

ORIGINAL ARTICLE

INDICATORS OF THE CELL-MEDIATED IMMUNITY IN SURGICAL PATIENTS WITH GASTRODUODENAL ULCERS COMPLICATED BY BLEEDING

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ABSTRACT

The aim: To determine the peculiarities of indicators dynamic of cellular level of immunity in patients with gastroduodenal ulcer complicated by bleeding and microbial contamination the periulcerous area.

Materials and methods: 80 patients with gastroduodenal ulcers complicated by bleeding were studied. Among patients in the main group, 18 (22.5%) cases were patients with bleeding gastric ulcer, 56 (70%) patients with bleeding ulcer of duodenum. The study of cellular immunity are B - CD19, Natural killers(NK) - CD16, T-helpers - CD4, T-regulatory - CD8.

Results: An active bleeding according to the Forrest classification in patients with gastric ulcer 81.7% and in patients with ulcer of the duodenum 88.3%. Depending on the endoscopic hemostasis, there was a marked decrease in T-lymphocyte indices with CD4 growth to the reference rates in cases of active bleeding (from $13 \pm 2.4\%$ to $26.4 \pm 1.2\%$ $p < 0.05$) and in hemostasis with high recurrence rate from $20.8 \pm 2.3\%$ to $31.3 \pm 2.9\%$ and from $22.6 \pm 1.3\%$ to $39.8 \pm 2.9\%$ $p < 0.05$, respectively.

Conclusions: The study found a decrease in CD16 from $3.6 \pm 1.1\%$ to $2.6 \pm 1.1\%$ ($p < 0.05$) and an increase in the level of CD4, CD8, CD19 to $37.4 \pm 1.1\%$, $4.4 \pm 1.1\%$ ($p < 0.05$) and $30.8 \pm 1.2\%$ ($p < 0.05$), respectively, indicating that there is a decrease in the immune response in patients with gastroduodenal bleeding of ulcerative genesis.

In patients with microbial contamination of the periulcerous area by *Streptococcus* β -haemolyticus (107 CFU) and *Klebsiella pneumoniae* (106 CFU), gastric ulcers decreased the level of CD8 population from $7.3 \pm 2.1\%$ to $7.3 \pm 2.1\%$ to $6.4 \pm 7\%$ ($p < 0.05$) and from $5.8 \pm 2.8\%$ to $4.1 \pm 2.3\%$ ($p < 0.05$), respectively, which may create favorable conditions for the development of recurrent bleeding.

KEY WORDS: gastric ulcer, duodenal ulcer, gastroduodenal bleeding

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INTRODUCTION

One of the current problems of urgent surgery is the problem of treating patients with peptic ulcer of the gastroduodenal zone according to Shepetko E.M., Trofimov M.V., Chukhriienko A.V. [1,2].

At the present stage of development of medicine, the use of endoscopic hemostasis in patients with ulcerative bleeding stomach and duodenum requires a more in-depth understanding of the features of the blood supply and atrophic changes of the mucous membrane of the gastroduodenal zone. Without evaluation of the cellular and humoral immunity level in surgical patients with gastroduodenal ulcers complicated by bleeding, it is impossible to make a comprehensive analysis of the morphological changes of these parameters [3].

THE AIM

To determine the peculiarities of dynamics of indicators of cellular level of immunity in patients with gastroduodenal ulcers and microbial contamination in the periulcerous area, complicated by bleeding.

MATERIALS AND METHODS

80 surgical patients with gastroduodenal ulcers with bleeding complications were examined and treated in SE "Dnipro City Hospital of Ambulance". The inclusion of patients into groups took place on a randomized system. The criterion for inclusion in the study is the ulcer of the gastroduodenal zone, which is complicated by the development of bleeding. The exclusion criterion was the presence of a confirmed malignant neoplasm of any localization in the patient. All patients gave their written agreement and permission to participate in the study. Patients of the experimental group on the first and third days of the disease in the morning on an empty stomach had their blood samples taken by puncture of the elbow vein. As a control group, we used indicators of the immune system of a group of people – healthy donors, who by age, gender, methods of determining the indicator were similar to the study group. Number of controls – 17 patients.

Among the studied patients, 18 (27.5%) cases were patients with bleeding gastric ulcer, 56 (70%) patients had duodenal bleeding ulcer and 6 (7.5%) patients were with combined bleeding gastric and duodenal ulcer.

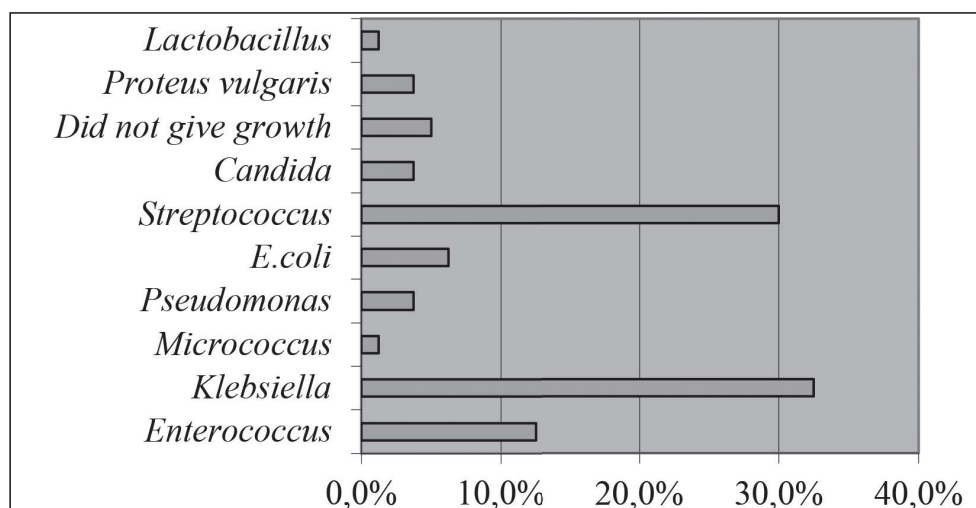


Fig. 1. The nature of the microbial picture in patients with bleeding gastroduodenal ulcer.

Having analyzed the distribution of patients by gender, we established the following distribution: men - 52 (65%) cases, women - 28 (35%) cases. The highest number of male patients was found in the group of patients with bleeding gastric ulcer - 12 (66.7%) patients, in the group of patients with bleeding ulcer of the duodenum, men accounted for 34 (60.7%) cases, and in the group of patients with combined gastric and duodenal ulcer and the patients of both sexes were equal - 3 (50%) patients.

As a result of the analysis of the distribution of patients by age, it was found that the average age of patients was 57.7 ± 2.7 years, the youngest patient was 18 years old, and the oldest patient was 91 years old.

The analysis of the distribution of patients by the degree of blood loss according to the classification of the American Association of Surgeons (1998) found that severe degrees of blood loss (large and massive) were determined in 42 (52.5%) cases. The most severe course of the disease was established in patients with bleeding ulcer of the duodenum - the number of patients with severe degrees of blood loss was 31 (55.4%) cases, in the group of patients with bleeding gastric ulcer this figure was 5 (41.6%), and in combined bleeding gastric and duodenal ulcer, this figure was 2 (33.3%) patients.

Microbiological examination of the biopsy material of the mucosa of the periulcerous area of the ulcer was also performed in patients.

Investigation of the microbiological content of the periulcerous zone was performed by analyzing the mucous membranes of the specified area. The selection of gastric mucosa and duodenum biopsy specimens for microbiological examination was performed with the exception of the ingress of foreign microflora into the material. Before the start of the examination, the outer surface of the endoscope and the instrument channel were treated with a solution of lysoformin. After 10-15 minutes, the instrument channel was washed three times with the 70% ethyl alcohol solution, and then three times with sterile distilled water. The cable with biopsy forceps was pre-immersed in a solution of lysoformin for 30 minutes. The outer opening

of the instrumental canal at its distal end was closed with a special sterile lid that prevented entry into the canal of mucus, saliva, gastric contents, which contained foreign microorganisms. The lid was inserted into the opening of the canal so tightly that it could be pushed out by a cable of forceps. Each biopsy was subsequently placed in a sterile test tube with 5 ml of sterile saline solution. The tube was shaken vigorously to wash from the surface of the microbiota biopsy specimen, and the bioplate was transferred to the next sterile tube. The procedure was performed 5 times. Five times the washed biopsies were collected with a sterile bacteriological loop, transferred into sterile bacteriological porcelain mortars and, following the aseptic rules, thoroughly homogenized using a pestle, adding 2-3 ml of broth. The homogenate from each esophageal or gastric mucosa biopsy was seeded sequentially with a Pasteur graded loop separately into different nutrient media. The content as then incubated in a thermostat at 37°C daily; they were further stained with Gram staining and examined under an Axioplan microscope (Carl Zeiss, Germany). The degree of bacterial contamination was determined using the quantitative method of sector content. Endo medium and 5% blood agar were used to isolate anaerobic microorganisms [4].

The study of the cellular immunity was carried out with the help of specific monoclonal CD sera against each type of lymphocytes: B - CD19, Natural killers (NK) - CD16, T-helpers - CD4, T-regulatory - CD8 produced by company Immunotech SA, Beckman Kulter Company (France). Determination of the type of immunocompetent cell was performed using laser flow cytometry using a FACS Calibur apparatus (BD, USA) [5,6]. As reference values, we used appropriate indicators from a healthy donor group that were similar in age, gender, and sampling methodology to the study group. Statistical analysis was performed using Statistica for Windows 13 (StatSoft Inc., No. JP-Z8041382130ARCN10-J). The normality of the distribution of quantitative traits was estimated using the Shapiro-Wilk W. test. Relative frequencies were given with standard error S (in %). The significance of the differences in the quanti-

Table I. Condition of CD-16 and CD-19 populations at different degrees of blood loss.

Degree of blood loss	CD-19 (%±S)		Control n=17	CD-16 (%±S)		Control n=17
	I sample	II sample		I sample	II sample	
Mild (n=27)	12.4±1.1 $p_k<0.05$	20.4±1.2 $p_k<0.05$, $p_l<0.05$	22.5±0.09	3.6±1.1 $p_k<0.05$	2.6±1.1 $p_k<0.05$, $p_l>0.05$	5.6±0.03
Moderate (n=11)	15.2±1.4 $p_k<0.05$	17.2±5.45 $p_k<0.05$, $p_l<0.05$		1.6±0.35 $p_k<0.05$	1.2±0.5 $p_k<0.05$, $p_l<0.01$	
Severe (n=28)	13.8±7.3 $p_k<0.05$	20.9±11.7 $p_k>0.05$, $p_l<0.05$		1.9±0.6 $p_k<0.05$	1.9±1.02 $p_k<0.05$, $p_l<0.05$	
Massive (n=14)	20.6±11.2 $p_k>0.05$	30.8±1.2 $p_k>0.05$, $p_l>0.05$		3.3±1.1 $p_k<0.05$	3.6±1.1 $p_k<0.05$, $p_l>0.05$	

Remarks:

- 1) p_k – is the reliability of the divergence of indicators compared to the control group;
- 2) p_l – is the reliability of the divergence of indicators compared to the first study;
- 3) % ± S – percent ± standard percentage error.

Table II. Condition of CD-16 population status at different local endoscopic hemostasis.

Conditions of the local endoscopic hemostasis	CD-16 (%±S)		Control
	I sample	II sample	
Forrest IA (n=21)	3.1±1.9 $p_k<0.05$	2.9±1.8 $p_k<0.05$, $p_l<0.05$	5.6±0.03
Forrest IB (n=3)	1.7±1.3 $p_k<0.05$	1.5±0.4 $p_k<0.05$, $p_l<0.05$	
Forrest IIA (n=30)	3.2 ± 1.3 $p_k<0.05$	1.7 ± 1.2 $p_k<0.01$, $p_l<0.05$	
Forrest IIB (n=19)	3.1 ± 1.3 $p_k<0.05$	2.8 ± 1.3 $p_k<0.01$, $p_l<0.05$	
Forrest IIC (n=7)	3.5±1.8 $p_k>0.05$	3.6±1.1 $p_k<0.05$, $p_l<0.05$	
Forrest III (n=7)	4.1±1.5 $p_k<0.05$	5.3±1.2 $p_k<1.9$, $p_l<0.05$	

Remarks:

- 1) p_k – is the reliability of the divergence of indicators compared to the control group;
- 2) p_l – is the reliability of the divergence of indicators compared to the first study;
- 3) % ± S – percent ± standard percentage error.

tative variables between the two independent groups was assessed using the Mann-Whitney test. The relationship between the two quantitative variables was analyzed by the Pearson pair correlation method. Difference of mean values of indices was considered reliable at $p<0.05$ [7].

RESULTS

Further investigation of CD4 in patients with gastroduodenal ulcers complicated by bleeding revealed that with a slight blood loss there was a decrease in CD4 from 20±2.4% to 17.4±1.4% ($p<0.05$), with massive blood loss observed, on the contrary, an increase from 23.4±1.6% to 37.4±1.1% ($p<0.05$). In the analysis of changes in the index of CD8 it was found that the index fell from 8.3±2.0% to 4.4±1.1% ($p<0.05$) with a slight blood loss from 3.1±1.3% to 1.4±1.1% ($p<0.05$) with the massive blood loss.

The study of changes in the level of CD16 in patients with gastroduodenal ulcers with complicated bleeding revealed a decrease in this indicator in patients with low blood loss from 3.6±1.1% ($p<0.05$) to 2.6±1.1% ($p<0.05$)

and normalization of CD16 level with more severe degrees of blood loss from 3.3±1.1% ($p<0.05$) to 3.6±1.1% ($p<0.05$). There was also an increase in the level of CD19 in patients with low blood loss from 12.4±1.1% ($p<0.05$) to 20.4±1.2% ($p<0.05$), in contrast to patients with massive blood loss, this index increased from 20.6±1.2% ($p<0.05$) to 30.8±1.2% ($p<0.05$). In patients with duodenal ulcer, bleeding marked manifestations of decrease in the immune response, which are within the reference range. The above data can be presented in the form of table I.

Depending on the state of the endoscopic hemostasis, a decrease in T-lymphocyte indices with CD4 growth was determined to the reference rates in cases of active bleeding (from 13±2.4% to 26.4±1.2% $p<0.05$) and with high-level hemostasis recurrence from 20.8±2.3% to 31.3±2.9% and from 22.6±1.3% to 39.8±2.9% ($p<0.05$), respectively.

In the analysis of changes in CD8 indices there was a decrease of this indicator, especially in patients with active bleeding (from 3.3±1.2% to 1.4±1.3% ($p<0.05$) and in cases of unstable hemostasis with low recurrence rate bleeding from 8.3±1.1% to 3.6±1.2% ($p<0.05$).

When analyzing changes in CD16, a decrease in their level was determined. This phenomenon was observed with active bleeding (from $3.1 \pm 1.9\%$ to $2.9 \pm 1.8\%$ ($p < 0.05$) and unstable local hemostasis from $3.2 \pm 1.3\%$ to $1.7 \pm 1.2\%$ ($p < 0.05$) and from $3.1 \pm 1.3\%$ to $2.8 \pm 1.3\%$, respectively. $4.1 \pm 1.5\%$ to $5.3 \pm 1.9\%$ ($p < 0.05$), which can be represented as table II.

CD19 levels were also increased to reference values in cases of unstable endoscopic hemostasis with high recurrence of bleeding from $9.8 \pm 2.4\%$ to $25.4 \pm 3.5\%$ ($p < 0.05$) and when a fresh clot covered a defect with $19.6 \pm 2.9\%$ to $35.4 \pm 3.4\%$ ($p < 0.05$). A slight decrease in CD19 was found from $28.6 \pm 3.6\%$ to $22.6 \pm 2.7\%$ ($p < 0.05$) in patients with unstable local hemostasis and low recurrence.

In cases of microbiological confirmation of presence on the mucous membrane of the periulcerous section of microorganisms *Streptococcus* β -haemoliticus (107 CFU) and *Klebsiella pneumoniae* (106 CFU), there was an increase in the level of CD-4 indices from $24.9 \pm 1.4\%$ to $25.9 \pm 2.7\%$ ($p > 0.05$) and from $16.4 \pm 2.6\%$ to $34.1 \pm 2.5\%$ ($p < 0.05$), respectively. By reducing the level of CD8 from $7.3 \pm 2.1\%$ to $6.4 \pm 1.7\%$ ($p < 0.05$) and from $5.8 \pm 2.8\%$ to $4.1 \pm 2.3\%$ ($p < 0.05$), respectively. There was an increase in the level of CD19 in cases of confirmation of the presence of the periulcerous section of microorganisms *Streptococcus* β -haemoliticus and *Klebsiella pneumoniae* on the mucous membrane from $16.1 \pm 3.2\%$ to $23.9 \pm 3.5\%$ ($p < 0.05$) and from $12.1 \pm 3.1\%$ to $29.1 \pm 2.9\%$ ($p < 0.05$), respectively. The level of CD16 remained equally low in all patients, which was $3.1 \pm 1.5\%$ ($p < 0.05$).

DISCUSSION

In the process of diagnosis and treatment of all patients we used classification of ulcerative bleeding by Forrest in the modification of Nikishaev V.I. and performed esophagogastroduodenoscopic examination with endoscopic hemostasis and its control every 4-6 hours [3]. An analysis of local endoscopic hemostasis revealed that active jet bleeding (F1a) was detected in 21 (26.5%) patients, blood flow (F1b) was detected in 3 (3.8%) patients. There is a significant proportion of patients with unstable local endoscopic hemostasis with a high risk of recurrent bleeding (FIIa, FIIb, and FIIc) - 49 (61.3%). Ulcerative defect with no signs of bleeding (FIII) was established in 7 (8.75%) patients.

At all stages of forming specific immune response of the body, the dominant role belongs to CD19, CD16, CD4, CD8 [4,6]. Increasing in the level of these markers (CD4, CD8, CD19) and decreasing of CD16 level is a decrease in the immune response in patients with gastroduodenal bleeding of ulcerative genesis. At the same time, over expression of mediators causes changes of physiological processes. The research of cytokines in patients with gastroduodenal hemorrhage has been carried out.

We found a significant microbial polymorphism with the dominance of pathogenic and pathogenic microflora in the periulcerous area in the studied patients. Thus, *Klebsiella pneumoniae* was detected in 26 (32.5%) cases, *Streptococcus* β -haemoliticus – in 24 (30%) patients. The saprophytic

flora in these patients presented *Enterococcus* in 8 (8.2%) observations, *E. Coli* was found in 10 (10.3%) patients. The above data can be presented as Figure 1.

It was found that in endoscopic picture of active jet bleeding (F1a) *Streptococcus* β -haemoliticus was detected in 45 (56.3%) patients with CFU 106 in 31 (38.8%) cases, *Klebsiella pneumoniae* in 19 (23.8%) patients with a quantitative rate of 106 CFU in 10 (12.5%) observations. *Klebsiella pneumoniae* is a gram-negative stick with a thick mucous capsule, characterized by high enzymatic activity and pathogenicity. *Klebsiella pneumoniae* is capable of decomposing urea to ammonia and may exist in acidic gastric environment due to its alkaline action. *Streptococcus* β -haemoliticus is a gram-positive coccus that has high enzymatic and pathogenic activity. This microorganism decomposes glucose to the formation of the lactic acid, which acidifies its environment, forms a variety of endotoxins and exotoxins, has the ability to bind individually to and break down hemoglobin of erythrocytes. Given the above, we can conclude that the contamination of the mucous membrane of the periulcerous area by these types of microorganisms causes a change in the pH of the mucous membrane, the development of atrophic processes in epithelial cells, suppression and destruction of normal saprophytic flora, impaired coagulation.

CONCLUSIONS

1. The study found a sharp decrease in CD16 from $3.6 \pm 1.1\%$ ($p < 0.05$) to $2.6 \pm 1.1\%$ ($p < 0.05$) in cases with low level of haemorrhage at normalization with more severe degrees of bloodloss and a rapid increase in the level of CD4, CD8, CD19 to $37.4 \pm 1.1\%$ ($p < 0.05$), $4.4 \pm 1.1\%$ ($p < 0.05$), and $30.8 \pm 1.2\%$ ($p < 0.05$), respectively, indicating that there is a decrease in the immune response in patients with gastroduodenal bleeding of ulcerative genesis.
2. In patients with microbial contamination of the periulcerous area with microorganisms such as *Streptococcus* β -haemoliticus (10^7 CFU) and *Klebsiella pneumoniae* (10^6 CFU) we can see a decrease in the CD8 population from $7.3 \pm 2.1\%$, to $6.4 \pm 1.7\%$ ($p < 0.05$) and from $5.8 \pm 2.8\%$ to $4.1 \pm 2.3\%$ ($p < 0.05$), respectively has been established, which may create favorable conditions for the recurrence of the bleeding.
3. The data obtained in the analysis of indicators of the cell-mediated immunity in surgical patients with gastroduodenal ulcers complicated by bleeding can improve the medical treatment program and predict the course of the disease.

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Conflict of interest:

The Authors declare no conflict of interest.

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