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Diagnostic value of serial measurement of C-reactive protein in serum and matrix metalloproteinase-9 in drainage fluid in the detection of infectious complications and anastomotic leakage in patients with colorectal resection

Dijagnostička vrednost serijskog merenja C-reaktivnog proteina u serumu i matriksne metaloproteinaze-9 u drenažnoj tečnosti za detekciju infektivnih komplikacija i dehiscencije anastomoze kod bolesnika sa kolorektalnom resekcijom

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Abstract

Background/Aim. Postoperative infectious complications are one of the most important problems in surgical treatment of colorectal cancer (CRC), being present in up to 40% of patients. The aim of this paper was to establish the significance of serial measurement of C-reactive protein (CRP) in serum and matrix metalloproteinase-9 (MMP-9) in drainage fluid for the detection of infectious complications and anastomotic leakage (AL) in patients with colorectal resection. **Methods.** CRP and MMP-9 values in serum and drainage fluid, respectively, were measured on the first, third, fifth, and seventh postoperative day (POD) in 150 patients with colorectal resection and primary anastomosis. The values obtained were compared between the patients without complicatons and those with surgical site and remote infections and AL. **Results.** Surgical site infections (SSIs) were observed in 41 (27.3%), and remote infections in 10 (6.7%) patients. Clinically evident AL was observed in 15 (10%) patients. In 82% of the patients with SSIs, serum CRP value on POD 5 exceeded 82 mg/L, with 81% specificity. AL was reported in 85% and 92% of the patients on PODs 5 and 7, respectively, with CRP values of 77 mg/L and 90 mg/L, respectively. The specificity was 77% for POD 5 and 88% for POD 7. All the patients with CRP values exceeding 139 mg/L on POD 5 had some of SSIs and/or AL. The mean values of MMP-9 were not statistically different between the group without complications (n = 99) and the group with AL (n = 15). **Conclusion.** Serial measurement of CRP is recommended for screening of infectious complications of colorectal resection. Patients with CRP values above 139 mg/L on POD 5 cannot be discharged from hospital, and require an intensive search for infectious complications, particularly AL. MMP-9 measurement in drainage fluid is not relevant in the detection of AL in patients with colorectal resection.

Key words:
c-reactive protein; matrix metalloproteinases; anastomosis surgical; postoperative complications; digestive system surgical procedures.

Apstrakt

Uvod/Cilj. Postoperativne infektivne komplikacije spadaju u najveće probleme hirurškog lečenja kolorektalnog karcinoma [*colorectal cancer* (CRC)] i nastaju kod 40% bolesnika. Cilj rada bio je utvrđivanje značaja serijskog merenja vrednosti C-reaktivnog proteina (CRP) u serumu i matriks metaloproteinaze-9 (MMP-9) u drenažnoj tečnosti za detekciju infektivnih komplikacija i dehiscencije anastomoze kod bolesnika sa resekcijom kolona i rektuma. **Metode.** Prvog, trećeg, petog i sedmog postoperativnog dana određivane su vrednosti CRP u serumu i MMP-9 u drenažnoj tečnosti kod 150 bolesnika sa kolorektalnom resekcijom i primarnom anastomozom. Dobijene vrednosti su upoređivane između grupa bolesnika bez komplikacija i onih sa specifičnim i udaljenim infektivnim komplikacijama i dehiscencijom anastomoze. **Rezultati.** Specifične infektivne komplikacije operativnog lečenja registrovane su kod 41 (27,3%), a udaljene kod 10 (6,7%) obolelih. Klinički manifestnu dehiscenciju anastomoze imalo je 15 (10%) bolesnika. Kod 82% bolesnika sa specifičnim komplikacijama vrednost CRP petog postoperativnog dana bila je veća od 82 mg/L, uz specifič-

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nost od 81%. Dehiscencija anastomoze registrovana je kod 85% bolesnika petog i kod 92% sedmog postoperativnog dana pri vrednostima CRP od 77 mg/L i 90 mg/L, respektivno. Specifičnost je bila 77% petog dana i 88% sedmog postoperativnog dana. Svi bolesnici sa vrednošću CRP preko 139 mg/L petog postoperativnog dana imali su neku od specifičnih infektivnih komplikacija i/ili dehiscenciju anastomoze. Srednja vrednost MMP-9 bila je bez statistički značajne razlike između grupe bolesnika bez komplikacija (n = 99) i grupe sa dehiscencijom anastomoze (n = 15). **Zaključak.** Serijsko merenje vrednosti CRP u serumu može da se preporuči za otkrivanje infektivnih komplikacija. Operisani bolesnici sa vrednostima CRP iznad 139 mg/L petog postoperativnog dana ne mogu se otpustiti iz bolnice i kod njih se mora tragati za infektivnim komplikacijama operativnog lečenja, prvenstveno dehiscencijom anastomoze. Određivanje MMP-9 u drenažnoj tečnosti nema značaj za detekciju dehiscencije anastomoze kod bolesnika sa kolorektalnom resekcijom.

Ključne reči:
c-reaktivni protein; matriks metaloproteinaze; anastomoza, hirurška; postoperativne komplikacije; hirurgija digestivnog sistema, procedure.

Introduction

Postoperative infectious complications are one of the most important problems in surgical treatment of colorectal cancer (CRC), being present in up to 40% of patients [1]. Leakage of anastomosis created during surgical treatment is the most severe treatment complication, posing dilemmas for surgeons as to the prevention, early detection, and appropriate further treatment. Not only that it significantly impacts postoperative morbidity and mortality [2, 3], quality of life [4], prolongs length of hospital stay and increases treatment costs [2, 5], but is largely correlated with local recurrence rates and reduced tumor-specific survival of patients [6]. The reported leak rate varies, between 3% to 19%, depending on the definition [2, 3, 7–9]. It is more common after rectal surgery, between 8% and 14% [3, 8, 10, 11], compared to the colon, ranging from 3% to 7% [12, 13]. Early detection of this, potentially most dangerous complication, in the absence of clear clinical manifestations, would make possible early introduction of appropriate therapeutic measures intended to alleviate or eliminate adverse effects. The possibility of anticipating a postoperative course without complications in the era of adoption of the Enhanced Recovery After Surgery (ERAS) [14] protocol, is of significance with regard to earlier patient discharge and shorter length of hospital stay.

C-reactive protein (CRP) is the most popular and most widely available marker of the acute inflammatory response. In recent years, the significance of association of elevated systemic inflammatory response and worse cancer specific survival of the patients with colorectal cancer has been emphasized, regardless of the tumor disease stage [15]. CRP is synthesized in the hepatocytes, being released during the acute phase of inflammatory response, after the stimulation with interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and interleukin 1 β (IL-1 β), originating in the infection site [16]. CRP values in serum rise even before the clinical signs of infection become apparent [17]. These properties make CRP an ideal predictor of postoperative infectious complications, and combined with the clinical presentation, can be a marker of postoperative course, including both surgical and non-surgical treatment complications [18]. Since it is a non-selective marker of the inflammatory process, before searching for more specific ones, it is necessary to exclude remote infectious complications [17].

Matrix metalloproteinases (MMPs) are the most important factor in the preservation of extracellular matrix homeostasis in numerous tissues, being involved in numerous processes such as reproduction, morphogenesis, embryonal development, bone remodeling, angiogenesis, and tissue healing [19]. These are endopeptidases by nature, and 23 members of the family have been described in humans. At the transcriptional level, their synthesis is induced by proinflammatory cytokines, growth factors, hormones, ultraviolet radiation, physical stress, cell-cell and cell-matrix interactions [19, 20]. They are secreted into the environment in their inactive form as zymogens or promatrix metalloproteinases, with the exception of neutrophils, macrophages, and Paneth cells, where they are stored in the granules [19]. They have a complex function, which depends on the relationship of numerous activators and inhibitors of tissue matrix metalloproteinases (TIMMPs) [20]. Increased MMPs activity in the immediate vicinity of an anastomosis in experimental studies, has given rise to the idea that increased MMP activity in the immediate postoperative course could be responsible for anastomotic leakage (AL) [21, 22]. According to our knowledge there have been only two clinical papers elaborating a possible association of MMPs and AL in patients with colorectal resection [23, 24].

The aim of this paper was to establish the significance of serial measurement of CRP in serum and MMP-9 in drainage fluid for the detection of infectious complications and AL in patients with colorectal resection.

Methods

This prospective analysis enrolled 150 patients with cancer of the left colon and rectum surgically treated at the Clinic for General Surgery, Military Medical Academy, Belgrade, in the period from April 2011 to November 2012.

Preoperatively, appropriate investigations were performed in all the patients in order to establish the diagnosis and degree of disease spread [clinical examinations, endoscopic examination with biopsy and histopathology, abdominal ultrasound (US), chest x-ray]. Additional investigations, such as multislice computerized tomography (MSCT) of the abdomen and/or chest in patients with colon cancer, were done only in those cases in which the baseline tests (US, chest x-ray) had aroused suspicion of disease dissemination. In those with cancer of the distal and middle third of rectum, MSCT of the small pelvis was performed in order to establish local cancer spread and the need for preoperative adjuvant therapy.

The parameters relevant for the outcome of surgical treatment were registered for all the patients: age, anemia, comorbid conditions (diabetes mellitus, chronic obstructive pulmonary disease, arterial hypertension), and body mass index (BMI).

The patients with clinical signs of infection or some other inflammatory condition present preoperatively, were excluded from the study.

All the operations were done at the Clinic for General Surgery, Military Medical Academy, by the surgeons with at least 30 similar surgical interventions performed *per* year.

Mechanical preoperative large bowel preparation was done only in those with rectal cancers.

The analysis involved only the patients in whom conventional elective, radical, or palliative surgical intervention was done, with colo-colonic or colorectal anasomosis, handsawn or stapled. The patients operated for tumor recurrence were excluded from the study. The creation of diverting ileostomy or transversocolostomy depended on the individual assessment of surgeons. Before the closure of laparotomy incision, abdominal cavity of the patients was routinely drained with at least one drain placed in the area of the pouch of Douglas or in the presacral area, in the region of colorectal anastomosis.

In the immediate postoperative course, within a month of surgery, all remote (pneumonia, urinary infection, infections caused by the central venous line) and surgical site (wound infection, anastomotic leakage, intra-abdominal abscess collections) infectious complications were registered. Redness, edema, and purulent secretion at the site of laparotomy wound were the clinical criteria establishing the presence of infection in the surgical incision site [18]. Clinical parameters of AL were defined by the presence of purulent or fecal content at the drain site, pelvic abscess, peritonitis, rectovaginal fistula, or the appearance of purulent content from the rectum (*per recti*) [25]. Routine, contrast-enhanced x-ray control of the anastomosis was not implemented, since the patients with asymptomatic leakage, were not relevant for the study. In patients with low colorectal anastomosis, digital rectal examination was an integral part of the examination to detect possible AL. Intraabdominal abscesses were detected by way of the presence of purulent secretion after surgical or percutaneous ultrasound-guided drainage of these collections [18]. Appropriate clinical presentation with a positive x-ray finding in pneumonia, urinary sediment and urine culture in urinary infection, and positive blood culture in infections caused by central venous line, defined the presence of individual remote infections.

On the first, third, fifth, and seventh postoperative day (POD), serum CRP values were measured, utilizing the method immunonephelometry on a SIEMENS autoanalyzer (Dade Behring BN II), and MMP-9 activity was measured in intraperitoneal drainage fluid utilizing the enzyme-linked immunosorbent assay (ELISA) method. These laboratory parameters were measured at the Institute of Medical Biochemistry, Military Medical Academy, Belgrade.

The usual descriptive statistic parameters were used in statistical analysis of the obtained results (mean value, standard deviation, range, 95% confidence interval, frequency of individual characteristics). Depending on the normality of distribution of the observed parameters and the number of groups among which statistical significance was sought for, out of parametric tests the Students *t*-test was used for independent characteristics, and out of non-parametric tests the Mann-Whitney *U*-test. The existance of a statistically significant difference between the frequency distributions of individual groups was validated using the χ^2-test. The sensitivity and specificity of relevant biochemical markers were analyzed using the receiver operating characteristic curve (ROC).

Commercially available statistical software package SPSS version 17 (USA) was used for statistical analysis.

Results

We analyzed 150 patients in total, 94 (66.7%) men and 56 (33.3%) women (male-to-female ratio, 1.7:1). The youngest operated patient was 33, and the oldest one 87 years of age. The average age of the patients was 65 ± 11 years. Table 1 presents some of the characteristics of our surgically treated patients.

Table1

Characteristics of the surgically treated patients

Patients characteristics	Values
Gender, n (%)	
male	94 (66.7)
female	56 (33.3)
Age (years)	
$\bar{x}$ ± SD	65 ± 11
range	33–87
median	65
Body mass index (kg/m^2)	
$\bar{x}$ ± SD	25.80 ± 4.14
median	25.18
Comorbidities, n (%)	
yes	85 (56.7)
no	65 (43.3)
Tumor site, n (%)	
splenic flexure	12 (8)
descendent colon	2 (1.3)
sygmoid colon	33 (22)
rectosigmoid junction	38 (25.3)
rectum – upper (10–15 cm)	9 (6)
rectum – middle (5–10 cm)	36 (24)
rectum – lower (< 5 cm)	20 (13.3)
Disease stage, n (%)	
T1	11 (7.3)
T2	14 (9.3)
T3	122 (81.3)
T4	3 (2)
N0	91 (60.7)
N1 (1–3)	41 (27.3)
N2 (4–6)	16 (10.7)
N3 (> 6)	2 (1.3)
M0	136 (90.7)
M1	14 (9.3)
Astler-Coller, n (%)	
A	10 (6.7)
B1	12 (8)
B2	66 (44)
C1	2 (1.3)
C2	46 (30.7)
D	14 (9.3)
Anastomosis, n (%)	
colo-colo	47 (31.3)
colo-recto	103 (68.7)
handsawn	63 (42)
stapled	87 (58)
Preoperative radiation, n (%)	
yes	28 (18.7)
no	122 (81.3)
Diverting stoma, n (%)	
transverse colostomy	45 (30)
ileostomy	4 (2.7)
no	101 (67.3)
Morbidity, n (%)	
total complications	51/150 (34)
surgical site infections	41/150 (27.3)
remote infections	10/150 (6.7)
Mortality	6/150 (4)

Comorbidities were present in 85 (56.7%) of the patients. Cancer of the left colon was present in 85 (56.7%), and rectal cancer in 65 (43.3%) of the patients. There were 122 (81.3%) of the patients with T3 tumors; metastatic lymph node involvement was present in 59 (39.3%) of the patients, and distant metastatic disease in 14 (9.3%) of the patients. There were 20 (13.3%) colo-colonic, and 130 (86.7%) colo-rectal anastomoses performed. In four patients, atypical segment resection of the liver with metastatic foci was performed, in two cases typical liver resection, and in eight metastatic deposits were only biopsied. There were 63 (42%) handsewn anastomoses, and 87 (58%) were created using staplers. Preoperative radiation therapy was administered in 28 patients, i.e. in 50% of those with cancers of the middle and distal portion of the rectum.

The overall morbidity rate associated with surgical treatment was 34%, and mortality rate 4%. Surgical site infections (SSIs) were observed in 41 (27.3%) and remote infections in 10 (6.7%) of the patients. In 99 surgically treated patients postoperative course was without any complications.

Clinically evident AL was observed in 15 of the patients – in two patients (4.2%) with left colonic surgery, and in 13 (12.6%) patients with colo-rectal anastomosis. Postoperative mortality rate associated with AL was 13%, and out of six fatal outcomes, leakage was the immediate cause of death in two. Table 2 shows the results of univariate and multivariate analysis of risk factors for the occurrence of AL.

Male patients with tumors of the middle and distal third of the rectum (≤ 10 cm), those undergoing preoperative radiation therapy, and those with created diverting stomas were at the highest risk of developing AL.

In Table 3 the mean CRP values in serum for the observed postoperative days in the group of patients without sur-

Table 2

Characteristics of patients with anastomotic leakage (AL) – univariate and multivariate analysis

Variable	Patients with AL (n = 15)	All patients (n = 150)	Univariate analysis	Multivariate analysis 95% CI			
				RR	lower	upper	*p*
Age (years), $\bar{x} \pm SD$	68 ± 14	65 ± 11	$t = 0.953$ $p = 0.342$	1.02	0.98	1.08	0.32
≤ 65, n (%) > 65, n (%)	5/77 (65) 10/73 (13.7)		$\chi^2 = 1.435$ $p = 0.231$	2.29	0.74	7.04	0.15
Gander, n (%) male	14/94 (14.9)		$\chi^2 = 5.322$	9.26	1.23	75.34	0.03
female	1/56 (1.6)		$p = 0.02$				
Body mass index (kg/m^2), $\bar{x} \pm SD$	25.35 ± 3.85	25.8 ± 4.14	$t = 0.404$ $p = 0.687$	0.97	0.84	1.11	0.65
Tumor site, n (%) left colon	2/47 (4.2)		$\chi^2 = 3.525$	7.72	0.99	60.55	0.052
rectum	13/103 (12.6)		$p = 0.060$				
Anastomosis (cm), n (%) > 10	2/85 (2.3)		$\chi^2 = 0.86$	10.37	2.25	47.84	0.003
≤ 10	13/65 /20)		$p < 0.001$				
Comorbidities, n (%) yes	10/85 (11.7)		$\chi^2 = 0.302$	1.17	0.39	3.48	0.78
no	5/56 (7.7)		$p = 0.583$				
Disease stage, n (%) T_1, T_2	3/25 (12)		$\chi^2 = 0.000$	1.28	0.33	4.93	0.71
T_3, T_4	12/125 (9.6)		$p = 1.000$				
N0	8/91 (8.8)		$\chi^2 = 0.112$	1.40	0.48	4.08	0.54
N+	7/59 (11.9)		$p = 0.738$				
Anastomosis, n (%) handsaw	3/63 (4.8)		$\chi^2 = 2.384$	3.20	0.86	11.86	0.08
stapled	12/87 (13.8)		$p = 0.123$				
Preoperative radiation, n (%) yes	8/29 (28.6)		$\chi^2 = 10.778$	153.14	16.74	1400.74	0.001
no	7/122 (5.7)		$p = 0.001$				
Divert stoma, n (%) yes	10/49 (20.4)		$\chi^2 = 7.126$	268.00	28.50	2520.20	0.001
no	5/101 (5)		$p = 0.008$				

***RR – relative risk; CI – confidence interval; χ^2 – chi square test; *t* – Student's *t*-test.**

Table 3

Correlation of serial serum C-reactive protein (CRP) (mg/L) measurement with infectious surgical treatment complications, by postoperative days (POD)

POD	No complications (n = 99)	SSIs (n = 41)		Remote infections (n = 10)		AL (n = 15)	
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	*p*	$\bar{x} \pm SD$	*p*	$\bar{x} \pm SD$	*p*
1	95.15 ± 37.97	109.39 ± 47.81	0.151	121.63 ± 27.78	0.014	102.11 ± 39.65	0.595
3	113.47 ±40.72	200.58 ± 69.46	< 0.001	145.20 ± 52.90	0.059	197.25 ± 75.76	0.001
5	57.10 ± 28.15	154.84 ± 69.96	< 0.001	103.91 ± 52.70	0.001	175.93 ± 72.51	< 0.001
7	49.71 ± 29.95	119.86 ± 72.59	< 0.001	102.17 ± 71.66	0.003	155.61 ± 77.49	< 0.001

***Mann-Whitney U-test, comparison with patients with no complications; SSIs – surgical site infections; AL – anastomotic leakage.**

gical treatment complications (n = 99) and those with SSIs (n = 41) and remote (n = 10) infectious complications, and those with AL (n = 15) are given. On POD 1, there were no statistically significant differences in CRP values between the group of patient without and those with surgical treatment complications.

Figure 1 illustrates the mean CRP values by the group of patients for the observed PODs.

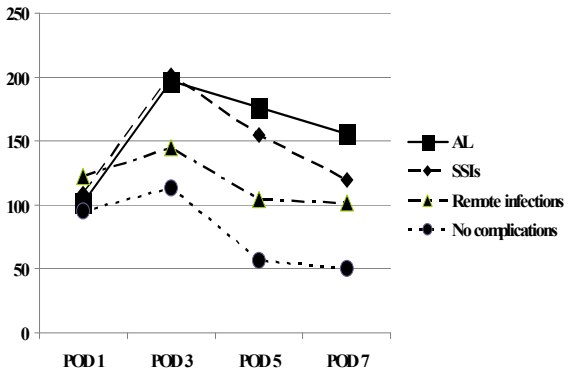

Fig. 1 – Serum C-reactive protein (CRP) values (mg/L) in the patients without, and those with surgical site infections (SSIs) remote infections, and anastomotic leakage (AL), by postoperative days (POD).

In all the patients there was the increase of CRP, with maximum values reported for POD 3. In those without complications, there was a descending tendency, significantly more evident compared to descending CRP values in those with SSIs and AL. While serum CRP values in patients with SSIs and AL remained high on PODs 5 and 7, in those with remote infections CRP values returned to the baseline on POD 7.

Sensitivity and specificity of serum CRP measurement in the detection of SSIs and AL were analyzed using the ROC curve and are shown in Table 4 and Figures 2 and 3. CRP values on POD 5 are most important in the detection of SSIs, and on PODs 5 and 7 in the detection of AL. The area under the ROC curve on POD 5 in the patients with SSIs was 0.926, and in those with AL 0.920 on POD 5, and 0.960 on POD 7. In 82% of the patients with SSIs, CRP values on POD 5 exceeded 82 mg/L, with 81% specificity. All the patients with CRP values above 139 mg/L on POD 5 had some of SSIs. AL was observed in 85% of the patients on POD 5 and in 92% on POD 7, with CRP values of 77 mg/L and 90 mg/L. The method specificity was 77% for POD 5, and 88% for POD 7. All the patients with AL had CRP values above 139 mg/L on POD 5.

Table 5 illustrates the results of cutoff CRP values in postoperative infectious complications in different authors' papers, including ours.

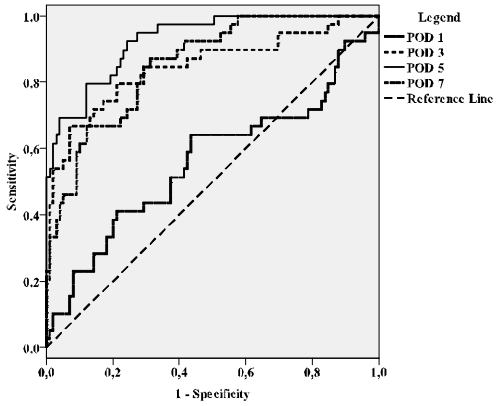

Fig. 2 – Diagnostic accuracy of serum C-reactive protein (CRP) in the detection of surgical site infections (SSIs) expressed as a receiver operating characteristic (ROC) curve. POD – postoperative day.

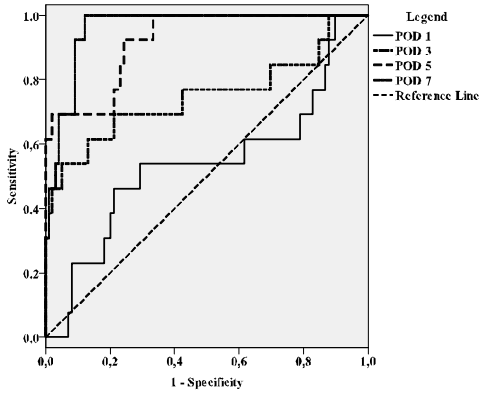

Fig. 3 – Diagnostic accuracy of serum C-reactive protein (CRP) in the detection of anastomotic leakage (AL), expressed as a receiver operating characteristic (ROC) curve. POD – postoperative day.

Table 4

Sensitivity and specificity of serum C-reactive protein (CRP) in the patients with surgical site infections and anastomotic leakage by postoperative days (POD), espressed as an area under the receiver operating chracteristic (ROC) curve (AUC)

POD	Cutoff value CRP (mg/L)	Sensitivity (%)	Specificity (%)	AUC (95% CI)	*p*
Surgical site infections (n = 41)					
1	100	51	63	0.567 (0.454 – 0.681)	0.28
3	150	73	84	0.847 (0.766 – 0.928)	< 0.001
5	82	82	81	0.926 (0.882 – 0.970)	< 0.001
7	82	67	88	0.854 (0.789 – 0.920)	< 0.001
Anastomotic leakage (n = 15)					
1	111	54	71	0.538 (0.348 – 0.729)	0.65
3	140	69	79	0.748 (0.567 – 0.929)	0.004
5	77	85	77	0.920 (0.849 – 0.991)	< 0.001
7	90	92	89	0.960 (0.925 – 0.994)	< 0.001

CI – confidence interval.

Table 5

Cutoff values of serum C-reactive protein (CRP) in postoperative infectious complications

Study	CRP (mg/L)
POD 1	
MacKay et al. [31]	82
Korner et al. [34]	89
Scepanovic et al. [36]	187
Warschkow et al. [38]	123
Kostic et al. (this study)	112
POD 2	
Welsch et al. [17]	140
MacKay et al. [31]	164
Scepanovic et al. [36]	164
Warschkow et al. [38]	173
POD 3	
Welsch et al. [17]	140
MacKay et al. [31]	195
Korner et al. [34]	190
Scepanovic et al. [36]	135
Warschkow et al. [38]	185
Kostic et al. (this study)	150
POD 4	
Welsch et al. [17]	140
MacKay et al. [31]	145
Ortega-Deballon et al. [32]	125
Scepanovic et al. [36]	116
Warschkow et al. [38]	123
POD 5	
MacKay et al. [31]	135
Korner et al. [34]	154
Scepanovic et al. [36]	114
Warschkow et al. [38]	83
Kostic et al. (this study)	82
POD 7	
Korner et al. [34]	215
Scepanovic et al. [36]	85
Kostic et al. (this study)	82

POD – postoperative day.

Table 6 shows the relationship of the mean MMP-9 values in drainage fluid for the observed PODs between the group of patients without surgical complications (n = 99) and the group of those with AL (n = 15). There were no statistically significant differences in MMP-9 values between the analyzed groups.

patients with AL in our study was 13%, and out of six deaths, leakage was the immediate cause of death in two. Rickert et al. [12], analyzing the treatment results in 1,731 patients with colon resection, have reported the AL rate of 3.5% and the mortality rate of 25% in those with complications. In 807 patients with colorectal resection, Buchs et al. [2] have reported the AL rate of 3.6% and the mortality rate of 13% in the affected. A half of the patients were surgically treated for CRC, and AL rate after rectal resection was 13%. Reporting the results of treatment of 739 patients with colorectal resection in a ten years' period (1997–2007), in a tertiary care institution in Netherlands, Komen et al. [8] stated that AL occurred in 64 (8.7%) patients, out of which nine (14%) died. The incidence of AL in patients with left hemicolectomy was 12%, with resection of sygmoid colon 8%, rectosygmoid colon 17%, and with low anterior resection and with total mesorectal excision 13%.

Numerous risk factors could be considered significant for the occurrence of AL, such as age [27, 28], gender [3, 7, 13], smoking [10], obesity [2, 8, 27], malnutrition [27, 29], American Society of Anesthesiologists (ASA) score [2, 7], laparoscopic access [13], duration of surgery [2, 8, 30], intraoperative blood transfusions [13], level of anastomosis [3, 7, 10], site of rectal cancer [2], preoperative steroid [30] and radiation therapy [7], diverting stoma [7, 9, 11], and prophylactic intraoperative abdominal drainage [8, 10]. Univariate and multivariate analysis of our patients demonstrated male gender, anastomosis created below 10 cm from the anal verge, preoperative radiation therapy, and diverting stoma to be significant risk factors for AL. Years of age, body mass index, comorbidities, tumor size, lymphonodal status, and type of anastomosis (handsewn or stapled) did not have an impact on the incidence of AL. Tumor site (colon *vs* rectum) could probably be a significant risk factor if the study included patients with cancer of the right colon, in view of a lower incidence of AL after resection of the right compared to the left colon [13]. In contrast to other authors' papers, where the incidence of AL was lower in those with intraoperatively created diverting stoma [9, 11, 25],

Table 6

Matrix metalloproteinase-9 (MMP-9) values in the drainage fluid in patients without complications compared to those with anastomotic leakage (AL)

POD	No complications (n = 99) $\bar{x} \pm SD$	AL (n = 15) $\bar{x} \pm SD$	Z adjusted	*p*
1	1239.09 ± 618.22	1296.10 ± 687.71	0.112136	0.91
3	1151.31 ± 975.58	1114.79 ± 715.96	0.479493	0.61
5	1147.17 ± 559.64	1166.43 ± 599.55	0.799385	0.42
7	1086.55 ± 466.47	948.16 ± 393.48	0.608981	0.54

***Mann-Whitney *U*-test; POD – postoperative day.**

Discussion

Anastomotic leakage (AL) is potentially the most serious surgical treatment complication in patients with CRC. It is the leading cause of postoperative mortality of patients with CRC, and an immediate cause of the one third of deaths after surgery [10, 12]. In the analysis of our patients, we used exclusively the clinical criteria to establish AL [26], and it was reported in 15 patients. The postoperative mortality rate of in our study AL was significantly more common in the patients with stoma. The explanation for this could be found in our patient selection criteria. In other authors' papers, stoma was most commonly created depending on the level of anastomosis, and patients with surgical treatment complications were excluded from the analysis. Diverting stoma did not prevent anastomotic leakage, but it reduced adverse septic consequences of leakage instead [7, 9]. In our patients, the creation of diverting transversocolostomy or ileostomy depended

exclusively on the surgeon's decision, and it was most commonly performed in patients with surgery-related problems, such as long lasting surgical procedure, intraoperative hypotension, bleeding and the need for blood transfusion, difficulties in the creation of anastomosis, incomplete rings in the anastomosis created with mechanical staplers, and similar, and these are the factors significantly contributing to the incidence of the complication [7].

The closer an anastomosis is to the anocutaneous junction, the higher is the risk of leakage. Trencheva et al. [3], in their prospective analysis of 616 patients with colorectal resection, with leakage incidence of 5.7%, have emphasized that anastomoses created below 10 cm from the anal verge were the most important prognostic risk factor, which was in accordance with our own results. Shiomi et al. [11] have recommended mandatory fecal diversion for the anastomoses below 5 cm from the anal verge, taking into consideration a significant difference in the incidence of clinically evident AL in this group of patients with and without defunctioning stoma (3.8% *vs* 12.7%). Similarly, Mathiessen et al. [9] have found a statistically significant difference in the incidence of AL in patients with anastomosis up to 7 cm from the anal verge, without and with diverting stoma (28% *vs* 10.3%).

Complications are an inevitable companion of any surgical treatment, and their prevention and/or earlier detection and timely therapeutic management are the prerequisite of improved overall treatment results. In the immediate postoperative course, often septic condition cannot be reliably distinguished from normal systemic inflammatory response to surgical trauma. Since clinical examination most often cannot be the sole approach in making the diagnosis, the need for selective and specific markers of infectious complications is essential, enabling their early detection and implementation of appropriate measures to improve treatment outcomes [17, 31]. The ability to predict a complication-free postoperative course in the era of adoption of the Enhanced Recovery After Surgery (ERAS) [14] protocol, is essential regarding earlier discharge and shorter length of hospital stay [32].

C-rective protein (CRP) is the protein of acute inflammatory phase and most commonly measured non-specific marker of systemic inflammatory response. Serum half-life of CRP is constant, being 19 hours, and its elevated concentrations are the consequence of increased synthesis in response to stimulus intensity, being the measure of acute inflammatory response [33]. What is even more important, serum CRP values increase even before the clinical signs of infection become evident, such as increased body temperature, rapid heartbeat, and pain [17]. These properties make CRP an ideal predictor of postoperative infectious complications. Since it is a non-selective marker of the inflammation process, before searching for specific ones it is necessary to exclude other infectious complications [17]. That is why we analyzed serum CRP values in the patient groups without, with surgical site, and remote infections, and especially in those with AL. In all the patient groups there was an increase of CRP after surgery, with maximum values observed on POD 3. After that, in those without complications there was a gradual decline, while high CRP values were maintained on PODs 5 and 7 in those with SSIs and AL (Figure 1 and Table 3).

Similar results can be found in papers of other authors [17, 34, 35]. Welsch et al. [17] have recorded infectious complications in 383 patients with cancer of the rectum in whom resection and primary anastomosis were performed. Serum CRP values, number of leukocytes, thrombocytes, and body temperature were recorded every day during twelve PODs. Two groups were compared – 48 patients with complications and 48 with normal postoperative course. The authors found that in patients without complications, after an initial rise of CRP values (with maximum mean value of 140 mg/L) on POD 2, there was a gradual decline of CRP values on days to follow. In those with complications, high CRP values persisted after POD 2. Korner et al. [34] analyzed the results of serum CRP values on PODs 1, 3, 5, and 7 in 231 patients with colorectal resection divided into three groups: those without complications, with intraabdominal complications, and with extra-abdominal infectious complications. On POD 1, CRP was elevated in all the patients. The authors found that the mean value of CRP was highest in patients with intra-abdominal complications, and that high CRP values persisted in this group on PODs 5 and 7. In those without complications, a decrease of CRP was observed after POD 3, while in the group of patients with extra-abdominal complications a small rise and more modest decline of mean values were observed. This finding is similar to our own results, since CRP values on POD 7 correspond to the values observed on POD 1 in the group of patients with remote infections.

The results of a prospective study in France on 133 patients with colorectal resection point out the significance of CRP measurement on PODs 2 and 4 in early identification of patients with AL. The incidence of AL was 15.5% in this patient series [32]. The relationship of AL and CRP values in 342 patients with colorectal resection was analyzed by Woeste et al. [35]. In contrast to the patients without complications, in 26 (7.6%) patients with AL there was not any decline of CRP values after the maximum reached on POD 3. What was most important was that these high values were measured before clinical evidence of AL. MacKay et al. [31] studied all postoperative infectious complications altogether, regardless of the type of complication. They concluded that there was a statistically significant difference in CRP values between the patients with and without infections starting from POD 2, and that CRP measurement was most relevant on POD 4 in view of the observed difference. Most authors today regard CRP measurement between PODs 3 and 5 as most important for the detection of infectious complications [17, 31, 32, 34, 36].

Serum CRP values significantly vary during postoperative course in both the patients with and without complications [17]. It is therefore necessary to monitor changes of CRP values in the immediate postoperative course utilizing serial measurements. Diagnostic accuracy of CRP values monitored as a constant variable depends significantly on the cutoff value. For this purpose, the statistical analysis with ROC curve is believed to be the most appropriate [37]. For any clinical assumptions to be drawn based on CRP values, one has to know that sensitivity and specificity are inversely related to the chosen cutoff value.

Considering the statistically assessed cutoff value of 123 mg/L on POD 4, Warschkow et al. [38] have reported sensitivity and specificity of 66% and 77%, respectively, in

the prediction of infectious complications. In view of the fact that it could not be used as a completely reliable diagnostic indicator of infection, the authors concluded that CRP values could be taken into consideration only within the clinical presentation context. According to Welsch et al. [17], the serum CRP value of 140 mg/L on PODs 3 and 4 was the cutoff value for the patients with infectious complications, with sensitivity of 80% and 54.3%, and specificity of 81% and 92.3%, respectively. For this CRP value on PODs 3 and 4, a positive predictive value for a postoperative course with complications was 80.7% and 90.5%, respectively. Korner et al. [34] found that the CRP value of 190 mg/L on POD 3 was a cutoff value indicating intra-abdominal infectious complications with sensitivity and specificity of 82% and 73%, respectively. The area under the ROC curve was 0.82. An identical diagnostic accuracy was established for CRP values measured on PODs 5 and 7. According to Ortega-Deballon et al. [32], measurement of CRP had the highest diagnostic accuracy out of all clinical and laboratory data. The values on PODs 2 and 4 had the highest predictive value in early detection of AL (the area under the ROC curve being 0.715 and 0.845, respectively), as well as other postoperative septic complications (the area under the ROC curve being 0.804 and 0.787, respectively). They concluded that the patients with CRP values above 125 mg/L on POD 4 could not be discharged from the hospital. MacKay et al. [31] found the positive and negative predictive value of 61% and 96% in the detection of infectious complications, with high sensitivity and specificity, for CRP values exceeding 125 mg/L on POD 4. Patients with CRP values below 135 mg/L on POD 3, according to Šćepanović et al. [36], were considered to be without infectious complications and could be safely discharged from the hospital.

Analysis of our results using the ROC curve for serum CRP values demonstrated that the values on POD 5 were most significant in the detection of SSIs, and on PODs 5 and 7 in the detection of AL. Area under the ROC curve on POD 5 for those with SSIs and AL was 0.926 and 0.920, respectively. In 82% of patients with SSIs, CRP values on POD 5 exceeded 82 mg/L, with 81% specificity. All the patients with CRP values above 139 mg/L on POD 5, had some of SSIs. Those with CRP values of 150 mg/L and 82 mg/L on PODs 3 and 7, had the highest risk of developing SSIs, with sensitivity and specificity of 73% and 84% for POD 3, and 67% and 88% for POD 7. AL was detected in 85% of the patients on POD 5 and 92% on POD 7, with CRP values of 77 mg/L and 90 mg/L, and specificity of 77% and 89%, respectively. AL was present in all the patients with serum CRP values above 139 mg/L on POD 5, and 150 mg/L on POD 7.

Increased serum CRP values can indicate the presence of infectious complications of surgical treatment, but the type of complication can not be established using this method, which is of utmost importance in further therapeutic management. We therefore tried to establish whether MMP-9 measurement in the peritoneal drainage fluid could be of use in early identification of the patients with AL as the most serious complication of surgical treatment. Biomechanical strength of an anastomosis depends primarily on the integrity of collagen-rich submucosal layer [39]. Experimental models have shown that in the immediate postoperative course, collagen decomposition prevails collagen synthesis, making an anastomosis most vulnerable in the first three to five days [40]. Studies on experimental animals have associated increased collagen decomposition with increased activity of MMPs [21, 22]. In general, healing represents a complex process of balancing of extracellular matrix components, involved in its synthesis and breakdown. Stumpf et al. [41] have compared in their prospective study a group of 15 patients with, and 104 patients without AL. Type I to type III collagen relationship was significantly lower in patients with AL. In these, tissue levels of MMPs 1, 2 and 9 were significantly higher, so that the analysis of biopsy samples of normal colonic wall in this group showed increased tissue expression of MMPs 1 and 2 in the mucosa, and MMPs 2 and 9 in the submucosa.

Drainage fluid is a complex mixture of different biological substances, involving different MMPs, both latent and active enzyme forms, and tissue inhibitors of metalloproteinase (TIMP) and their complexes. Preservation of extracellular matrix structure depends on the activity of MMPs and their endogenous inhibitors, and increased activity of MMP-8 and MMP-9 in the immediate vicinity of anastomosis has prompted the idea in experimental studies that increased MMP activity in the immediate postoperative course could be responsible for AL [21, 22]. Both active form and total (active and latent) MMP concentrations can be measured in the drainage fluid [42].

According to our knowledge, only two clinical papers have been published referring to the association of MMP with AL in patients with colorectal resection [23, 24]. Pasternak et al. [23] have analyzed the drainage fluid samples in 29 out of 30 patients in whom low anterior resection of the rectum was performed for cancer. The total morbidity was 41%, and symptomatic AL was detected in 10 (34%) patients. Such a high rate of AL was explained by a small patient sample. Drainage fluid was sampled only once, immediately following the surgery (after 2–6 hours), and the MMP levels were determined using multiplex flow cytometry. The levels of MMP-8 and MMP-9 were statistically significantly higher in the group with AL. The mean value of the difference was 259 ng/mL for MMP-8 ($p = 0.02$) and 1,180 ng/mL for MMP-9 ($p = 0.03$). The observed values for other MMPs (1, 2, 3, 7, and 13) were without any significant difference between the groups. As the shortcomings of the study, the authors reported small number of the patients, the absence of multiple testing and multivariate analysis of the results. In view of the strong observed association of MMP-8 and MMP-9 levels with AL, they suggested further studies in the area that would certainly contribute to earlier detection of AL and timely therapeutic intervention. However, one should not forget that while MMP-8 measurement is a routine, bedside procedure [43], it is not the case with MMP-9, and flow-cytometry, although highly sensitive, is a costly method requiring highly trained personnel.

The paper of Baker et al. [24] describes the measurement of MMP and TIMP levels in 58 patients undergoing various

surgical procedures, from right hemicolectomy to low anterior rectal resection. Samples of peritoneal drainage fluid were taken daily during the postoperative course, and MMP and TIMP measurements were done using the ELISA method. The result analysis showed a significant positive correlation of MMP-2 levels (POD 3), total MMP-2 (POD 6), and total MMP-9 (PODs 6 and 7) with surgical complications, while there was a negative correlation with TIMP-1 (POD 7) and TIMP-2 (PODs 2 and 3). The highest values of active and total MMP-9 were observed on POD 1, and gradually decline till POD 7. High MMP-9 values immediately after the surgery are the consequence of its increased release from neutrophils and monocyte cells, leading to an increased breakdown of stromal and basal membrane components, facilitating the infiltration of these cells in the area of the wound. The shortcomings of the study were heterogeneous groups of patients and the lack of data about the type of postoperative complications.

In our patients, MMP-9 values were determined using the ELISA method in the samples of drainage fluid taken on PODs 1, 3, 5, and 7. In contrast to Pasternak et al. [23], we did not find any statistically significant difference between the average, mean values of MMP-9 levels in the group of patients without surgical complications and those with AL for the studied days. A declining tendency in the average MMP-9 levels was observed in both groups of our patients, which was in agreement with the results of Baker et al. [24]. A possible explanation of the differences in the results in our study compared to the cited papers could be possibly found in the manner and time of analysis. MMP-9 tests used by us were adjusted by the manufacturer to test a larger number of samples, so that analysis was not done immediately; the collected samples were stored at -70ºC until the sufficient number of samples was collected. Such a procedure of keeping the samples for a period of time could have an impact on the composition and relationships of various biological materials in a complex mixture such as drainage fluid.

Conclusion

Serial measurement of C-reactive protein in serum in the immediate postoperative course makes possible an early detection of patients with surgical site infectious and anastomotic leakage and those with normal postoperative course. Patients with C-reactive protein values above 139 mg/L on postoperative day 5 can not be discharged from the hospital and require an intensive search for infectious complications, particularly anastomotic leakage. Early detection of this, potentially most dangerous complication, in the absence of clear clinical manifestations, would make possible an early introduction of appropriate therapeutic measures intended to alleviate or eliminate adverse effects. Patients without complications can be early released from the hospital afer a shortened length of hospital stay and with lower costs of treatment. Matrix metalloproteinase-9 measurement in drainage fluid is not relevant in the detection of anastomotic leakage in patients with colorectal resection.

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