MOLECULAR AND EXPERIMENTAL BIOLOGY IN MEDICINE

ORIGINAL ARTICLE

PERITONEAL INTERLEUKIN-6 AND TUMOR NECROSIS FACTOR-ALPHA AS MARKERS FOR EARLY DETECTION OF ANASTOMOTIC DEHISCENCE FOLLOWING SURGERY FOR COLORECTAL CANCER

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Abstract: Anastomotic dehiscence is one of the most serious complications following surgery for colorectal cancer, and early detection of anastomotic dehiscence is critical to minimize mortality and morbidity. The aim of this study was to determine the value of peritoneal interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) as markers for preclinical detection of anastomosis dehiscence following colorectal surgery. Concentrations of IL-6 and TNF-α were measured in drain fluid obtained from 58 patients on days 1 to 4 following surgery for colorectal cancer. Five out of 58 patients developed anastomosis dehiscence. Patients who developed anastomosis dehiscence had significantly higher concentration of IL-6 on day 1 after surgery, and TNF-α on day 1, 2 and 4 after surgery. Interleukin-6 on day 1 was predictive for anastomosis dehiscence with specificity of 83%, sensitivity of 80%, positive predictive value (PPV) of 31% and negative predictive value (NPV) of 98%. TNF-α was predictive for anastomosis dehiscence on day 1 (specificity 92%, sensitivity 80%, PPV 50%, NPV 98%), day 2 (specificity 94%, sensitivity 80%, PPV 57%, NPV 98%), and day 4 (specificity 83%, sensitivity 100%, PPV 27%, NPV 100%). Our study indicates the potential use of peritoneal cytokines IL-6 and TNF-α as additional diagnostic tool for early detection of anastomosis dehiscence following colorectal surgery.

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INTRODUCTION

Surgical cancer treatment is most often the first stage and also the most important part of treating patients with colorectal cancer. Despite progress in surgical techniques, perioperative procedure morbidity and mortality after surgery for colorectal cancer continues to be problematic.[1, 2] Complications occurring after surgery are mainly caused by anastomosis failure. Dehiscence of colorectal anastomosis remains one of the worst complications after the cancer is surgically removed.[2] The colorectal anastomosis is formed to establish continuity of the gastrointestinal tract after surgical resection of the colon. It is critical to anticipate and diagnose anastomosis leakage as early as possible in order to reduce the potential morbidity and mortality of this complication. However, dehiscence detection is difficult and is delayed until the patient's health is endangered by peritonitis and sepsis. Today anastomosis leakage is diagnosed by endoscopy, CT scan, biochemical scintigraphy, and the clinical picture.[3-5] These methods are not always satisfying, and their limitations need to be kept in mind. This creates the need for new methods that would be more effective.[2, 3, 6]

There are several studies that investigate pro-inflammatory cytokines in peritoneal fluid for early detection of anastomosis dehiscence. Prior to the occurrence of systemic symptoms such as fever and leukocytosis, localized infection occurs at the sites of anastomosis, involving immune cells and cytokines. Pro-inflammatory cytokines such as IL-6 (interleukin 6) and TNF-α (tumor necrosis factor-α) mediate this inflammatory response. The surgical procedure itself affects the levels of cytokines; however, elevated cytokine levels are considered to indicate surgery complications including anastomotic dehiscence.

Several studies have shown that increased concentrations of peritoneal cytokines can predict early complications of anastomosis.[3, 7, 8]
The aim of this study was to determine the value of IL-6 and TNF-α in peritoneal fluid as markers for preclinical detection of anastomosis dehiscence following colorectal surgery.

MATERIAL AND METHODS

Patients

From December 2015 until July 2017, 58 patients who underwent surgery for colorectal cancer at the Department of surgery University Hospital Sestre milosrdnice were included in this study. Criteria for anastomosis dehiscence were the presence of a leak of luminal contents from a surgical join between 2 hollow viscera diagnosed by any of the following methods: radiologically with the presence of intra-abdominal collection adjacent to the anastomosis, clinically as evidence of extravasation of bowel content or gas through a wound or drain; endoscopically or intraoperatively.

Sample collection

Intra-abdominal fluid (drainage fluid) was collected on days 1, 2, 3, and 4 after the surgery. Approximately 10 ml of drainage fluid was transferred into a vacutainer tube, and samples were centrifuged at 3000 x g for 15 min at 4°C. Supernatant was stored at -80°C until analysis.

Cytokines measurements

Interleukin-6 and TNF-α were measured with an automated immunometric chemiluminescence assay (Immulite 1000, Simens, Germany) in the diagnostic laboratory at the Department of Oncology and Nuclear Medicine, Sestre milosrdnice University Hospital Center. The calibration of IL-6 ranged from 4 to 1000 pg/mL and in the case of TNF-α from 2 to 1000 pg/mL. Samples with concentrations above the upper limit of calibration were reanalyzed after adequate dilution with specific sample diluent.

Statistical analysis

Quantitative data were tested for normality of distribution using the Kolmogorov-Smirnov test. In case of normal distribution, they were presented as means and standard deviation and analyzed using the Student's t test. If the distribution was not normal, data were analyzed using the Mann-Whitney test and presented as median and interquartile range. To test the predictive power of cytokines as markers for anastomosis dehiscence, the receiver operating curve (ROC) analysis was performed. The results of the ROC analysis were presented as area under curve, specificity, sensitivity, positive predictive value and negative predicted value. MedCalc version 10.4.0.0 (MedCalc Software bvba; Mariakerke, Belgium) was used for the analysis, and a P value of ≤0.05 was considered statistically significant.

RESULTS

Patient characteristics

A total of 58 patients were included in the study. Median age at the time of surgery was 71 years, the youngest patient was 24 years old and the oldest patient was 91 years old. Out of 58 patients, 27 (47%) were female and 31 (53%) male. Five out of 58 patients (9%) developed anastomosis dehiscence following surgery, one patient on day 3, one on day 4, one on day 7, and two patients on day 10 after surgery.
No statistically significant association between the development of anastomosis dehiscence and gender was observed (Chi-square test, χ^2=0.026, p=0.87). Among 27 female patients, 2 (7.4%) developed anastomosis dehiscence. Among 31 male patients, 3 (9.7%) developed anastomosis dehiscence. No statistically significant association between the development of anastomosis dehiscence and patient age was observed (Student's t test, t=1.38, p=0.17).

Table 1. Comparison of cytokine concentration in drain fluid between patients who developed anastomosis dehiscence and ones who did not develop anastomosis dehiscence.

Cytokine	Concentration of cytokine (pg/mL)		P value
	NO anastomosis dehiscence	Anastomosis dehiscence	
IL-6 day 1	32926 ± 23596*	71600 ± 28521*	**0.001*****
IL-6 day 2	12800 (7275 – 24800)**	26200 (12175 – 100000)**	0.09
IL-6 day 3	7150 (5000 – 12300)**	43750 (5350 – 90650)**	0.33
IL-6 day 4	5600 (2640 – 14800)**	10600 (6550 – 77650)**	0.20
TNFα day 1	47 (29 – 75)**	647 (166 – 1765)**	**0.006**
TNFα day 2	34 (24-46)**	228 (79 – 963)**	**0.03**
TNFα day 3	32 (22 – 42)**	694 (204 – 1300)**	0.07
TNFα day 4	32 (25 – 52)**	906 (315 – 977)**	**0.02**

IL-6 – interleukin 6; TNFα – tumor necrosis factor α; * mean ± standard deviation; ** median (interquartile range); *** Student's t test (all other P values were calculated using Mann-Whitney test)

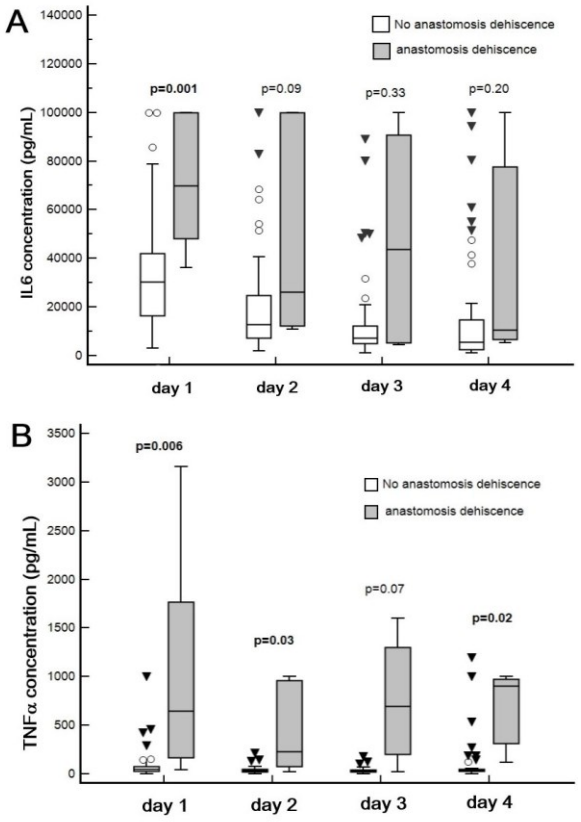

Figure 1. Concentrations of cytokines IL6 (A) and TNFα (B) in drain fluid of patients who developed and patients who did not develop anastomosis dehiscence measured on days 1, 2, 3, and 4 after surgery.

Cytokine concentrations in patients

Concentrations of IL-6 and TNFα were measured in drain fluid obtained from patients on day 1, day 2, day 3 and day 4 following surgery.

Patients who developed anastomosis dehiscence had significantly higher values of IL-6 on day 1 after surgery (Student's t test, t=3.4, p=0.001) (Table 1) (Figure 1). No statistically significant association was observed between the development of anastomosis dehiscence and IL-6 values measured on post-operative day 2 (Mann Whitney test, z=1.6, p=0.09), day 3 (Mann Whitney test, z=0.9, p=0.33), and day 4 (Mann Whitney test, z=1.3, p=0.20) (Table 1) (Figure 1).

Patients who developed anastomosis dehiscence had significantly higher values of TNFα on day 1 after surgery (Mann Whitney test, z=2.7, p=0.006), day 2 after surgery (Mann Whitney test, z=2.3, p=0.03) and day 4 after surgery (Mann Whitney test, z=2.4, p=0.02) (Table 1) (Figure 1). No statistically significant association was observed between the development of anastomosis dehiscence and TNFα values measured on post-operative day 3 (Mann Whitney test, z=1.8, p=0.07) (Table 1) (Figure 1).

Predictive value of cytokines

A ROC analysis was performed to evaluate diagnostic accuracy of IL-6 and TNFα in drain fluid as markers for predicting the development of anastomosis dehiscence. Only values that have shown significant difference between patients who developed anastomosis dehiscence and the ones who did not (IL-6 on day 1 and TNFα on days 1, 2, and 4) were analyzed. For IL-6 on day 1 the optimal cut-off value was 50900 pg/mL, with the area under the curve of 0.87 (95% confidence interval (CI): 0.75-0.94), specificity of 83%, sensitivity of 80%, positive predictive value of 31% and negative predictive value of 98% (Figure 2). For TNFα on day 1 the optimal cut-off value was 154 pg/mL, with the area under the curve of 0.87 (95% CI: 0.76-0.95), specificity of 92%, sensitivity of 80%, positive predictive value of 50% and negative predictive value of 98% (Figure 2). For TNFα on day 2 the optimal cut-off value was 74 pg/mL, with the area under the curve of 0.81 (95% CI: 0.69-0.90), specificity of 94%, sensitivity of 80%, positive predictive value of 57% and negative predictive value of 98% (Figure 2). For TNFα on day 4 the optimal cut-off value was 54 pg/mL, with the area under the curve of 0.92 (95% CI: 0.80-0.98), specificity of 83%, sensitivity of 100%, positive predictive value of 27% and negative predictive value of 100% (Figure 2).

DISCUSSION

In this study, IL-6 and TNF-α in peritoneal fluid were analyzed as potential markers for preclinical detection of anastomosis dehiscence following colorectal surgery. Anastomosis dehiscence after colon surgery is still one of the most difficult complications. Our results show that the peritoneal values of pro-inflammatory cytokines are significantly higher in the first few days after surgery in patients who had anastomosis dehiscence, indicating their diagnostic value. Pro-inflammatory cytokines are mainly derived from macrophages and neutrophils that are present in the anastomotic region. These cells locally release cytokines at the site of anastomosis dehiscence, and these cytokines cause an inflammatory reaction. Due to such local production, these cytokines are present at higher concentrations in the peritoneal fluid than in blood.[9-11] Therefore, pro-inflammatory cytokines IL-6 and TNF-α in peritoneal fluid could be important for early detection of anastomosis dehiscence.[3, 9, 12, 13]

Today there is no definition of anastomosis dehiscence, and most studies define dehiscence of anastomosis by clinical signs such as pain, fever, tachycardia, peritonitis. Our results show elevated values of peritoneal pro-inflammatory cytokines on postoperative days 1-4. In some cases, dehiscence does not show obvious clinical signs until the 8th or even the 20th day following surgery.[14] Early diagnosis is crucial for treating patients and reducing mortality. In our study

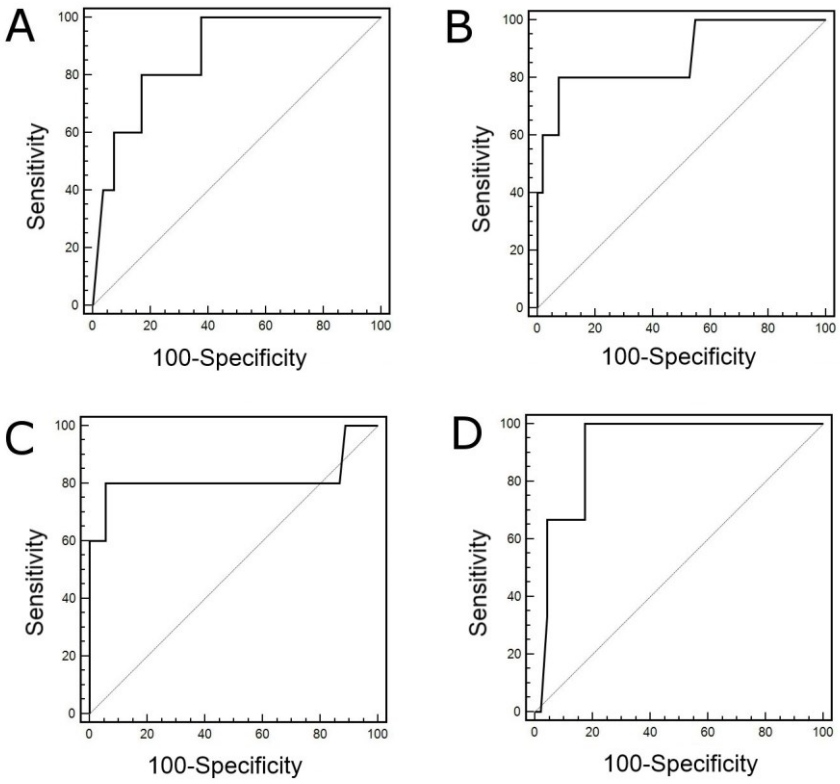

Figure 2. ROC curves representing diagnostic value of cytokine concentration in drain fluid as marker for development of anastomosis dehiscence (A – IL6 on day1, B – TNFα on day 1, C – TNFα on day 2, D – TNFα on day 4).

IL-6 values were higher in patients with dehiscence compared to those without dehiscence on the first postoperative day, indicating the possibility of early detection of dehiscence of anastomosis by measuring peritoneal cytokines. TNF-α was significantly elevated on days 1, 2 and 4 after surgery, while on the 3rd day the difference was not statistically significant. In one large study on 206 patients IL-6 and TNF-α were analyzed together with IL-10 and IL-8 on the first postoperative day and peritoneal IL-6 was shown to be significantly elevated in patients with anastomosis dehiscence, which is consistent with our results, but unlike our study, they did not show significantly higher TNF-α concentration in patients who developed anastomosis dehiscence.[7] This difference regarding TNF-α may be due to the significantly larger number of patients analyzed in this study compared to our study (206 vs 58 patients). In that study, cytokine concentrations in plasma were also analyzed and were shown to be significantly lower compared to peritoneal concentrations and with no predictive value for anastomosis dehiscence, which justifies the design of our study in which only peritoneal cytokines were analyzed. This is in line with several studies showing that intra-peritoneal cytokine response following abdominal surgery is both significantly more pronounced and clinically more important compared to systemic cytokine response.[15, 16] In another study on 137 patients IL-6 and TNF-α were analyzed on days 1, 3, and 7 following major abdominal surgery and IL-6 was significantly higher in patients with surgery complications on day 1, while TNF-α was shown to be statistically significantly higher in patients with surgery complications only on day 7 following surgery.[12] Several other smaller studies have also shown potential predictive value of peritoneal IL-6 and TNF-α for predicting anastomosis dehiscence.[3, 17-19]

In our study, IL-6 concentration peaked on day 1 after surgery and decreased on the following days, while TNF-α concentration was higher on day 4 compared to day 1 after surgery. Similar dynamics of Il-6 and TNF-α were shown in some other studies including the largest previous study that measured these cytokines on different days following surgery.[12, 17] However, several other smaller studies have shown different dynamics of these cytokines following surgery. Bertram et al have shown a continuous increase in TNF up to the 7th day after surgery, while IL-6 remained constant without significant change over time.[1] Yamamoto et al have shown an increase in both IL-6 and TNF-α from day 1 to day 3 in patients who developed peritonitis following colorectal surgery.[20] Ugras et al have shown an increase in both IL-6 and TNF-α from day 1 to day 5 in patients who developed anastomosis dehiscence following colorectal surgery.[10]

The major limitation of our study is the relatively small number of patients. Anastomosis dehiscence was confirmed in 9% of patients from our study, which is comparable with other studies. Still, with that rate of anastomosis dehiscence, the absolute number of patients who developed anastomosis dehiscence was only 5.

Differences in cytokine concentrations from literature may be due to different dehiscence definitions that vary between the subclinical and the one requiring surgical intervention, and the non-standardized inclusion criteria that differed from study to study. Drain location can affect the composition of the peritoneal fluid. Cytokine half-life is also very important for reliable measurement. In the analysis of peritoneal fluid, different methods were used. Unlike other cytokines, IL-6 has a longer half-life, which results in more reliable measurement.[21] In our study both IL-6 and TNF-α have shown predictive value as early markers of anastomosis dehiscence. However, due to its longer half-life and more reliable measurement, IL-6 has more potential to become part of routine clinical assessment.

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