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PREDICTIVE BIOLOGICAL MARKERS FOR ANASTOMOTIC LEAKAGE AFTER CURATIVE SURGERY FOR COLORECTAL CANCER

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Abstract: *anastomotic leakage is one of the most important postoperative complications for colorectal cancer patients undergoing curative surgery. Early recognition of patients in risk would be essential for preventing the high mortality rates associated with these complications. C reactive protein (PCR), tumour necrosis factor (TNF-alpha), cortisol levels and granulocyte/lymphocyte ratio (G/L) were compared in this period in patients who developed leakage vs patients without this complication. Material and methods: 52 colorectal cancer patients who underwent elective surgery in a private clinical hospital were evaluated preoperatively and postoperatively for the patients. Results: 14 patients (26,9%) developed clinically significant anastomotic leakage. The best cut-off value for preoperative G/L ratio of 5,8 had sensitivity of 71,43% and specificity of 73,68%. Conclusions: Preoperative G/L ratio can be used as a largely available tool for identifying the colorectal cancer patients at high risk for anastomotic leakage.*

INTRODUCTION

Anastomotic leakage is a severe complication after colorectal surgery associated with a high perioperative mortality and morbidity, prolonged hospital admittance and higher care expenses. This complication is also an independent risk factor for a reserved prognosis in patients undergoing curative colorectal surgery procedure, resulting in a higher chance of local recurrence and general survival. There are multiple associated risk factors for anastomotic leakage, and as a consequence it is complicated to predict this complication for a certain patient.

Despite evolution in understanding risk factors for anastomotic leakage and surgical technique development, anastomotic leakage remains an important complication that occurs in some patients without an evident cause and without any known risk factors. Early diagnosis of anastomotic leakage should be possible as a way to reduce morbidity and associated mortality.

The role of cytokine and Alpha-TNF, interleukin 1 and interleukin 6 in stimulation of production of acute phase protein is well-known and its release in the postoperative phase could be correlated with surgical stress extension and increased rate of complications.

Reactive C protein (PCR), is an acute phase protein - synthesized in the liver- which was intensively studied as a predictive marker for postoperative complication in abdominal surgery(1).

Due to relatively short half-life (19 h), PCR is a trustful marker for predicting systemic inflammatory response secondary to surgical procedures and even for complications, with a sharp drop in his rates as the patient recovers.(2) As for anastomotic leakage secondary to curative colorectal surgical procedure it is well known that increasing PCR levels in 2 – 3 days in postoperative period is associated with higher risk of developing this complication.(3)

Another marker for systemic inflammatory response could be leukocyte formula.(4) It is known that leukocytes express cholinergic and adrenergic receptors.(5) As a consequence, changes in the vegetative nervous system, as in stress inflammation may affect leukocytes that carry cholinergic and adrenergic receptors.(6) It is believed that during operation, neutrophils and lymphocytes are differently affected so they intermediate different actions.

An inborn immune answer against different stimuli has granulocytosis as response, and lymphopenia also observed in patients with cancer in advanced phases.(7) Granulocytes and lymphocytes show changes as a response to biochemical mediators and stress hormones equally in quantity and quality. Improving clinical status after surgical procedures also concur increasing lymphocyte level and decreasing granulocyte levels at the same time.(8)

Based on these observations it is reasonable to presume its clinical value for granulocytes / lymphocytes rates.

AIM

Under the circumstances we proposed that biomarkers identification which permit rapid patient check up for high risk of developing anastomotic leakage after a curative surgical procedure for colorectal cancer.

Analysing the current literature and the current routine available biological markers in our surgery department we proposed the next following markers in pre- and post-operative day 1: alpha TNF ,serum cortisol levels, PCR levels and the granulocytes/lymphocytes rates (G/L).

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MATERIALS AND METHODS

Our clinical study, a prospective observational cohort study, has been conducted in the Surgery Department of Oradea Pelican Clinical Hospital between January 2015-August 2019. The research has been analysed and approved by the hospital's ethics committee and in case of identifying an eligible patient, we proceeded to present and sign an informed consent protocol. Patients' inclusion criteria for colorectal cancer confirmed after colonoscopy and histological confirmed results.

Exclusion criteria: Age under 18, pregnant patient, evident distant metastasis, any pre-existing inflammatory bowel disease, or rheumatoid arthritis, clinical evident preoperative infection, postoperative infection from another proved source different form anastomotic leakage. The blood samples were taken a-jèun, by puncturing a peripheral vein. The blood sampling was made by blood prelevation from a vein in a vacutainer with anticoagulant for blood samples for whole blood count-leukocytes formula (for G/L ratio), and in vacutainer without anticoagulant in case of biochemical determinations such as alpha-TNF, serum cortisol levels and CPR levels. The following analysing methods were applied are automated analyser on fluorescent principle in cytometric flux using semiconductor LASER, and hydrodynamic focusing for complete blood count for leukocytes formula, chemiluminescent immunochemical detection method for alpha-TNF, latex immunoturbidimetric method for CRP, and immunochemical detection method by electrochemiluminescence for serum cortisol levels. Besides these lab findings, the study file has been completed with the following date for each patient enrolled: age, sex, environment, tumoral stage (Duke's classification), type of surgical procedure- classic or laparoscopic approach- and tumoral site. Anastomotic leakage was diagnosed by clinical findings of a peritonitis and/or evident free fecaloide liquid in abdominal cavity or on the drain tube confirmed through abdominal and pelvic CT with IV contrast substance or anorectal. The two groups were built based on this factor. Group A with anastomotic leakage and Group B without anastomotic leakage diagnosed.

Statistical analyses: Continuous variables will be presented under an arithmetic average, respectively geometrical one with a standard deviation - in brackets - having normal distribution some of them after a logarithmic transformation. Categorical variables will be described by number of observations and percentages in brackets-significant differences from statistical point of view where considered at lower values then 0.05 off zeros hypothesis(p). Statistical tests were done with the help of MedCalc° version 12.5.0.0 (MedCalc° Software, Mariakerke, Belgium). Comparison of the two groups for categorical variables has been done with chi square cast and chi square cast with Yates correction when used in table 2×2; and for continuous variables with the help of student test for independent groups (with or without logarithmic transformation as needed after case). For correlation check-up in between biological markers it has been used.

Pearson correlation factor. ROC curves (receiver operator characteristic) and the area under the curves (AUC) were used to compare diagnostic tests and for determination of limit values that indicates the risk of anastomotic leakage. ROC curves are points terminated by real positive values (sensitive) and false positive one (1-specificity) for each value detected (level of PCR or G/L rate).

Limitations of the study: The power of the study statistically depends on the number of cases for each group and the number of patients with postoperative complication. Fortunately, this number was not high, but this aspect of the study limits the importance of the conclusion referring to risk factors. Monitoring the evolution of biological markers during several days in the post-operative period would increase the precision of predictors for anastomotic leakage but also the correlation in between pre-and postoperative biological markers. It is possible that some patients may have a subclinical anastomotic leakage undiagnosed which may imply inevitable statistical error. But this subclinical anastomotic leakage usually has favourable evolution without treatment and the purpose of our study is to identify the patients with complications which in turn implies increased morbidity and mortality.

RESULTS

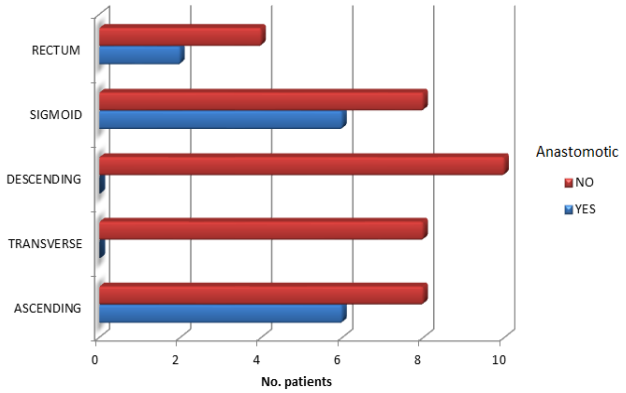
14 patients (26,9%) from 52 patients enrolled in the study, presented anastomotic leakage in the postoperative period. The main characteristic for the two groups are presented in the table no. 1.

Table no. 1. Main characteristic for two groups

Basic features	Lot A n=14	Lot B n=38	p (statistical significance)
Sex (M/F)	10/4	26/12	0,8964
Age (year) – average (±DS)	62,42 (±12,04)	70,26 (±11,31)	**0,0342**
Resident (U/R)	4/10	16/22	0,5697
Tumor stage (%) Dukes A Dukes B Dukes C	 2 (14,4%) 6 (42,8%) 6 (42,8%)	 11 (28,9%) 17 (44,8%) 10 (26,3%)	0,4070
Type of surgery Laparotomy Laparoscopy	 10 (71,4%) 4 (28,6%)	 14 (36,9%) 24 (63,1%)	0,0567
Tumor location Ascending colon Transverse colon Colon descending Sigmoid colon Recto-sigmoid colon	 6 (42,9%) 0 (0,0%) 0 (0,0%) 6 (42,9%) 2 (14,2%)	 8 (21,1%) 8 (21,1%) 10 (26,3%) 8 (21,1%) 4 (10,5%)	**0,0346**
M = male, F = female, DS = standard deviation, U = urban, R = rural			

The data presented indicates that neither the sex of a patient nor the environment does not influence the risk of developing anastomotic leakage, in post-operative period it is maintained the prevalence of male patients and patients from rural areas. Elderly patients seem to benefit from their age, this complication being more frequent in the young patients in our groups. The stage of developed cancer among the groups did not influence the postoperative complications. complication. In contrast, the tumour site has presented significant differences between patients who developed anastomotic leakage compared two those who did not have it: surgical procedures performed for tumours located on transverse and descending colon have not developed postoperative complications at all in this group (figure no. 1).

Figure no. 1. Incidence of anastomotic leakage for different locations of tumour



Determination of biological markers have been giving us the results in the two groups of patients (table no. 2). We can

easily observe, from a statistical point of view, that significant differences have been registered in determination of the postoperative period for PCR and the G/L rate in the preoperator period. All the other markers have not been valuable in identifying the patients with risk of developing postoperative anastomotic leakage.

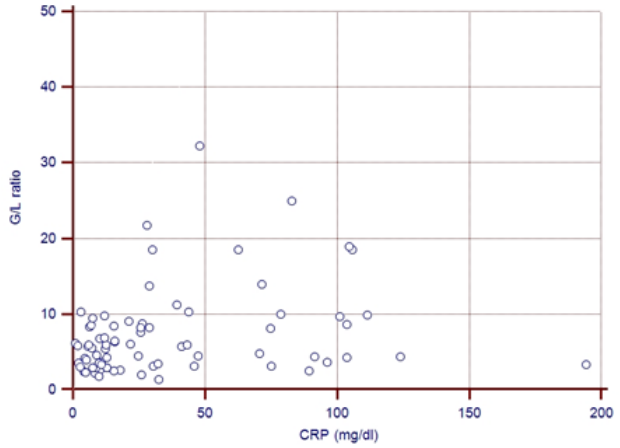
Knowing the role of PCR in identifying patients at risk for anastomotic leakage after surgical procedures and seeing the values of the results, we proceeded in analysing the correlation between the modification found in the WBC formula and the perioperative PCR value evolution considering all 104 prelevated biological blood samples. In these conditions we succeeded to demonstrate a strong linear correlation between these two values with an important statistical significance (r=0,3083, p=0,0015) (figure no. 2).

Table no. 2. Values of biological markers

Biological markers	**Lot A n=14**	**Lot B n=38**	**p (statistical significance)**
TNF-α – average (±DS) pg/ml			
preoperatively	14,41 (±3,2)	13,89 (±11,4)	0,2046
postoperatively day 1	8,87 (±1,6)	9,75 (±8,9)	0,5714
Cortisol – average (±DS) nmol/l			
preoperatively	153,99 (±86,3)	143,63 (±137,2)	0,7942
postoperatively day 1	119,40 (±70,0)	133,24 (±150,8)	0,7440
PCR – average (±DS) mg/dl			
preoperatively	21,66 (±11,5)	17,80 (±21,2)	0,5232
postoperatively day 1	87,67 (±53,8)	54,69 (±37,7)	**0,0165**
G/L ratio – average (±DS)			
preoperatively	10,09 (±7,3)	4,68 (±2,1)	**0,0031**
postoperatively day 1	10,94 (±6,6)	9,34 (±7,8)	0,1945

TNF-α = tumour necrosis factor alpha, DS = standard deviation, PCR = C-reactive protein, G/L = granulocytes/lymphocytes

Figure no. 2. Correlation between pcr and g/l rate in perioperative period



Comparison of the two diagnostic tests for postoperative anastomosis leakage has been accomplished by comparing the two ROC curves (receiver operating characteristic) and AUC (area under curve), meaning the curve for post operative G/L rate and postoperative day 1 PCR level. The results indicated that these two tests are almost identical for G/L rate =0,692, as for PCR= 0, 691 (p=0,9999). For a more accurate identification of the patients with an increased risk for postoperative complication, we have tried to identify some limiting values for these two markers. Analysing ROC curves for both determinations, we found the following limits with best values for sensibility and specificity: for PCR the limit of 71 mg/dl with an sensibility of 71,43% (IC 95%: 41,9-91,6) and specificity of 68,42% (IC95%: 51,3-82,5); for G/L rate the 5,8 units with sensibility of 71,43% (IC 95%:41,9-91,6) and specificity of 73,68% (IC 95%: 56,9-82,5).

Moreover, we made the observation that all patients with GL rate above 10 units in the postoperative period developed anastomotic leakage. Combining the two criteria (G/L rate above 5,8 and postoperative day 1 PCR above 71 mg/dl) does not come with benefits in the sense of increased sensibility, but it becomes an excellent excluding test with a 96,97% of specificity (IC 95%: 84,24-99,92%) and negative predictive value of 80% (IC 95%:64,3590,95).

DISCUSSIONS

The incidence of anastomotic leakage after colorectal cancer surgery varies between 1% and 40% depending on the definition of leakage and the type of resection.(8,9) The incidence observed in our study is 26.9% which is in the average range. This complication is frequently associated with a high mortality rate between 4% and 15% and under these circumstances the early diagnosis is very important. But an early diagnosis of an anastomotic leakage is not always easy in immediate postoperative period due to reduced clinical evidence in this period, a fact that may contribute to an increased mortality. The presence of respiratory, neurological and abdominal symptoms will not allow the early diagnosis of anastomotic leakage, because these symptoms usually appear with the beginning of the 4th day after surgery (11) and fever and abdominal sensibility are not specific signs for anastomotic leakage being frequently present due to other causes in the immediate postoperative period.(8) According to Alves & Amp (12) a late diagnosis of anastomotic leakage (after the 5th day of surgery) is associated with a mortality of 18%, but diagnosed and treated earlier, mortality could decrease under 1%. As a consequence, early detection and treatment of anastomotic leakage is essential and makes the early biological markers become very useful. Tissue ischemia of the suture line at the level of the anastomosis appears to be responsible for early inflammatory response with a release of acute phase proteins (such as PCR).(13,14) Decrease of pH at the level of mucosa in the anastomotic suture line in the first 24-hours after the surgery increases the risk of dehiscence (15) and sustains the theory that inadequate perfusion in the anastomosis appears in the early phase and increases the risk of complications.

Exponential increase of PCR level in 2-3 days after surgery indicates an increased risk of complications at the level of the suture in patients with other excluded infectious causes (respiratory, urinary or suture cause) (3), but we wanted an earlier marker detection even in the preoperative period which could predict an unwanted evolution of the anastomosis. We have found that G/L rate increases in the preoperative period can serve as an indicator for anastomotic leakage with a comparable power as the levels of PCR in post-operative period: a sensitivity of 70 to 80% and a specificity of 80 to 86% for PCR above of 140 mg/dl in the postoperative 3rd day(3,13) versus sensibility of 71,43% and specificity of 73.68% for G/L rate above 5,8 unites postoperative.

Considering observations tied to modified WBC formula in different types of stress and inflammation correlated with results of our study we can presume that an increased preoperative G/L rate could exist in the presence of a local subclinical inflammation that marks the evolution of

anastomosis in the postoperative period.

CONCLUSIONS

Early identification of patients with colorectal cancer exposed to anastomotic leakage risk may be careful check-up of biological markers in the immediate postoperative period and early treatment considerably decreasing the mortality due to this complication. Our study brings attention to the importance of GL rate as a marker of inflammation immediately available and accessible. Reprogramming patients seems reasonable if a G/L rate is greater than 10 before surgery.

Conflict of interest: *No conflict of interests.*

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