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Original Scientific Paper

COMPARISON OF C-REACTIVE PROTEIN LEVELS IN SERUM AND PERITONEAL FLUID IN EARLY DIAGNOSIS OF ANASTOMOTIC LEAKAGE AFTER COLORECTAL SURGERY

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SUMMARY – In colorectal surgery, anastomotic leakage is a serious complication, leading to higher postoperative morbidity and mortality. The aim of this study was to evaluate the accuracy of serum and intraperitoneal C-reactive protein (CRP) in early diagnostics of anastomotic leakage on the first four postoperative days after colorectal surgery. From January to October 2019, fifty-nine patients with colorectal carcinoma were operated on, with formation of primary anastomosis. Anastomotic leakage was diagnosed in eight patients. Comparing the levels of serum and intraperitoneal CRP, our study showed that serum CRP was a better predictor of anastomotic leakage. Serum CRP levels lower than 121 mg/L on postoperative day 4 were predictive of good healing of anastomosis.

Key words: *Colorectal carcinoma; Colorectal surgery; Anastomotic leakage; C-reactive protein*

Introduction

The reality of elective colorectal surgery is the risk of anastomotic leakage after colon resection with primary anastomosis. Despite intensive research of risk factors associated with anastomotic leakage, improvements in surgical tehnique and perioperative management of patients, anastomotic leakage remains a major complication, contributing to higher postoperative morbidity and mortality, higher incidence of local recurrence, and decreased survival in patients with colorectal carcinoma[1,2]. In order to reduce the incidence of morbidity and mortality associated with anastomotic leakage, early detection and appropriate treatment are essential.

Unfortunately, early clinical signs of anastomotic leakage can be very subtle and therefore can cause delay in diagnosis and treatment of anastomotic leakage. Some previous research has shown that serum C-reactive protein (CRP) is a reliable marker for early prediction of anastomotic leakage after colorectal surgery. In most studies, serum CRP levels lower than 124-172 mg/L on postoperative day 3 or 4 were a positive predictive marker of good anastomotic healing[3-6]. In contrast to evaluation of serum CRP, very little research has been done regarding CRP levels in intraperitoneal fluid as a marker of anastomotic leakage.

The aim of this study was to evaluate and compare the utility of serum and intraperitoneal levels of CRP in early diagnostics of anastomotic leakage after colorectal surgery.

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Material and Methods

Patients

This study was a single center, prospective study conducted at the Department of Surgery, Sestre milosrdnice University Hospital Center, Zagreb, between January 2019 and October 2019. Fifty-nine patients with colorectal carcinoma, in which colon resection with primary anastomosis was planned, were included prospectively. Exclusion criteria for this study were age younger than 18 years, neoadjuvant chemoradiotherapy, patients operated in emergency settings, and patients on steroid therapy. All surgical procedures were performed by the same surgical team. All patients underwent bowel prepration a day before surgery and were administered cefuroxim and metronidazole for antibiotic prophylaxis. All anastomoses were performed with stapling devices. An intraperitoneal drain was placed in all patients, near the anastomosis, and it was generally removed after postoperative day 4 except for patients with postoperative complications.

Diagnosis of anastomotic leakage was suspected in patients with clinical signs of peritonitis or with purulent or fecal content within the drain. On clinical suspicion, diagnosis of anastomotic leakage was confirmed by abdominal and pelvic computed tomography scan with intravenous contrast. Other postoperative infectious complications that could lead to elevation of serum levels of CRP were closely monitored, diagnosed and treated if needed, e.g., pneumonia, urinary tract infection, surgical site infection, and central line infection.

Sample collection

Intra-abdominal fluid (from intraperitoneal drain) and blood samples were collected on postoperative day 1 (24 hours after surgery), day 2, day 3 and day 4. Six mL of blood and 9 mL of intraperitoneal fluid were collected for evaluation of CRP levels. Serum and intraperitoneal levels of CRP were measured by latex immunoturbidimetry, in the routine manner, in our hospital laboratory.

Patients were compared by age, gender, preoperative CRP and protein levels, and mean levels of serum and intraperitoneal CRP on postoperative days 1-4.

The study was approved by the institutional review board (Sestre milosrdnice University Hospital Center Ethics Committee). Signed informed consent was obtained from all patients or their legal representative before surgery.

Statistical analysis

Descriptive statistics was used to describe differences between groups and were evaluated based on the χ^2-test for dichotomic variables. In case of normal distribution, they were expressed as mean and standard deviation and analyzed using Student's t-test. If the distribution was not normal, data were analyzed using Mann-Whitney test and presented as median and interquartile range. The repeated-measures ANOVA was used to compare differences between the groups of patients with and without anastomotic leakage at every point of measurement. To test the predictive power of CRP as a marker for anastomotic dehiscence, the receiver operating curve (ROC) analysis was performed. The results of the ROC analysis were presented as area under the curve, specificity, sensitivity, positive and negative predicted values. ROC curves of CRP were used do determine the best cut-off values for anastomotic dehiscence. The level of statistical significance was set at $p<0.05$.

Results

Patient characteristics

A total of 59 patients were enrolled in our study, 27 (45%) of them female and 32 (55%) male. Median age was 68 years, ranging from 25 to 91 years. Right hemicolectomy was performed in 23, left hemicolectomy in 18, and low anterior rectal resection in 18 patients. Eight (13.6%) patients were dignosed with anastamotic leakage. Anastomotic leakage occurred from postoperative day 2 to postoperative day 21. In all of the patients with anastomotic leakage, low colorectal (4 patients) or coloanal (5 patients) anastomosis was performed, and there was no anastomotic leakage reported in patients after right hemicolectomy. Eight out of nine patients with anastomotic leakage had relaparotomy, with drainage of the abscess and converting stoma. In one of the patients, anastomotic leakage resolved with enteral rest and parenteral antibiotics. One of the patients with anastomotic leakage died from septic shock and multiorgan failure (postoperative mortality 1.69%). Anastomotic leakage was reorded in three of 27 (11.1%) female patients and in five of 31 (15.6%) male patients. Considering age, we

divided patients in three groups as follows: <60 years (28.8%), 61-80 age group (50.8%) and >81 (20.3%). Anastomotic leakage occurred in 5.9% of patients aged <60 and in 13.3% of those in the 60-80 age group. In patients older than 81, anastomotic leakage occured in 25% of patients. Median preoperative levels of CRP was 18 mg/L, range 4.5-192 mg/L (normal values <5 mg/L). Preoperative protein levels were from 52 g/L to 84 g/L, with median value of 67 g/L (normal values 66-81 g/L). There was no statistically significant difference between the groups of patients with and without anastomotic leakage according to gender (χ^2=0.255, df=1; p=0.614) (Table 1) and age (χ^2=2.196, df=2; p=0.334) (Table 2). We also evaluated preoperative serum CRP levels and total serum protein levels in patients with and without anastomotic leakage using T-test for independent variables, and there was no statistically significant difference between these two groups of patients. For preoperative CRP levels, p value was 0.756, and for preoperative serum protein level p value was 0.635 (Table 3).

Table 1. Dehiscence and gender crosstabulation

		Gender		
		Male	Female	Total
Dehiscence	Yes	15.6%	11.1%	13.6%
	No	84.4%	88.9%	86.4%
Total		100.0%	100.0%	100.0%

χ^2=0.255, df=1; p=0.614

Table 2. Dehiscence and age crosstabulation

		Age (years)			
		<60	61-80	>81	Total
Dehiscence	Yes	5.9%	13.3%	25.0%	13.6%
	No	94.1%	86.7%	75.0%	86.4%
Total		100.0%	100.0%	100.0%	100.0%

χ2=2.196, df=2; p=0.334

Table 3. Significance of preoperative levels of C-reactive protein and total serum protein in anastomotic leak

	Dehiscence	n	Mean	SD	p
Preoperative CRP level	Yes	8	13.9625	28.80719	0.756
	No	51	18.6314	40.58310	
Preoperative total serum protein	Yes	8	66.00	6.908	0.635
	No	51	67.18	6.421	

CRP = C-reactive protein; SD = standard deviation

C-reactive protein levels in serum and intraperitoneal fluid

Repeated measures ANOVA showed a statistically significant change in serum CRP levels on four postoperative days ($F_{(1.6,55)}$=6.107; p=0.006). There was a statistically significant difference between the two groups of patients ($F_{(1,55)}$=11.071; p=0.002), being higher in the group with anastomotic leakage. Also, there was a statistically significant interaction between the group of patients and change in serum CRP ($F_{(1.6,55)}$=12.743; p<0.001), which could be explained by the increase of serum CRP in patients with anastomotic leakage and decrease in patients without anastomotic leakage (Fig. 1).

Considering intraperitoneal CRP levels, repeated measures ANOVA showed a statistically significant change in intraperitoneal CRP on four postoperative days ($F_{(1.8,53)}$=40.761; p<0.001). There was a statistically significant difference between the two groups of patients ($F_{(1,53)}$=5.068; p=0.029), being higher in the group with anastomotic leakage. Also, there was a statistically significant interaction between the group of patients and change in intraperitoneal CRP ($F_{(1.8,53)}$=14.162; p<0.001), which could be explained by stronger increase of intraperitoneal CRP levels in

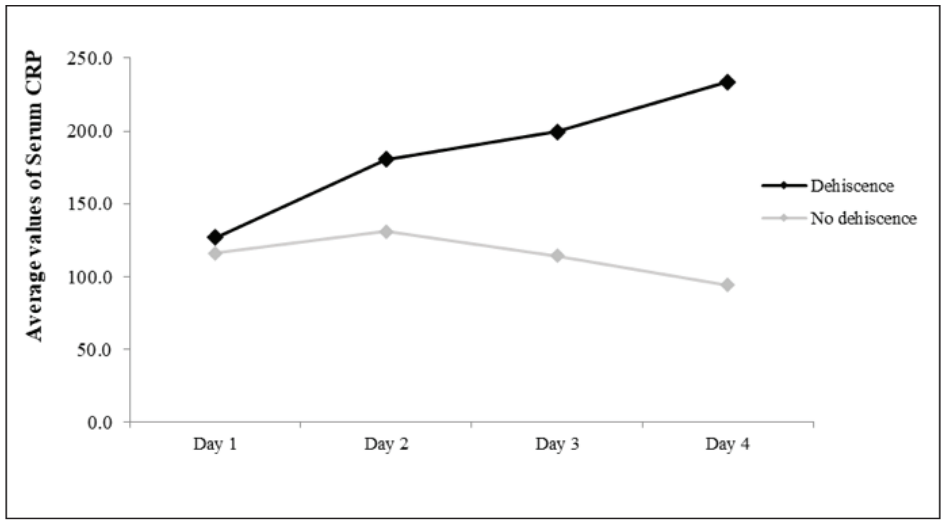

Fig. 1. Mean values of serum C-reactive protein (CRP) on four postoperative days in patients with and without dehiscence.

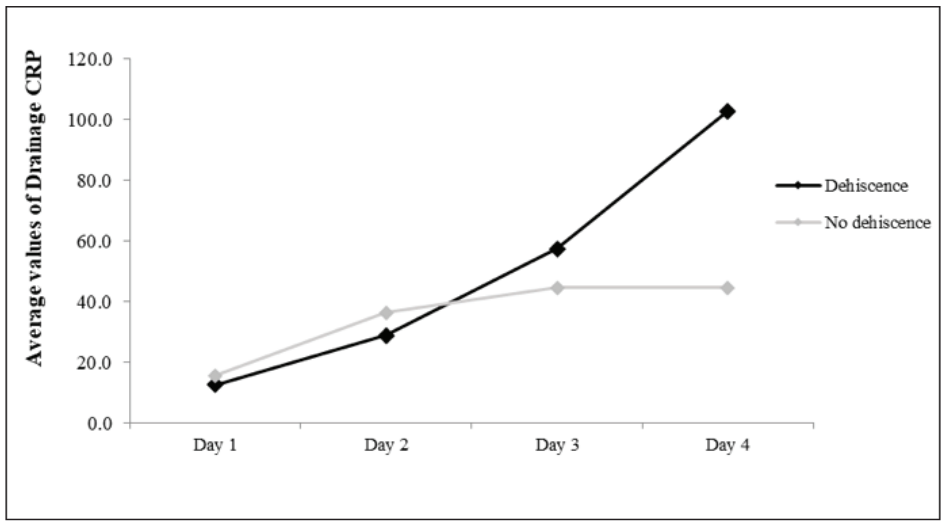

Fig. 2. Mean values of drainage C-reactive protein (CRP) on four postoperative days in patients with and without dehiscence.

patients with anastomotic leakage, and rather small increase (in plateauing) in patients without anastomotic leakage (Fig. 2).

Predictive value of C-reactive protein levels in serum and intraperitoneal fluid

A ROC analysis was performed to evaluate accuracy of serum and intraperitoneal fluid CRP levels as markers for prediction of anastomotic leakage. For serum CRP, statistically significant values were detected on postoperative days 2, 3 and 4. Most accurate in diagnosis of anastomotic leakage was serum CRP level on postoperative day 4. If a patient had a serum CRP level of 121 and higher on the postoperative day 4, there was a 85.7% chance of anastomotic leakage (Fig. 3, Table 4).

For intraperitoneal fluid CRP, only levels on postoperative day 4 were important in detecting anastomotic leakage, with accuracy of 71.4% of predicting anastomotic leakage with CRP levels of 55.20 and higher (Fig. 4, Table 5).

To determine which of the CRP measurements (serum or intraperitoneal fluid) is a better predictor

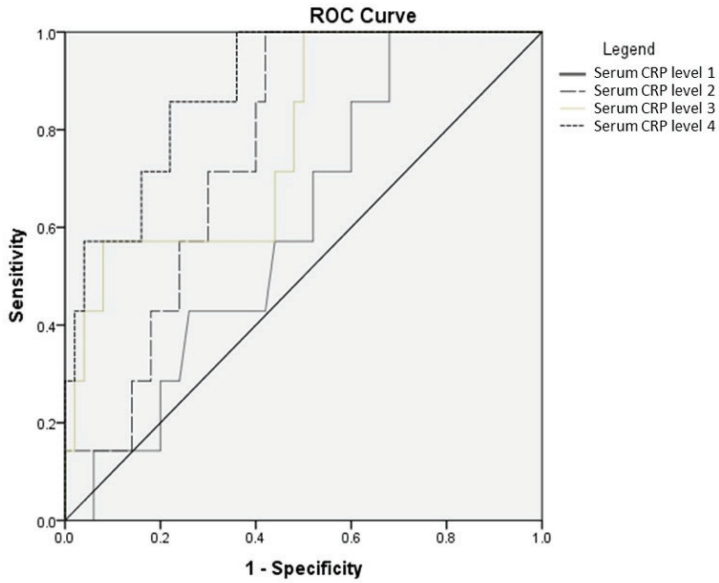

Fig. 3. Diagnostic accuracy of serum C-reactive protein (CRP) values in detecting anastomotic dehiscence (receiver operating curve).

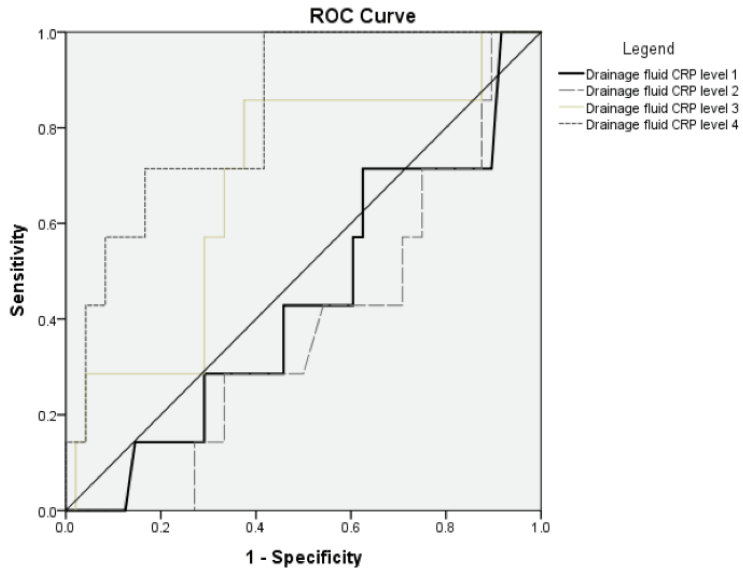

Fig. 4. Diagnostic accuracy of drainage C-reactive protein (CRP) values in detecting anastomotic leakage (receiver operating curve).

of anastomotic leakage, a binary logistic regression model was used. It showed that on postoperative day 4, serum CRP levels were statistically significant in predicting anastomotic leakage. On postoperative day 4, patients with serum CRP higher than 121 mg/L (cut-off value) had 67 times more chance of having an anastomotic leakage than patients with lower serum CRP levels (OR=67.383, 95% CI 2.482-1829.202) (Table 6).

Discussion

The incidence of anastomotic leakage after restorative colon and rectal surgery varies between 2% and 7%, depending on the site of colonic anastomo-

Table 4. Cut-off values, sensitivity, specificity and area under curve with corresponding confidence interval for serum CRP by postoperative days

	Cut-off value	Sensitivity	Specificity	AUC	95% CI	P
Serum CRP 1	112.85	62.5	56.9	0.609	0.423-0.794	0.356
Serum CRP 2	147.05	75.0	66.7	0.760	0.622-0.898	**0.027**
Serum CRP 3	121.55	57.1	56.0	0.777	0.600-0.954	**0.018**
Serum CRP 4	121.00	85.7	78.0	0.886	0.776-0.996	**0.001**

AUC = area under curve; CI = confidence interval; CRP = C-reactive protein

Table 5. Cut-off values, sensitivity, specificity and area under curve with corresponding 95% confidence interval for intraperitoneal CRP by postoperative days

	Cut-off value	Sensitivity	Specificity	AUC	95% CI	p
Drainage CRP 1	9.90	37.5	44.0	0.439	0.219-0.659	0.605
Drainage CRP 2	31.10	37.5	41.2	0.378	0.180-0.576	0.300
Drainage CRP 3	45.75	71.4	67.3	0.682	0.469-0.894	0.123
Drainage CRP 4	55.20	71.4	71.4	0.833	0.693-0.974	**0.005**

CRP = C-reactive protein; AUC = area under curve; CI = confidence interval

Table 6. Serum and intraperitoneal CRP predicting dehiscence in a binary logistic regression model

		p	OR	95% CI	OR
	sCRP (1)	**0.012**	67.383	2.482	- 1829.202
	dCRP (1)	0.255	0.170	0.008	- 3.605
a. Variable(s) entered on step 1: scrp4.r, dcrp4.					

sCRP = serum C-reactive protein; dCRP = intraperitoneal C-reactive protein; CI = confidence interval; OR = odds ratio

sis[7-9]. Ileocolic anastomosis has the lowest incidence of anastomotic leakage (1%-3%), whereas coloanal anastomosis has the highest incidence, ranging from 10% to 20%[10]. Anastomotic leakage leads to higher morbidity and mortality of patients, higher permanent stoma rates, and increased local recurrence rates[1,2]. The leading cause of postoperative death after colorectal surgery is anastomotic leakage, with mortality rates of 6%-22%[11]. In our study, mortality rate after anastomotic leak was 1.69% (one out of 59 patients) and there were no postoperative deaths in the group of patients without anastomotic leakage. Some reports have shown that a delayed diagnosis of anastomotic leakage, after postoperative day 5, is associated with higher mortality rates in comparison with anastomotic leakage treated earlier[12].

In our study, we aimed to determine if repetitive measurement of serum and intraperitoneal levels of CRP could be a good predicitve marker for early diagnosis of anastomotic leakage and to determine which of these measurements (serum or intraperitoneal) was more accurate in predicting anastomotic leakage.

Every major surgical procedure induces systemic stress response, which includes a wide range of metabolic, endocrinologic, immune and hematologic effects. From the site of the injury activated leukocytes, fibroblasts and endothelial cells produce various cytokines. Some of the most important cytokines are in-

terleukin-1, interleukin-6 and tumor necrosios factor alpha. The main cytokine responsible for inducing the acute phase response is interleukin-6, secreted by activated leukocytes and neutrophils from the site of surgery. One of the features of acute phase response is production of acute phase proteins in the liver, such as CRP, fibrinogen, alpha 2 macroglobulin, and other anti-proteases[13,14]. The physiological role of CRP is to bind to lysophosphatidylcholine expressed on the surface of the dead or dying cells and some type of bateria in order to activate complement system, promoting phagocytosis by macrophages[15]. In healthy adults, the normal concentracion of CRP is lower than 5.0 mg/L. After stimulus, serum CRP levels start to rise very quickly (6 hours after stimulus) and peak at 48 hours. The plasma half-life of CRP is 19 hours, it is constant in all medical conditions, and the only factor that determines serum levels of CRP is the rate of its production. Without stimulus, serum levels of CRP start to decline very quickly. From prevoius studies, we knew that repetitive measurement of serum CRP could be beneficial for surgeons in an attempt to diagnose anastomotic leakage earlier, before evident clinical signs, but we did not have sufficient information for accuracy of measurement of CRP levels in intraperitoneal fluid in early diagnosis of anastomotic leakage. Given the physiological role of CRP (binding to dead cells and some type of bacteria), we wanted to investigate if the measurement of CRP levels from the site of surgery or the site of anastomosis could give us more precise information about anastomotic healing. The rise of both serum and intraperitoneal CRP levels in the first 48 hours after surgery reflects mostly the degree of tissue damage from the anastomosis site[16]. If tissue damage progresses and leads to anastomotic leakage with massive bacterial spillage into the abdominal cavity, both serum and intraperitoneal levels of CRP will continue to rise. In patients with good anastomotic healing, from postoperative day 2, the levels of serum CRP will start to decrease rather quickly, with slower decline of intraperitoneal CRP level[17]. In our study, serum levels of CRP below 121 (cut-off value) on postoperative day 4 was a good predictor of good anastomotic healing. For intraperitoneal fluid, only levels on postoperative day 4, with a cut off of 55.2, had a predictive value, but lower than serum CRP levels. In addition, in patients with anastomotic leakage, serum CRP levels start to rise earlier (from postoperative day 2) than intraperitoneal CRP levels. One posssible explanation for lower levels and delay in rise of intraperitoneal CRP lies in the fact that peritoneal fluid is an ultrafiltrate of plasma. In comparison with some other researsch, in our study, gender, age, preoperative levels of total serum protein and CRP were not statistically significant risk factors for anastomotic leakage. Although not statistically significant, 25% of patients older than 81 had anastomotic leakage, in comparison with younger age groups.

Our study results show that repetitive measurements of CRP in serum and intraperitoneal fluid on the first four postoperative days can give us relevant information about anastomotic healing. Measurement of serum CRP levels in early postoperative period has a higher sensitivity and accuracy in predicting anastomotic leakage compared to intraperitoneal CRP measurement. If there is no decline in the levels of serum and intraperitoneal CRP after postoperative day 2, high suspicion of anastomotic leakage development should be raised, with continued monitoring of the patient and exclusion of other causes of infection in early postoperative period.

Conclusion

According to the results of our study, measurements of serum and intraperitoneal CRP levels from the first to fourth postoperative day, especially on postoperative day 4, can give colorectal surgeons valuable information about the severitiy of inflammatory process in the abdominal cavity after colorectal surgery. Consecutive measurements of serum CRP levels can be a particularly useful tool in identifying those patients who need more vigilant postopertive clinical and radiological monitoring in order to diagnose anastomotic leakage as early as possible.

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Sažetak

USPOREDBA VRIJEDNOSTI SERUMSKOG I INTRAPERITONEALNOG C-REAKTIVNOG PROTEINA U RANOJ DIJAGNOSTICI DEHISCENCIJE ANASTOMOZE NAKON KIRURGIJE DEBELOGA CRIJEVA

D. Mužina Mišić, M. Zovak, M. Kopljar, S. Čiček i *Z. Bilić*

U kirurgiji debelog crijeva dehiscencija crijevne anastomoze je ozbiljna komplikacija koja dovodi do povećanja pobola i smrtnosti nakon operativnog zahvata. Cilj ove studije bio je utvrditi točnost serumskog i intraperitonealnog C-reaktivnog proteina u ranoj dijagnostici dehiscencije crijevne anastomoze u prva četiri poslijeoperacijska dana nakon operacije debelog crijeva. Od siječnja do kolovoza 2019. godine operirano je 59 bolesnika s rakom debelog crijeva uz uspostavu primarne crijevne anastomoze. Kod osam bolesnika dijagnosticirana je dehiscencija crijevne anastomoze. Uspoređujući vrijednosti serumskog i intraperitonealnog C-reaktivnog proteina naša studija je pokazala da je serumski C-reaktivni protein bolji biljeg u predviđanju dehiscencije crijevne anastomoze. Vrijednosti serumskog C-reaktivnog proteina manje od 121 mg/L četvrtog poslijeoperacijskog dana pokazatelj su dobrog cijeljenja anastomoze.

Ključne riječi: *Kolorektalni karcinom; Kolorektalna kirurgija; Dehiscencija anastomoze; C-reaktivni protein*