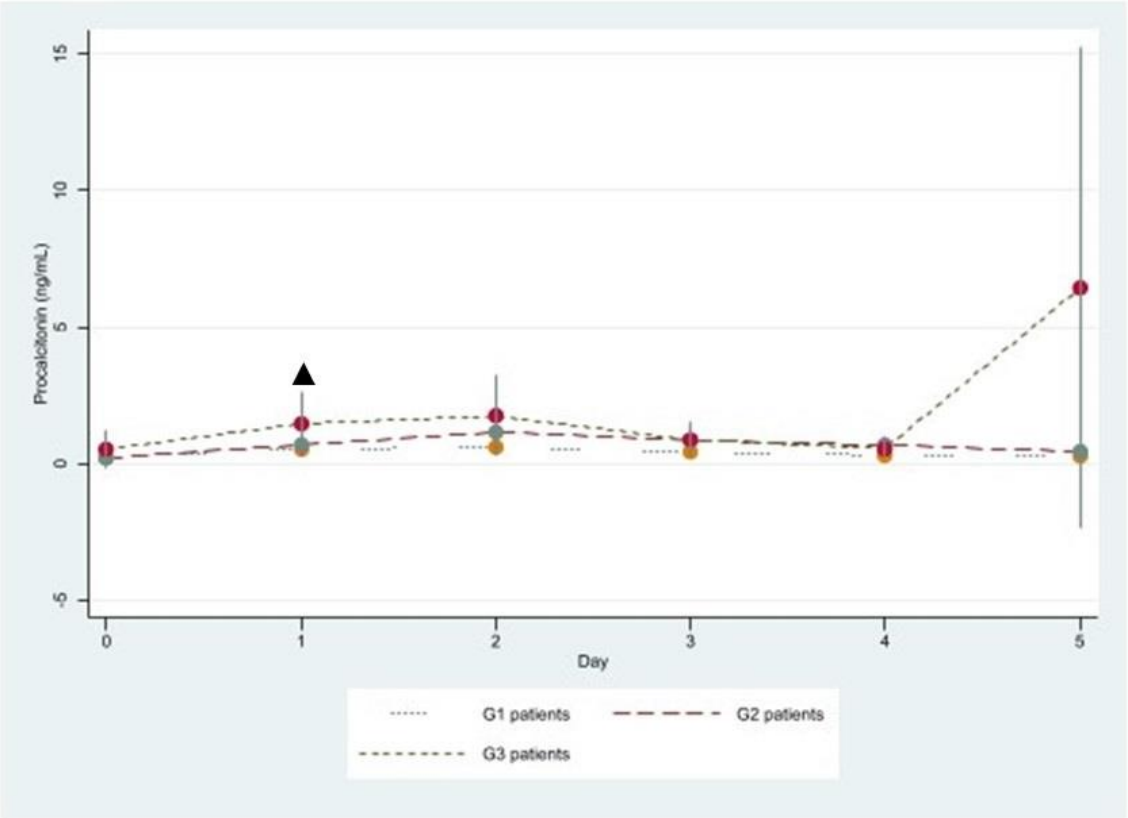

Figure 5 Procalcitonin levels. Values are the mean ± standard error. G1: No complications; G2: Complications not related to colorectal anastomotic leak (CAL); G3: CAL group. ▲, *P* statistically significant ($P < 0.05$).

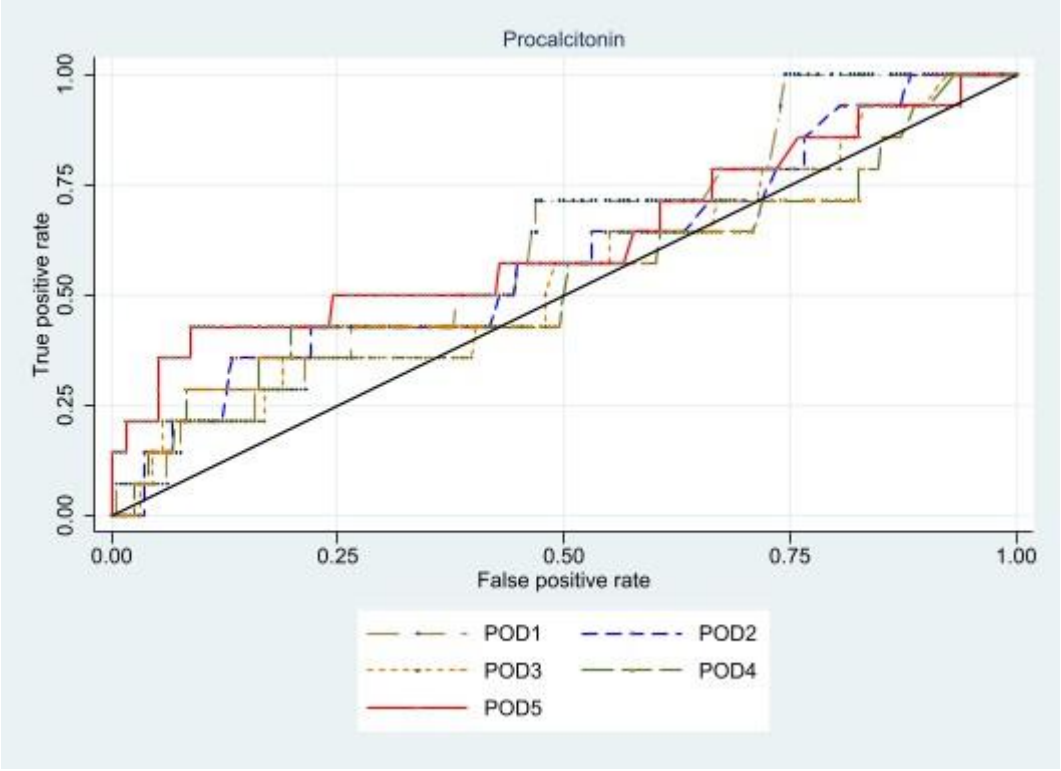

Figure 6 Area under the receiver operating characteristic curve of colorectal anastomotic leak for procalcitonin from postoperative day (POD) 1 to POD5.