| | |
|---|---|
| Permission granted<br>Date (D/M/Y): 23.12.2019<br>Protocol number 6<br>Assigned number 30 | Chairman of the Bioethics committee<br>NJSC "KMU" Visternichan O.A<br>PhD, associate professor<br>Date (D/M/Y): 14.01.2022<br>Signature |

**Karaganda Medical University**

# CLINICAL TRIAL PROTOCOL

"Prognostic significance of bacterial translocation markers as predictors of infectious and inflammatory complications in acute mechanical bowel obstruction"

Researcher: A.V. Ogizbayeva, doctoral student in the specialty 6D110100 "Medicine"

Scientific supervisors: Y.M.Turgunov, MD, Professor

Name and address of the organization, where the research is conducted: NJSC Karaganda Medical University, Gogol str., 40, Karaganda, Kazakhstan

Karaganda 2019

## I. The aim of the study and introduction

**The aim of the study:**
Determine the diagnostic and prognostic significance of bacterial translocation as a predictor of the complications development in patients with malignant and benign acute bowel obstruction by assessing the relationship of biomarkers in the systemic circulation (LBP, sCD14-ST) with the detection of microorganism genes (16s rRNA) in mesenteric lymph nodes.

**Research objectives:**
1. Compare the level and dynamics of changes in the biomarkers of bacterial translocation (LBP, sCD14-ST) in the blood serum of operated colorectal cancer patients with and without acute bowel obstruction, as well as those who operated on for benign acute bowel obstruction.
2. Determine the effect of clinical and morphological characteristics of a colon tumor on the frequency of bacterial translocation development.
3. Evaluate the clinical and laboratory parameters of operated colorectal cancer patients with and without acute bowel obstruction, as well as those who operated on for benign acute bowel obstruction, depending on the presence of bacterial translocation and systemic inflammatory response syndrome.
4. Identify the correlation between 16s rRNA in mesenteric lymph nodes and LBP, sCD14-ST in blood serum.

**Introduction**
Despite modern approaches to the diagnosis and treatment of acute bowel obstruction (ABO), postoperative mortality ranges from 5 to 32%, and complications occur up 23% of cases [1, 2, 3]. One of the formidable infectious and inflammatory complications of ABO is sepsis. In ABO caused by colorectal cancer, sepsis occurs from 1.7 to 10.5% of cases, and in benign ABO from 3 to 10.29% [4, 5, 6, 7]. The main component of the development of sepsis in ABO is bacterial translocation (BT) [8].
Bacterial translocation is the migration of intestinal bacteria or their products through the intestinal mucosa into the mesenteric lymph nodes and further into normally sterile tissues and organs. For the first time, Durwanding [9] described the alleged migration of bacteria from the intestine in 1881. R.D. Berg and A.W. Garlington first introduced the term “bacterial translocation” in 1979. The immune system of a healthy body quickly responds to the invasion of pathogenic microorganisms, thereby preventing their migration from the intestine into the systemic circulation [10]. Since severely ill patients are usually accompanied by systemic immunodeficiency or immunosuppression, the immune system is not able to eliminate pathogenic bacteria, and it leads to uncontrolled BT. In clinical practice, the study of the BT phenomenon is difficult, due to the inability to study the material of various tissues and organs for bacteriological research. Moreover, this method has low sensitivity, allows determining for the most part the aerobic flora and can give false negative results [11]. Therefore, other reliable BT markers are needed, the search for which can be carried out in this clinical study.
Today there are several methods for detecting BT:
1. direct method – the detection of 16s rRNA (ribosomal ribonucleic acid) in mesenteric lymph nodes (MLN);
2. indirect method - the detection of serum lipopolysaccharide-binding protein (LBP) and presepsin (Soluble CD14 subtype or sCD14-ST).
Mesenteric lymph nodes and vessels are probably the most important pathway for bacteria to spread from the intestines to the blood and other organs. Since MLN is usually sterile, the presence of viable bacteria in them is a marker of increased permeability of the intestinal barrier and bacterial translocation [12, 13].
LBP plays an important role in recognizing the main component of the bacteria outer cell wall - lipopolysaccharide (LPS). LBP sensitizes macrophage, monocyte and neutrophil receptors to LPS

of bacteria, thereby activating the inflammatory signaling pathway [14, 15]. A number of studies have shown that LBP is a reliable biomarker of microbial translocation and sepsis [16, 17].
sCD14-ST has a high affinity for the lipopolysaccharides of the cell walls of gram-negative bacteria and peptidoglycans of gram-positive bacteria [18,19]. Presepsin has been identified as a biomarker of the early phase of sepsis and its level is a significant prognostic factor in outcomes in patients with sepsis [20, 21, 22].
When analyzing the studies in the publication databases, it was found that more than 60% of the publications about BT is experimental, and most of the clinical studies were conducted in patients with HIV infection and cirrhosis. BT in ABO is still not well understood, as is the role of microbial translocation in the development of SIRS and infectious and inflammatory complications. The questions of the diagnostic value of the proposed biomarkers, their study in dynamics, as well as the relationship between direct and indirect BT markers, remain unresolved. These unresolved problems require further in-depth study. Therefore, for the early diagnosis of infectious and inflammatory complications, it is necessary to study LBP, sCD-14 and 16sRNA as BT markers in patients with malignant and benign ABO, as well as in patients after planned surgical intervention for colon tumors. Based on changes in BT biomarkers in the blood serum, it's suggested that patients with researched pathology can be stratified according to the risk level of developing infectious and inflammatory complications. It will be possible to take preventive measures to reduce the frequency and severity of these complications and mortality.

**II. Criteria for the selection of research participants**

1. Number of participants: 150 participants.
2. Sex: all.
3. Gender based: no.
4. Age: from 18 years old.
5. Nationality: the racial and ethnic distribution of the participants is unimportant, so people with different nationalities and ethnicities will be included in the study.
6. Criteria for inclusion: - patients with malignant acute bowel obstruction,
   - patients with benign acute bowel obstruction,
   - colorectal cancer patients without acute bowel obstruction (planned operations).
8. Criteria for exclusion: - age less than 18,
   - pregnancy,
   - patients with paralytic acute bowel obstruction,
   - patients with HIV infection, liver cirrhosis,
   - patient with an infectious process due to another pathology.

**III. Methods and procedures**

**Methods**

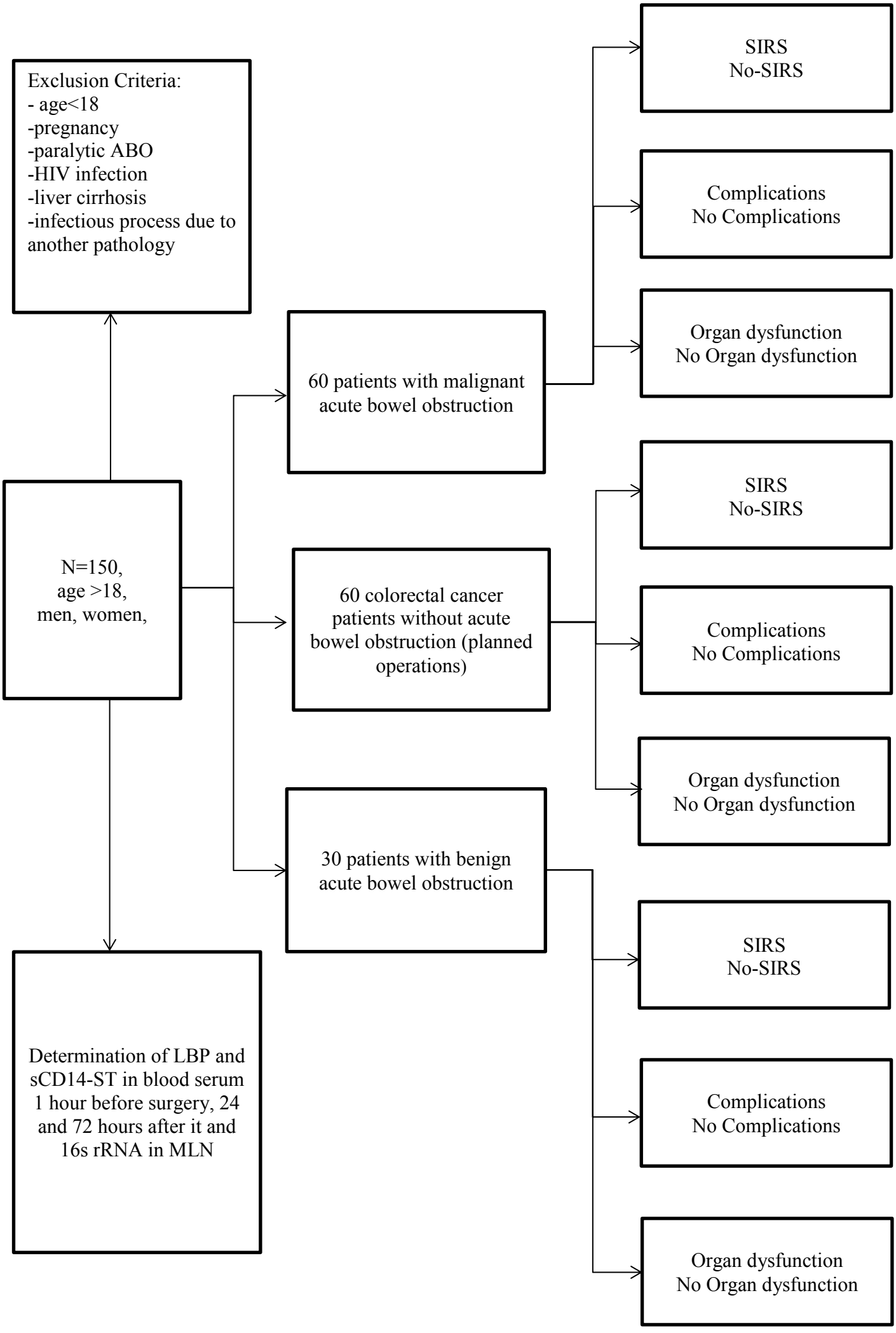
The study materials are blood serum and (MLN).
Venous blood sampling will be performed 1 hour before surgery, 24 and 72 hours after it. Venous blood will be collected in 5 ml vacutainers with a coagulation activator and a serum gel separator. It will be centrifuged for 20 minutes at 1000 x g, after which the gel completely separates the serum from the clot, forming a tight barrier. The obtained sample of freshly prepared serum will be stored at $-20^0$ to $-80^0$C for up to 2 mesenteric lymph nodes months, in order to avoid any loss of biological activity and contamination. No repeated freeze/thaw cycles is allowed. ELISA Kit for Lipopolysaccharide Binding Protein (LBP, Human) and for Presepsin (sCD14-ST, Human), from Cloud-Clone Corp. will be used to determine any presence of LBP and sCD14-ST. The analysis will be performed according to the manufacturer's instructions for an ELISA EVOLIS robotic system from BioRad.

The operating surgeon will perform a MLN sampling in sterile conditions during surgery after resection of the intestine from the mesentery of the gross specimen. MLN will be placed in a sterile tube without any fillers. The DNA will be extracted by the GeneJET Genomic DNA Purification Kit manufactured by Thermo Fisher Scientific, USA, in accordance with the manufacturer's instructions. The 16s rRNA bacteria in MLN will be detected by using real-time PCR and BIO-RAD CFX96 amplifier with 16s rRNA direct and reverse primers (U16SRT-F FACTCCTACGGGAGGGAGGCAGGT and U16SRT-R TATTACCGCGGCTGCTGGGC).

During the implementation, the resources of the Collective Use Laboratory of Research Center NJSC "Karaganda Medical University" will be used.

| **Arms** | **Assigned Interventions** |
|---|---|
| Group 1<br>60 patients with malignant acute bowel obstruction | Diagnostic Test: LBP<br>Determine any presence of LBP in blood serum by ELISA method 1 hour before surgery, 24 and 72 hours after it.<br>Diagnostic Test: sCD14-ST<br>Determine any presence of sCD14-ST in blood serum by ELISA method 1 hour before surgery, 24 and 72 hours after it.<br>Diagnostic Test: 16s rRNA<br>Determine any presence of 16s rRNA in mesenteric lymph nodes by PCR method. |
| Group 2<br>60 colorectal cancer patients without acute bowel obstruction (planned operations) | Diagnostic Test: LBP<br>Determine any presence of LBP in blood serum by ELISA method 1 hour before surgery, 24 and 72 hours after it.<br>Diagnostic Test: sCD14-ST<br>Determine any presence of sCD14-ST in blood serum by ELISA method 1 hour before surgery, 24 and 72 hours after it.<br>Diagnostic Test: 16s rRNA<br>Determine any presence of 16s rRNA in mesenteric lymph nodes by PCR method. |
| Group 3<br>30 patients with benign acute bowel obstruction | Diagnostic Test: LBP<br>Determine any presence of LBP in blood serum by ELISA method 1 hour before surgery, 24 and 72 hours after it.<br>Diagnostic Test: sCD14-ST<br>Determine any presence of sCD14-ST in blood serum by ELISA method 1 hour before surgery, 24 and 72 hours after it.<br>Diagnostic Test: 16s rRNA<br>Determine any presence of 16s rRNA in mesenteric lymph nodes by PCR method. |

All groups will be stratified in subgroups by the presence or absence of postoperative infectious and inflammatory complications, systemic inflammatory response syndrome (SIRS) and organ dysfunctions according to the SOFA criteria (Sequential Organ Failure Assessment).


Exclusion Criteria:
- age<18
-pregnancy
-paralytic ABO
-HIV infection
-liver cirrhosis
-infectious process due to another pathology
N=150,
age >18,
men, women,
60 patients with malignant acute bowel obstruction
60 colorectal cancer patients without acute bowel obstruction (planned operations)
30 patients with benign acute bowel obstruction
SIRS
No-SIRS
Complications
No Complications
Organ dysfunction
No Organ dysfunction
SIRS
No-SIRS
Complications
No Complications
Organ dysfunction
No Organ dysfunction
SIRS
No-SIRS
Complications
No Complications
Organ dysfunction
No Organ dysfunction
Determination of LBP and sCD14-ST in blood serum 1 hour before surgery, 24 and 72 hours after it and 16s rRNA in MLN

ABO- acute bowel obstruction; HIV - human immunodeficiency virus; LBP - lipopolysaccharide-binding protein; MLN - mesenteric lymph nodes; N – number of participants; sCD14-ST - Soluble CD14 subtype (presepsin); SIRS - systemic inflammatory response syndrome.

**Primary Outcome Measures:**
Postoperative infectious and inflammatory complications.

**Secondary Outcome Measures:**
- LBP level in serum blood.
LBP levels (1 hour before surgery, 24 and 72 hours after it) will be compared between groups/ subgroups and in each group/subgroup in dynamic.
- sCD14-ST level in serum blood.
sCD14-ST levels (1 hour before surgery, 24 and 72 hours after it) will be compared between groups/ subgroups and in each group/subgroup in dynamic.
-16s rRNA in mesenteric lymph nodes.
Presence or absence of 16s rRNA in mesenteric lymph nodes will be compared between groups/subgroups.

**Study plan**

| **Scope and summary of the work** | **Deadline** |
|---|---|
| **Theoretical work** | |
| Preparation of articles (abstracts) for publication with a literature review and analysis of the data obtained on the problem under study | During the execution of research work |
| **Experimental work** | |
| Set of material for research | During the execution of research work |
| Statistical analysis | During the execution of research work |
| **Design of research work** | |
| Completion of the writing of the main sections of the research work, registration of the study, publication | November 2021 - October 2023 |

**Data analysis and monitoring**
The statistical analysis will be carried out using IBM SPSS Statistics 20.0 program. The data will be presented as mean (M), standard deviation (SD), median ($M_e$) and interquartile range (IQR). Testing of statistical hypotheses for dependent groups (between marker values before and after surgery on the 1st and 3rd day in each of the groups) will be carried out using the nonparametric Wilcoxon T-test. For independent groups, statistical hypotheses will be tested using the nonparametric Kruskal-Wallis test. To compare the decrease or the increase in markers levels in dynamic, depending on the presence of SIRS and postoperative complications, the Fisher exact test will be used. To determine the critical values of biomarkers and determine the sensitivity and specificity of the tests, ROC analysis will be applied. To identify the correlation relationship, the Spearman correlation coefficient will be calculated. Results at $p < 0.05$ will be considered statistically significant. Moreover, $\alpha = 0.05$, $1-\beta = 80\%$.

**Data storage and confidentiality**
Confidentiality will be ensured by encrypting the personal data of the participants with a digital code. The storage of information about the participants will be carried out in writing in the registration journal of the research topic, in electronic form on a computer in the databases of Microsoft Excel and SPSS Statistics 20.0. The supervisor and performer of the research will have access to the data. The research results, without specifying personal data, will be used for writing a doctoral dissertation, the research results will be published in print and electronic publications

and reported at conferences.

**IV. Assessment of the risk / benefit ratio**

1. Degree of risk. In a study, the benefit outweighs the risk.
2. Potential risk. In a study, the benefit outweighs the risk.
3. Protection against risk. In a study, the benefit outweighs the risk.
4. Potential benefits for the participant. The results of the study can be applied in surgical practice in predicting infectious and inflammatory complications in acute bowel obstruction.
5. Alternatives for the participant. The patient has the right to refuse the proposed diagnostic method. The patient cannot be included in the study without written consent.

**V. Identification of research participants, recruitment and consent**

Before each examination, the participant is explained the goals and objectives of the examination, the examination procedure is brought up, his right to refuse the examination is given, and a written consent to the examination is obtained.

1. Methods for identifying participants and their recruitment. Study participants will be sampled according to the inclusion / exclusion criteria.
2. The process of obtaining consent. The process of obtaining consent will be carried out by the researcher in the form of written consent after explaining to the subject the objectives and methods of the study, as well as all potential risks.
3. The state of the participant. If not all participants are able to give informed consent, then they will not be included in the study.
4. Understanding. Before each examination, the patient is explained the goals and methods of the study, potential risks, the examination procedure and his right to refuse the examination are explained, and written consent to the study is obtained.
5. Consent Forms. An informed consent form will be drawn up according to the recommendations of the Bioethics Committee.
6. Documenting consent. The responsible executor is responsible for obtaining and documenting IP from all entities.
7. Participation price. The examinations are free of charge for the participants, since this study is carried out within the framework of the research work granted by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (Grant No. AP09260597).
8. Participation fee is not provided.

**VI. References:**


1. Gore RM, Silvers RI, Thakrar KH, et al. Bowel obstruction // Radiologic Clinics of North America. – 2015. V.53(6). – P.1225–40.
2. Taylor MR, Lalani N. Adult small bowel obstruction // Academic Emergency Medicine. - 2013. V.20. – P.528–544.
3. Roses RE, Folkert IW, Krouse RS. Malignant Bowel Obstruction // Surgical Oncology Clinics of North America. - 2018. doi:10.1016/j.soc.2018.05.010.
4. Shwaartz C, Fields AC, Prigoff JG, Aalberg JJ, Divino CM. Should patients with obstructing colorectal cancer have proximal diversion? // The American Journal of Surgery. -2017. – V.213(4). – P. 742–747. doi:10.1016/j.amjsurg.2016.08.005.
5. Chiu H-C, Lin Y-C, Hsieh H-M, Chen H-P, Wang H-L, Wang J-Y. The impact of complications on prolonged length of hospital stay after resection in colorectal cancer: A retrospective study of Taiwanese patients // Journal of International Medical Research. – 2017. – V.45(2). – P.691–705. doi:10.1177/0300060516684087.
6. Simillis C, Kalakouti E, Afxentiou T, Kontovounisios C, Smith JJ, Cunningham D, Adamina M, Tekkis PP. Primary Tumor Resection in Patients with Incurable Localized or Metastatic Colorectal Cancer: A Systematic Review and Meta-analysis // World Journal of Surgery. – 2019 Jul. – V.43(7). P.1829-1840. doi: 10.1007/s00268-019-04984-2.

7. Wancata LM, Abdelsatta ZM, Suwanabol PA, Campbell DA, Hendren S. Outcomes After Surgery for Benign and Malignant Small Bowel Obstruction // Journal of Gastrointestinal Surgery. – 2016. – V.21(2). – P. 363–371. doi:10.1007/s11605-016-3307-8.
8. Stubljar D, Skvarc M. Effective Strategies for Diagnosis of Systemic Inflammatory Response Syndrome (SIRS) due to Bacterial Infection in Surgical Patients // Infection Disorder Drug Targets. – 2015. –V.15(1). - P.53- 56.
9. Flexner S. Peritonitis caused by the invasion of the micrococcus Lanceolatus from the intestine // Johns Hopkins Hospital bulletin. – 1895. – V.6. – P.64-67.
10. Levy M, Kolodziejczyk AA, Thaiss CA, Elinav E. Dysbiosis and the immune system // Nature Reviews Immunology. - 2017. – V.17. – P.219-232.
11. Piton G, Capellier G. Biomarkers of gut barrier failure in the ICU. // Current Opinion in Critical Care. - 2016 Apr. – V.22(2). – P.152-160.
12. Tsujimoto H, Ono S, Mochizuki H. Role of Translocation of Pathogen-Associated Molecular Patterns in Sepsis // Digestive Surgery. – 2009. – V.26(2). - P.100–109. doi:10.1159/000206143.
13. MacFie J, Reddy BS, Gatt M, et al: Bacterial translocation studied in 927 patients over 13 years // British Journal of Surgery. – 2006. – V.93. – P.87–93.
14. Fang L, Xu Z, Wang GS. Directed evolution of an LBP/ CD14 inhibitory peptide and its anti-endotoxin activity // PLoS One. – 2014. – V.9(7). - e101406.
15. Stehle JR, Leng X, Kitzman DW, Nicklas BJ, Kritchevsky SB, High KP. Lipopolysaccharide-Binding Protein, a Surrogate Marker of Microbial Translocation, Is Associated With Physical Function in Healthy Older Adults // The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. - 2012. – V.67(11). – P.1212–1218. doi:10.1093/gerona/gls178.
16. Kell DB, Pretorius E. On the translocation of bacteria and their lipopolysaccharides between blood and peripheral locations in chronic, inflammatory diseases: the central roles of LPS and LPS-induced cell death // Integrative Biology. – 2015. – V.7(11). – P.1339–1377. doi:10.1039/c5ib00158g.
17. Mierzchala M, Krzystek-Korpacka M, Gamian A, Durek G. Quantitative indices of dynamics in concentrations of lipopolysaccharide-binding protein (LBP) as prognostic factors in severe sepsis/septic shock patients — Comparison with CRP and procalcitonin // Clinical Biochemistry. -2011. – V.44(5-6). – P.357–363. doi:10.1016/j.clinbiochem.2011.01.012.
18. Mussap M, Noto A, Fravega M, Fanos V. Soluble CD14 subtype presepsin (sCD14-ST) and lipopolysaccharide binding protein (LBP) in neonatal sepsis: new clinical and analytical perspectives for two old biomarkers // The Journal of Maternal-Fetal & Neonatal Medicine. -2011. – V.24(2). – P.12–14.
19. Iris van Maldeghem, Charlotte M, Douwe H. Visser Nusman. Soluble CD14 subtype (sCD14-ST) as biomarker in neonatal early-onset sepsis and late-onset sepsis: a systematic review and meta-analysis // BMC Immunology. - 2019. doi: 10.1186/s12865-019-0298-8.
20. Hosomi S, Yamagami H, Itani S, Yukawa T, Otani K, Nagami Y, Tanaka F, Taira K, Kamata N, Tanigawa T, Shiba M, Watanabe T, Fujiwara Y. Sepsis Markers Soluble IL-2 Receptor and Soluble CD14 Subtype as Potential Biomarkers for Complete Mucosal Healing in Patients With Inflammatory Bowel Disease // Journal of Crohn's and Colitis. - 2018 Jan. – V.5:12(1). – P.87-95. doi: 10.1093/ecco-jcc/jjx124.
21. Masson S, Caironi P, Fanizza C, Thomae R, Bernasconi R, Noto A, Oggioni R, Pasetti GS, Romero M, Tognoni G, Latini R, Gattinoni L. Circulating presepsin (soluble CD14 subtype) as a marker of host response in patients with severe sepsis or septic shock: data from the multicenter, randomized ALBIOS trial // Intensive Care Medicine. - 2015 Jan. – V.41(1). – P.12-20. doi: 10.1007/s00134-014-3514-2. Epub 2014 Oct 16.
22. Endo S, Suzuki Y, Takahashi G, Shozushima T, Ishikura H, Murai A, Okamura, Y. Presepsin as a powerful monitoring tool for the prognosis and treatment of sepsis: A multicenter

prospective study // Journal of Infection and Chemotherapy. – 2014. V.20(1). – P.30–34. doi:10.1016/j.jiac.2013.07.005.