**Aim of the study:** In this pilot study lipopolysaccharide-binding protein (LBP) levels were assessed as a possible risk factor for development of systemic inflammatory response syndrome (SIRS) and infectious and inflammatory complications in colorectal cancer (CRC) patients after surgery.

**Material and methods:** For LBP determination venous blood was taken 1 hour before the surgery and 72 hours after it. All patients were stratified by the presence or absence of acute bowel obstruction (ABO), SIRS and complications.

**Results:** 36 patients with CRC participated in the study. The LBP level before surgery was 879.8 ± 221.8 ng/ml (interquartile range (IQR) 749.3–1028.8); on the 3rd day it was 766.5 ± 159.4 ng/ml (IQR 669.5–847.6), which was a statistically significant decrease (p = 0.004). A decrease in LBP level by more than 280 ng/ml increases the probability of SIRS and complications in operated CRC patients (OR 6.6, 95% CI: 1.1–40.9 and OR 12.0, 95% CI: 1.8–80.4, respectively). In patients with ABO in the presence of SIRS, the LBP value decreased more than in those without SIRS (p = 0.046).

**Conclusions:** This study demonstrated that the LBP level in the operated CRC patients tends to decrease on the 3rd day after surgery. A bigger decrease in LBP level increases the probability of SIRS and postoperative infectious and inflammatory complications. Therefore, further studies with larger numbers of patients are required.

**Key words:** lipopolysaccharide-binding protein, colorectal cancer, bowel obstruction, bacterial translocation, SIRS, complications.

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**Lipopolysaccharide-binding protein as a risk factor for development of infectious and inflammatory postsurgical complications in colorectal cancer patients**

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**Introduction**

Colorectal cancer (CRC) is one of the major public health problems worldwide. It is one of the most common malignant diseases and one of the most important causes of cancer-related mortality (4th place in the world) [1]. Over 66% of CRC patients are urgently admitted due to complications [2]. Acute bowel obstruction (ABO) is the most common complication, which accounts for 80–85% of emergency surgical admissions in these patients [3]. There are still high rates of postoperative infectious and inflammatory complications (46–50% of cases) and mortality (up to 32% of cases) [4, 5].

These days, bacterial translocation (BT) takes one of the main roles in the development of infectious and inflammatory complications. Bacterial translocation is the invasion of intestinal bacteria and bacterial endotoxins through the intestinal mucosa into the systemic circulation and normally sterile tissues and internal organs afterwards [6]. Thus, for the early diagnosis of such complications, as well as the detection of BT, some appropriate markers in the blood serum are to be determined. One such marker is lipopolysaccharide-binding protein (LBP).

Lipopolysaccharide-binding protein is an acute-phase protein, which is responsible for transporting endotoxin (lipopolysaccharide – LPS) to immune effector cells (macrophages, monocytes, and neutrophils). Also, LBP binds to other bacterial products such as glycolipids and lipopeptides [7]. After binding to CD14 on the surfaces of immune effector cells, the LBP/LPS complex activates the inflammatory signaling pathway [8, 9]. The production and release of inflammatory mediators can result in the occurrence of systemic inflammatory response syndrome (SIRS) [10].

Today LBP research in CRC patients is of interest to scientists around the world. The research is not only focused on inflammatory response indicators, but also on risk factors for development of CRC. In the study conducted by Chen et al., the functional G allele in the LBP rs2232596 single nucleotide polymorphism showed a significant association with high risk of CRC [11]. On the other hand, Citronberg’s study did not find an association between LBP and CRC, or any association with body mass index (BMI), saturated fat intake, dietary fat intake, cancer site, or cancer condition [12]. However, in Cibulska-Stopa’s study, a higher BMI is associated with a statistically significantly better prognosis in patients with CRC [13].

Currently, anti-inflammatory therapy is considered not only for the treatment of infectious-inflammatory complications, but also as part of antican-
Lipopolysaccharide-binding protein as a risk factor for development of infectious and inflammatory postsurgical complications in colorectal cancer patients.

Cancer therapy. Therefore, numerous scientists are exploring the role of various inflammatory factors and their dependence on cancer staging. One study used the Human Circulation Biomarker panel, which included a large number of inflammatory markers: interleukin (IL)-6, IL-8, macrophage migration inhibition factor, fibroblast growth factor (FGF-2), stem cell factor (SCF), transforming growth factor (TGf), tumor necrosis factor (TNF), TNF apoptosis-inducing ligand (TRAIL). Only IL-6 demonstrated a substantial increase, but there was no dependence on CRC stage [14].

As for the study of LBP any alterations in the intestinal mucosa in CRC patients contribute to the translocation of bacteria and their endotoxins into the systemic circulation [15]. Since LPS and bacterial endotoxins trigger the production of LBP, it can be a reliable biomarker for endotoxemia associated with barrier dysfunction and BT [16, 17]. LBP concentrations in serum are suggested to be assessed as a valid marker of BT, SIRS and systemic infectious complications [18–21].

The aim of this pilot study is to analyze the dynamic changes in LBP level in blood serum and to assess it as a possible risk factor for the development of SIRS as well as infectious and inflammatory postsurgical complications in CRC patients.

Material and methods

Patients’ records. The study was conducted from September 2020 to February 2021 in two hospitals in Karaganda, Kazakhstan (Multidisciplinary Hospital No. 1 and No. 3). This pilot study included 36 patients with CRC (15 males and 21 females aged 38–89 years). The median age was 65.7 ± 12.4 years (interquartile range (IQR) 60–75 years). By localization, sigmoid colon cancer prevailed in men (7 patients – 47%), sigmoid colon cancer (8 patients – 38%) and colon cancer (7 patients – 33%) in women. All patients’ medical records are shown in Table 1. Patients under the age of 18, pregnant women, those having liver cirrhosis or HIV infection, and those who refused to participate were excluded. All patients underwent clinical, instrumental and laboratory research methods in hospitals in accordance with clinical protocol No. 60 “Acute bowel obstruction” issued by the Ministry of Health of the Republic of Kazakhstan. The clinical diagnosis was made after the surgery and on the basis of the obtained historical results. The presence of SIRS was assessed by the occurrence of at least two of the following criteria: fever > 38.0°C or hypothermia < 36.0°C, leukocytosis > 12×10^9/l or leucopenia < 4×10^9/l, tachycardia > 90 beats/minute, tachypnea > 20 breaths/minute. Venous blood was taken 1 hour before the surgery and 72 hours after it (on the 3rd day) to determine the LBP level. To compare the change in the LBP level on the 3rd day, the patients were divided into two groups with either a decrease or an increase of LBP on the 3rd day. To evaluate the association of LBP levels with the presence of ABO and SIRS, all patients were stratified by the presence or absence of ABO, SIRS or no-SIRS.

Blood collection

Venous blood was sampled 1 hour before the surgery and on the 3rd day after it. Venous blood was collected in 5 ml vacutainers with a coagulation activator and a serum gel separator. It was centrifuged for 20 minutes at 1000 x g, after which the gel completely separated the serum from the clot, forming a tight barrier. The obtained sample of freshly prepared serum was stored at –20°C to –80°C for up to 2 months, in order to avoid any loss of biological activity and contamination. No repeated freeze/thaw cycles were allowed.

Lipopolysaccharide-binding protein determination

ELISA Kit for LBP (Human) from Cloud-Clone Corp. was used to determine any presence of LBP. The analysis was performed according to the manufacturer’s instructions for an ELISA EVOLIS robotic system from BioRad. The microplate provided in this kit was pre-coated with biotin-conjugated antibodies specific for LBP. The standards and patient serum samples were added to appropriate wells of the microplate and incubated at 37°C. Subse-

| Table 1. General medical records of the patients |
| --- |
| **Age** | 65.7 ± 12.4 years (IQR 60–75 years) |
| **Gender** | |
| Male | 15 (41.7%) |
| Female | 21 (58.3%) |
| **Cancer staging** | |
| I | 5 (13.9%) |
| II | 17 (47.2%) |
| III | 9 (25.0%) |
| IV | 5 (13.9%) |
| **Tumor localization** | |
| Rectum | 5 (13.9%) |
| Sigmoid junction | 3 (8.3%) |
| Sigmoid colon | 15 (41.7%) |
| Colon | 10 (27.8%) |
| Cecum | 3 (8.3%) |
| **ABO on admission** | |
| Absence | 23 (63.9%) |
| Presence | 13 (36.1%) |
| **SIRS** | |
| Absence | 23 (63.9%) |
| Presence | 13 (36.1%) |
| **Complications** | |
| Absence | 26 (72.2%) |
| Presence | 10 (27.8%) |

SIRS – systemic inflammatory response syndrome, ABO – acute bowel obstruction, IQR – interquartile range
sequently, some avidin conjugated with horseradish peroxidase was added to each well and incubated at 37°C once again. After addition of the TMB substrate solution, only those wells containing LBP changed color. These changes were measured spectrophotometrically at a wavelength of 450 nm ± 10 nm. The LBP levels in the samples were then determined by comparing the absorbance of the samples with a standard calibration sample.

Statistical analysis

The number of the examined patients was calculated according to the recommendations for sample calculations for pilot studies [22]. The statistical analysis was carried out using STATISTICA v8.0 (StatSoft). The data are presented as mean (M), standard deviation (SD) and IQR. The Shapiro-Wilk test indicated that LBP values did not follow a normal distribution; consequently, non-parametric statistical tests were applied. The Wilcoxon nonparametric test was used to compare the LBP values before and after the surgery. The comparison between the two independent groups was performed using the Mann-Whitney U test. To compare the decrease or the increase in LBP level on the 3rd day, depending on the presence of SIRS and complications, the Fisher exact test was used. The significance level was set at \( p < 0.05 \).

Results

Measurement of lipopolysaccharide-binding protein level in real-time mode

LBP levels were measured 1 hour before the surgery and 72 hours after it (on the 3rd day). The value of LBP before the surgery was 879.8 ± 221.8 ng/ml (IQR 749.3–1028.8) and 766.5 ± 159.4 ng/ml (IQR 669.5–847.6) on the 3rd day, which was a statistically significant decrease (\( p = 0.004 \)). In patients with ABO, the LBP level before the surgery was 963.8 ± 195.2 ng/ml (IQR 810.7–1044.1) and 808.4 ± 208.4 ng/ml (IQR 644.9–1031.8) on the 3rd day, which was also a statistically significant decrease (\( p = 0.023 \)). In patients without ABO, no statistically significant difference was found. The LBP decreased in 28 patients on the 3rd day, and only 8 patients had an increase in LBP level. 12 patients with ABO and 16 without ABO had a decrease in LBP level with only 1 patient with ABO and 7 patients without ABO demonstrating an LBP increase. There was no statistically significant association between the change in LBP level on the 3rd day and the presence of ABO (\( p = 0.122 \)).

There were no significant differences in gender or LBP levels either before the surgery or after it on the 3rd day (\( p = 0.76 \) and \( p = 0.19 \), respectively). There were 3 men (20%) and 2 women (9%) with stage I, 8 men (53%) and 9 women (43%) with stage II, 4 men (27%) and 5 women (24%) with stage III, 5 women (24%) and no men with stage IV. Furthermore, there were no differences in the cancer stage and LBP levels before and after the surgery on the 3rd day (\( p = 0.49 \) and \( p = 0.75 \), respectively).

Association of lipopolysaccharide-binding protein with clinical presence of systemic inflammatory response syndrome

In no-SIRS patients (\( n = 23 \)), the LBP level before the surgery was 871.9 ± 205.6 ng/ml (IQR 724.7–976.6) and 791.5 ± 156.1 ng/ml (IQR 681.8–933.6) on the 3rd day. In SIRS patients (\( n = 13 \)), the LBP value before the surgery was 893.9 ± 256.3 ng/ml (IQR 786.1–1044.1) and 722.4 ± 161.5 ng/ml (IQR 577.3–792.3) on the 3rd day. It was also found that an LBP level decrease of more than 280 ng/ml increases the probability of SIRS (OR 6.6, 95% CI: 1.1–40.9) (Table 2). In patients with ABO in the presence of SIRS, the LBP value decreased more than in those without SIRS (\( p = 0.046 \)). In 9 patients with SIRS and 19 patients without SIRS, the LBP level decreased on the 3rd day, and only 2 patients with SIRS and 6 patients without SIRS had an increase in the LBP level. There was no significant association between the change in LBP level on the 3rd day and the presence of SIRS (\( p = 0.382 \)).

Association of lipopolysaccharide-binding protein with development of postoperative infection and inflammatory complications

In patients without postoperative complications, the LBP level before the surgery was 876.3 ± 200.3 ng/ml (IQR 767.7–976.6) and 783.2 ± 155.5 ng/ml (IQR 681.8–866) on the 3rd day. In patients with postoperative complications, the LBP value before the surgery was 887.8 ± 275.4 ng/ml (IQR 730.9–111.7) and 728.6 ± 169 ng/ml (IQR 552.8–792.3) on the 3rd day. In 8 patients with complications and 20 patients without them, the LBP level decreased on the 3rd day. Two patients with complications and 6 patients without them had an increase in the LBP level. There was no significant association between the increase/decrease in LBP level on the 3rd day and the presence of complications (\( p = 0.532 \)). It was found that a decrease in LBP level by more than 280 ng/ml increases the probability of postsurgical complications in CRC patients (OR 12.0, 95% CI: 1.8–80.4) (Table 2). There was no difference in the incidence of complications between patients with or without ABO (30.8% and 30.4% respectively).

| Number of patients with a decrease in LBP level by more than 280 ng/ml on the 3rd day after surgery | Number of patients with a decrease in LBP level by less than 280 ng/ml or its increase on the 3rd day after surgery |
|-----------------------------------------------|---------------------------------------------------------------|
| SIRS                                         | 5                                                             |
| no-SIRS                                      | 8                                                             |
| Complications                                | 2                                                             |
| No complications                             | 21                                                            |
| Number of patients with a decrease in LBP level by less than 280 ng/ml or its increase on the 3rd day after surgery | 2                                                                 |
| 5                                                             | 5                                                             |
| 2                                                             | 24                                                            |

LBP – lipopolysaccharide-binding protein, SIRS – systemic inflammatory response syndrome
Lipopolysaccharide-binding protein as a risk factor for development of infectious and inflammatory postsurgical complications in colorectal cancer patients

Discussion

The aim of the study was to analyze the dynamics of the LBP level as a potential risk factor for the development of SIRS and infectious and inflammatory complications in CRC patients. CRC patients still have a high risk of complications in the postoperative period. In this study, infectious and inflammatory complications, including sepsis, occurred in 11 patients (30.6%), sepsis in 3 patients (8.3%), which are quite high rates.

The main cause of postoperative infectious and inflammatory complications in CRC patients is the violation of the intestinal barrier, which occurs at the site of the tumor growth, as the tumor causes dysplasia of the epithelium. In the case of ABO presence, some alterations additionally occur above the obstruction, where disturbances in the microcirculation of the intestinal wall, its ischemia and hypoxia take place. These disorders in the intestinal wall result in translocation of bacteria and their endotoxins into the systemic circulation and production of a large number of pro-inflammatory mediators. The inflammatory response increases, and oxidative stress occurs. These changes contribute to the death of enterocytes and disruption of intercellular tight junctions, which increases the intestinal wall permeability. Bacteria or their endotoxins penetrate the damaged intestinal mucous barrier and further enhance the immune response, which becomes systemic, and ultimately leads to SIRS and infectious and inflammatory complications, including sepsis [23].

Komen et al. [24] analyzed the LBP levels in drain fluid of the operated CRC patients during the first five postoperative days. The LBP levels were higher on days two, three and four. The authors found that an increase from the average initial value at the first postoperative day with one SD increased the risk of colorectal anastomotic leakage by 1.6 times. In Scheepers et al.’s study on patients undergoing laparoscopic open or transthiatal esophagectomy during the observation period, significantly higher plasma LBP levels were observed after laparoscopic surgery ($p = 0.009$). The scientists came to the conclusion that the persistently elevated abdominal pressure resulted in the loss of the intestinal barrier function, leading to BT [25]. In patients with ABO, abdominal pressure is also increased, so BT also occurs with ABO.

In research by Mierzchala et al., the LBP value was assessed in patients with sepsis and septic shock. The serum levels of LBP were significantly higher in the patients with SIRS, sepsis and septic shock, as compared with the levels in the healthy controls. Additionally, the LBP level was higher in non-survivors compared to survivors. High levels of LBP were present at admission and gradually decreased on the following days [21, 26, 27]. On average, the decrease in LBP level in the septic patients was 12.8 mg/l (95% CI: −22.4 to −3.2). A decrease in LBP level over time was observed in 71.3% of survivors and in 38.5% of non-survivors [27]. It should be noted that the LBP response appeared to exhibit a decreased magnitude in the septic patients [26].

In Opal’s study, LBP levels were measured in patients with severe sepsis. The authors also found that the LBP values were elevated in the patients with sepsis compared with the normal control values. Nevertheless, the authors witnessed significantly worse outcomes in the patients with less highly elevated levels of LBP in contrast to the previous studies. The authors concluded that the patients with rapidly progressive sepsis could not adequately synthesize LBP, lacking time to adequately respond to some systemic microbial infection [28].

Previously, in patients with CRC, LBP has not been thoroughly studied in real-time mode in serum plasma, depending on the presence of ABO upon admission or the occurrence of SIRS and infectious and inflammatory complications. This study showed that the pre-operative LBP level in the operated CRC patients was 879.8 ± 221.8 ng/ml (IQR 749.3–1028.8) and 766.5 ± 159.4 ng/ml (IQR 669.5–847.6) on the 3rd day, which was a statistically significant decrease ($p = 0.004$).

In this study, the patients with ABO also had a statistically significant decrease in the LBP concentration ($p = 0.023$). In these patients, in addition to the changes in the intestinal wall due to obstruction and tumor, an increase in the intraabdominal pressure occurs, which increases BT. It can be assumed that the dynamic decrease in the LBP level might be due to the normalization of intraabdominal pressure, elimination of obstruction, and removal of the tumor itself, which all together caused BT.

In previous studies, a difference in the change of the LBP concentration in real-time mode has not been observed anywhere. For the first time ever, this study observed that the decrease in the LBP level by more than 280 ng/ml increased the probability of developing postoperative infectious and inflammatory complications in the operated CRC patients (OR 12.0, 95% CI: 1.8–80.4). Moreover, the most frequent postoperative complication was an anastomotic leakage (6 patients; 16.7%). A combination of several complications was observed in 5 patients, mostly anastomotic leakages and abdominal abscesses. Only 3 patients had sepsis (8.3%). The LBP value before the surgery in the patients with complications ranged from 448.35 to 1308.2 ng/ml, and from 503.6 to 1062.5 ng/ml on the 3rd day, which showed the lowest levels in total in all the patients. Also, the group of patients with complications had the largest decrease in the LBP concentration (448.4 ng/ml). Moreover, such a decrease in the LBP level by more than 280 ng/ml increases the probability of SIRS (OR 6.6, 95% CI: 1.1–40.9).

As in the previous studies, the mean LBP level tended to decrease on the 3rd day. Yet, significantly worse results (the presence of SIRS and complications) were observed in the patients with a lower LBP level on the 3rd day and with a more significant decrease in its dynamics. This is at odds with the previous studies, except for Opal’s study. The LBP may contribute to the protection and elimination of pathogenic bacteria and their endotoxins [29, 30]. According to the previous studies, the LBP level should increase with SIRS and occurrence of infectious and inflammatory complications. However, this study resulted in demonstrating that the patients with lower LBP levels on the 3rd day after the surgery and with a bigger decrease in LBP level are more prone to developing SIRS and infectious and inflammatory complications. As mentioned above,
the alterations in the intestinal wall in the CRC patients result in the translocation of bacteria and their endotoxins into the systemic circulation. One study found that the presence of low endotoxin (LPS) levels in the patients with chronic disease leads to a constant state of low-grade inflammation that prevents the normal healing process [31], which can explain the high rate of postoperative complications in the operated CRC patients. Also, a decrease in the LBP level in the operated CRC patients is possible due to immunodeficiency and inability to produce an appropriate immune response to infectious stimuli, which can lead to a poor prognosis (SIRS and infectious and inflammatory complications). Similar to the study of Citronberg [12], the present study observed that the association of LBP and CRC was not stronger in patients with a later stage. This is a pilot study and hence it has some limitations. The statistical power of the study is limited (36 patients only). Nevertheless, to our best knowledge, this is the first study that assesses the dynamics of LBP levels in operated CRC patients, depending on the presence of ABO upon admission and the occurrence of postoperative infectious and inflammatory complications. Compared to most previous studies, a poor prognosis is observed in the patients with a large decrease in the LBP levels from baseline.

Conclusions

This study demonstrated that the LBP level in the operated CRC patients tends to decrease on the 3rd day after the surgery. A bigger decrease in the LBP level increases the probability of SIRS and postoperative infectious and inflammatory complications. Therefore, further studies with larger numbers of patients are required.

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The study was conducted in accordance with the Ethical Guidelines for Medical Research with Human Subjects as established by the Ministry of Health of the Republic of Kazakhstan and with the guidelines outlined in the Helsinki Declaration and its amendments. This study was approved by the Ethics Committee of the NJSC “Karaganda Medical University” (Protocol No. 6 with the assigned number No. 30). Informed consent was obtained from all participants of the study.

The authors declare no conflict of interests.

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Lipopolysaccharide-binding protein as a risk factor for development of infectious and inflammatory postsurgical complications in colorectal cancer patients

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