ENGLISH VERSION: PATHOPHYSIOLOGICAL MECHANISMS OF DEHISCENCE OF ANASTOMOSE SUTURES IN PATIENTS WITH ONCOPATHOLOGY*

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It should be recognized that the creation of optimal conditions for healing of intestinal sutures is the main reserve for improving the results of surgical intervention on the organs of the gastrointestinal tract. The urgent issues of pathophysiology associated with oncopathology remain the inability of seams of anastomosis and stomaty. The purpose of the work is to determine the pathophysiological mechanisms and criterion-significant indicators of inability to sew anastomosis. In the study of this issue, an analysis of the immediate results of treatment of 74 patients, which was a test group, which operative treatment was performed in a radical volume. Patients were divided into two groups according to the anastomosis failure: Group Ia - patients who had been operated on for gastric cancer and who had anastomosis failure - 16 patients, II group 58 patients - who had been surgically inoperative and had not been diagnosed insolvency The patients of each group were divided into four subgroups, depending on the presence of instability of microsatellites in the locuses of the VAT-25, VAT-26. The presence of Streptococcus Bovis stomach cancer patients may be an early marker of disease progression. The leading metabolic profile of microbiocenosis in the development of stomach cancer is the significant accumulation of biogenic amines. The analysis of the results of the study shows that the failure of anastomosis in patients with gastric cancer is accompanied by profound disorders of the metabolism of connective tissue and is confirmed by increased activity of elastase, collagenolithic activity of blood serum and its content of glycosaminoglycans. The activity of elastase and glycosaminoglycans may be a prognostic criterion for insufficiency of seams in the course of treatment.

Key words: microsatellite instability, inability of seams of anastomosis, microbiocynosis.

Introduction

The urgent issues of pathophysiology associated with oncopathology remain the dehiscence of sutures of anastomoses. This complication is observed in 2-3.5% of cases with surgical interventions for gastric cancer in 9% with operations on thin and in 5-25% of cases with operations on the colon [1-4]. The probability of failure increases with the formation of anastomoses in conditions of the altered intestinal wall, which is observed in oncopathology of the intestinal-gastrointestinal tract. It should be recognized that the creation of optimal conditions for healing of intestinal sutures is the main reserve for improving the results of surgical intervention on the organs of the gastrointestinal tract [5, 6].

The integrity of surgical sutures depends on a number of reasons, both from the organs themselves and from non-organic changes. It is possible to distinguish between three main groups of causes that affect the integrity of anastomosis and stomata in oncopathology: the state and pathomorphological processes that occur in the organs subject to anastomosis; the adverse factors upon which these sutures are superimposed or the adverse factors that arise in the postoperative period; technical features of suture joints.

The first group of causes is, of course, the leading in terms of pathophysiology, because the vitality of the organ's wall primarily affects the ability of seagrass and anastomoses. These include: active tissue inflammation; intravenous and general circulation disruption; increased intracranial pressure; hypoproteinemia; local infection.

In experimental studies [7], devoted to the study of healing of anastomosis, an important role of collagen in the formation of a community was determined. Thus, in the first days after surgical intervention there is a massive collagen lysis in the anastomosis zone, and the processes of its synthesis are suppressed. Therefore, "collagen equilibrium" is crucial for maintaining the integrity and tightness of the intestinal suture. Infection of the suture zone leads to a significant increase in collagen lysis and dehiscence.

Another important factor that reduces the strength of the gastric and intestinal soybeans is the infection of the tissue itself that is anastomosis [5, 8, 9]. Infection occurs as a result of the contact of the suture channels and suture material (ligaturn infection) with the lumen of the body and its contents, which causes the penetration of the microflora into the thickness of the sewn tissue with the subsequent development of inflammatory and necrotic processes in them.

In the area of anastomosis in the early stages, there are always favorable conditions for the development of the microflora – the presence of ischemia, nutrient medium in the form of blood balances, changes in pH, oxidation-reducing potentials. Therefore, the infection of the anastomosis zone is a natural process and depends on the type of intestinal sutures and concentration of microbes, their type and interaction in the organ's lumen.

The **purpose** of the work is to determine the pathophysiological mechanisms and criterion-significant indicators of anastomosis sutures dehiscence.

Materials and methods

In the study of this issue, an analysis of the immediate results of treatment of 74 patients, which was a test group, which operative treatment was performed in a radical volume. Distribution of patients depending on the stage – T2-3 N0 M0 – 17.5%, T2-3 N1-2M0 – 73.2%, T4 N1-2M0 – 9.3%. Most patients (77%) had adenocarcinoma. Squamous cell carcinoma has been found in 13.5% of cases. In all patients, the diagnosis of cancer was morphologically verified before surgery.

Patients were divided into two groups according to the anastomosis failure: Group Ia – patients who had been operated on for gastric cancer and who had anastomosis failure – 16 patients, II group 58 patients – who had been surgically inoperative and had not been diagnosed with dehiscence. The patients of each group were divided into four subgroups, depending on the presence of instability of microsatellites in the locuses of the VAT-25, VAT-26 (Table 1).

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Table 1
Distribution of patients depending on the stability of microsatellites VAT-25, VAT-26 and the state of anastomosis sutures.

Group patients n	Stability of microsatellites, n							
	VAT-25 – VAT-26 – n=35		VAT 25 – VAT 26 + n=10		VAT 25 + VAT 26 – n=20		VAT 25 + VAT 26 + n=5	
	χ^2=5.85*		χ^2=2.74		χ^2=3.52		χ^2=0.31	
I-group n=16	8 (62.86%)	3 (14.29%)	1 (10.00%)	2 (20.00%)	1 (5.00%)	0 0	1 (20.00%)	0 0
II-group n=58	8 (8.57%)	14 (14.29%)	17 (60.00%)	6 (10.00%)	0 0	12 (20.00%)	0 0	1 (5.80%)

The studies were carried out on dewaxed sections with a thickness of 4-5 microns, with the previous antidamask decontamination in citrate buffer (pH 6.0) in a microwave oven with a power of 600 W for 10 minutes. An EnVision system (Dako Cytomation, Denmark) was used to visualize the reaction products.

Microsatellite instability was evaluated using a polymerase chain reaction using two quasimomorphic monocellular markers, VAT-25 and VAT-26.

Polymerase chain reaction was carried out according to the standard scheme on the thermocycler program Tercyk-2 of DNA production.

The following primers for the Wat-26 microsatellite sequence were used: 5'-TGA CTA CTT 'TGG ACT TCA GCC-3';

5'-AAC CAT TCA ACA TTT TTA ACC C-3 ', and

5'-TCG CCT CCA AGA ATG TAA GT-3 'and

5'-TCT GCA TTT TAA CTA TGG CTC-3 'for VAT-25:

The PCR result was evaluated in 8% polyacrylamide gel, followed by coloring in a solution of bromide ethidium at a concentration of 1 mg/ml. As a marker of molecular weight, DNA plasmids puc19, hydrolyzed with the enzyme HpaII, were used.

Criteria for the hypothesis: – there are no statistically significant differences in the effectiveness of the drug and there are: statistically significant differences in the effectiveness of the drug and present.

Examination of hypotheses is carried out by comparing the value of the criterion calculated by the formula with the critical value determined by the table for a given number of degrees of freedom and equal significance p <0.05. If at that, then the hypothesis about the independence of treatment outcome from therapy with a 95% reliability is rejected. Otherwise, this hypothesis is accepted.

The blood plasma determines the content of glycosaminoglycans, the activity of the enzyme elastase, and the collagenolytic activity (CLA) of the plasma, which can provide useful information on the state of structural and metabolic and metabolic processes in the connective tissue. Blood plasma CT scan was performed on the total amount (in enzymatic hydrolysis) of free and peptide-bound oxyproline in a diagnosed sample. To do this, they found, according to the calibration curve, the optical density of the value of the amount of oxyproline at a wavelength of 570 mk. The blood plasma levels were expressed in micromoles of oxyproline by 1 liter of blood plasma for 1 hour (μmol/l g) [12-15].

Total glycosaminoglycans (sour mucopolysacharides) were determined in blood serum using trichloroacetic acid and a known carbazole reaction, which provides violet-pink coloration. The photometric measurements of the samples under investigation were carried out at a wavelength of 530 nm. The content of GAG in serum was expressed through hexuronic acids in micromoles / liter. (μmol/L) [16].

Blood elastase was tested by immunoassay using monoclonal antibodies and a set of reagents (Human PMN Elastase Elisa RD 191021100) according to the instructions of the company Biovendor, added by Germany.

In order to find out the degree of intestinal dysbiosis, common bacteriological methods were determined in feces of bifidobacteria, bacteroids, lactobacilli, escherichia, proteins, sinus spider, staphylococci, enterococci, clostridia, yeast mushrooms, and peptostreptococci [11-12]. Metabolites of intestinal microbiocenosis (acetic, propionic, oily, lactic, oxalic-acetic, β-ketoglutaric acid, phenylpropionic acid, n-cresol, skatole, indole) were studied by gas-liquid chromatography on a chromatograph "Color 1000". Determination of metabolites in the extract of feces was were studied by the method of immunoenzymatic analysis using the immune enzyme kits of the company "Labor Diagnostika Nord" (Germany), provides a high specificity of extraction, which is a necessary condition for the specificity of the analysis itself.

Statistical hypotheses about the presence or absence of significant differences in the results of drug treatment for different variants of the biological status of patients were verified using a four-cell – criterion [14].

Results and discussion

We believe that an important pathophysiological factor in the failure of anastomoses, reducing the strength of the gastric and intestinal anastomosis is infection of the tissue zones that anastomoses. In this regard, the purpose of this subsection study was to investigate microbiological profile intestine in patients with gastric cancer and its prognostic value in the diagnosis, treatment and prevention of cancer morbidity.

Results of the study of intestinal microbiocenosis in patients with gastric cancer, shown in Table 2.

Table 2
The state of microbiocenosis in the intestine in patients with stomach cancer.

Types of microorganisms KUO for 1 g feces (Lg)	Patients group (n= 74)		Group health patients (n=30)
	I – group (n= 16)	II- group (n= 58)	
Bifidobacterium	3.06±0.31 *	4.27±0.45 *	9.85±0.54
Bacteroides	1.80±0.25 *	3.25±0.50 *	10.22±0.64
Lactobacillus	2.58±0,18 *	4.08±0.20 *	6.95±0.50
Escherichia:	12.00±0.65	10.04±0.65	9.25±0.61
Lacto-positive	9.15±0.38 *	7.63±0.40 *	6.45±0.35
hemolitical	3.72±0.24 *	2.83±0.27 *	1.15±0.12
Enterobacteriaceae	2.15±0.22 *	2.95±0.21 *	4.27±0.25
Pseudomonas aeruginosa	3.75±0.28 *	1.20±0.17 *	0
Enterococcus:	8.05±0.41 *	6.95±0.54 *	5.67±0.33
hemolitical	2.80±0.33 *	1.65±0.28 *	0
Staphylococcus:	8.25±0.94 *	6.55±0.53 *	2.54±0.31
Coagulo-positive	3.55±0.34 *	1.74±0.29 *	0
Streptococcus bovis	4.07±0.20 *	2.22±0.18 *	0
Peptostreptococcus	7.28±0.52 *	6.45±0.37 *	4.37±0.29
Clostridium	8.36±0.78 *	5.12±0.60 *	3.26±0.38
Candida albicans	3.66±0.23 *	2.05±0.21 *	0
Helicobacter pylori	1.97±0.23 *	1.25±0.23 *	0

Note: Lg is the decimal graph of the KUO / g

It was found that there was a significant decrease (p≤0.05) in the reduction of lactobacter bifidobacteria, bacteroids and citrate synthesizing enterobacteria (Table 2), respectively, by 6.3%, 19.8%, 65.1% and 53.7%, which form normal micro-flora of the intestinal-gastrointestinal tract in all patients with cancer of the stomach.

This dynamics of the process is trodden against the background of an increase in the total number of escherichia coli by 29.3%, the overwhelming majority of which were hemolytic forms. Of non-fermenting microorganisms, an increase in coca flora was observed – Staphylococcus at 185.2%, Peptostreptococcus by 41.4%, Enterococcus by 30.5%; Clostridium by 113.3% and Candida albicans by 273.6%.

In patients with gastric cancer among the conditionally pathogenic microorganisms the leading role is played by fungi of the genus Candida albicans, hemolytic Enterococcus, Pseudomonas aeruginosa, coagulase-positive Staphylococcus (Table 2).

When comparing microbiocinosis in gastric cancer patients undergoing surgical intervention, a significant difference was found between the groups. Thus, in patients of Group II, a significant decrease (p≤0.05) in the reduction of lactobacter bifidobacteria, bacteroids and citrate synthesizing enterobacteria, and an increase in hemolytic Enterococcus, Pseudomonas aeruginosa.

Particular attention should be paid to the fact that in patients with gastric cancer, the detection of Helicobacter pylori (92.6%) is significantly (p≤0.05). Also, the determination of 82.5% of Streptococcus bovis stomach cancer patients may be an early indication of carcinogenesis and anastomosis failure. The data we receive are consistent with studies, including clinical ones, by many scientists who have been aimed at the detection of microorganisms involved in the development of gastrointestinal cancer, which resulted in the identification of a number of bacteria potentially involved in the development of the disease: Streptococcus bovis, Helicobacter pylori and others.

Determination of metabolic indicators of microbiocenosis in patients with gastric cancer revealed a significant (p≤0.05) decrease in the content of carboxylic acids: acetic, propionic, oily and lactic acid, respectively, at 72.3%, 57.8%, 64.1% and 75, 6%, which is consistent with the decrease in the amount of anaerobic intestinal microflora (lactobacter bifidobacteria, bacteroids). A reliable (p≤0.05) decrease in the levels of β-ketoglutaric and oxalic acetic acid was found to be 49.8% and 60.6%, which confirms the weak biochemical activity of aerobic and anaerobic bacteria, especially in relation to digestion of carbohydrates (Table 3) .

Table 3

Indicators of metabolic activity of microbiocenosis in patients with stomach cancer

Indicators of metabolic activity (mg/L)	Group patients, M±m		
	Research group (n= 74)		Group health patients (n=30)
	I- group (n= 16)	II-group (n= 58)	
Carbonil acid: - acetic	345.62±15.38*	557.65±18.20*	1425.10±19.35
-propionic	69.65±5.35*	80.14±8.25*	200.85±15.71
-olio	54.27±4.52*	60.33±3.54*	151.34±7.23
-dairy	86.33±7.14*	108.11±5.75*	390.45±11.82
Carboxylic acids: -e-ketoglutarate	68.34±4.92*	80.14±4.23*	145.20±7.92
-shaft-acetic	8.33±6.15	10.42±5.65	20.05±1.98
Aromatis: - n-crezol	5.44±0.16*	3.13±0.12*	1.12±0.05
-indol	6.07±0.13*	4.27±0.15*	1.34±0.04
- scatol	6.53±0.14*	3.60±0.13*	1.29±0.03
-phenylpropionic acid	4.96±0.37*	2.85±0.29*	1.15±0.012
Amins: -metilamin	2.53±0.22*	1.28±0.19*	0.32±0.014
- histamine	2.66±0.18*	1.17±0.10*	0.28±0.018
-serotonin	8.24±0.57*	4.03±0.36*	1.62±0.15

*Note: * the difference is likely to be P <0.05*

The profile of intestinal metabolites was characterized by an increase of more than 3.2 times in the total amount of indole, skatole, n-cresol, and phenylpropionic acid in patients of the 1st group. The total amount of aromatics increased by 278%, n-cresol by 315%, indole by 365%, and phenylpropionic acid by 254%. This process dynamics should be taken into account when choosing a surgical intervention tactic, especially in the formation of a thin-intestinal reservoir or anastomosis, as these changes may lead to the development of general intoxication and the occurrence of complications such as dehiscence of sutures.

The research program included studying the state of connective tissue in patients with gastric cancer in order to determine the pathophysiological mechanisms for the formation of the anastomosis failure, given that it plays a crucial role in maintaining the integrity and tightness of the intestinal sutures.

The study of the parameters of connective tissue showed a significantly higher activity of elastase in patients of the 1st group on stomach cancer, regardless of microsatellite instability (94.1±14.4 pg/ml), exceeding the conditionally healthy observation group, respectively, in 4 times. However, when comparing the levels of elastase in the groups, it was found that in the 1st group this figure was increased by 2.8 times as compared with the control group and had a significant difference when compared with patients in the second group, in which there were no phenomena of anastomosis failure (Table 4).

At the same time, it should be noted that GAG was greatly increased in blood plasma in the I group of patients with stomach cancer (72.9±4.0 micromol/L), which was by 2.2 times higher, respectively, the value of the group conditionally-healthy patients. In patients of the 2nd group with gastric cancer, GAG levels did not increase significantly, although they were significantly different as compared to the conditionally healthy group.

The collagenolytic activity of the blood plasma had a similar dynamics and did not depend on the levels of cryoglobulins and microsatellite instability. It had a direct strong correlation with the indicators of elastase activity. The value of CLA in patients with gastric cancer was 68.5±3.4 μmoles oxyproline /l/g, blood glucose levels in excess of conditionally healthy patients by more than 5 times, which provided an important diagnostic and prognostic value for this indicator.

Table 4

Dynamics of biochemical parameters of the state of connective tissue in patients with gastric cancer, depending on the condition of anastomosis sutures.

Research group	Indicators, M±m		
	Elastase (pg / ml)	GAG (micromol/L)	CLA(μmoles oxyproline /l/g)
I group – the presence of dehiscence of sutures (n= 16)	94.1±14.4 *	72.9±4.0 *	68.5±3.4*
II-g group – with the absence of signs of dehiscence of sutures (n= 58)	71.6±4.8*	42.9±2.2*	49.7±3.2*
Group of healthy patients (n=30)	27.3±1.8	35.84±1.2	men. – 7.3±0.56* fem. – 7.6±0.43*

*Note: * probability is reliable P <0.05*

Conclusions

Taking into account the aforementioned, it can be concluded that gastric cancer patients have intestinal dysbiosis characterized by inhibition of protective and activation of opportunistic microflora against the background of digestion disorders of carbohydrates, fats, proteins and accumulation of toxic exchange products, which are an important pathogenetic factor of activation, induction, proliferation and metaplasia of the tumor tis-

sue. The presence of Streptococcus bovis stomach cancer patients may be an early marker of disease progression.

The leading metabolic profile of microbiocenosis in the development of stomach cancer is the significant accumulation of biogenic amines, which in turn may have a prognostic value for diagnosis, and the determination of pathogenetic therapy in patients with gastric cancer. The results of the study of intestinal microbiocenosis in patients with gastric cancer testify to the violation of interspecific ratios of the microflora that populate the intestine in normal conditions. Reducing the level of lactobifid bacteria and bacteroids, which in the process of life form a milk, acetic, anthraquine, succinic acid, may be one of the most important causes of changes in the trophic, protective, metabolic and immunological function of the gastrointestinal tract, due to the change in intestine of pH medium.

The analysis of the results of the study shows that the failure of anastomosis in patients with gastric cancer is accompanied by profound disorders of the metabolism of connective tissue and is confirmed by increased activity of elastase, collagenolithic activity of blood serum and its content of glycosaminoglycans. The activity of elastase and glycosaminoglycans may be a prognostic criterion for dehiscence of sutures in the course of treatment.

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