

# IMMUNOLOGICAL AND MICROBIAL LANDSCAPE OF A WOUND IN PATIENTS WITH A PURULENT INFECTION IN DIABETES CONDITION

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**Summary.** This study is aimed at characterizing the "microbial picture of purulent lesions and leukocyte Phagocytic activity of neutrophils during antibiotic treatment in combination with local processing ozonized salt solution.

**Key words:** resistance, local treatment, phagocytosis, antibiotic therapy, neutrophils, purulent hearth.

According to WHO, data currently in the world there are more than 177 million diabetics, and by 2025 the number will reach 300 million. Diabetes mellitus (DM), developing in adults, represents a heterogeneous group of disorders of carbohydrate metabolism. However, thanks to modern research, it becomes apparent that type I diabetes occurs much more frequently than previously thought, and makes about 20% of the total number of cases over the age of 35 years. Depending on the severity of the autoimmune process and the rate of b-cells destruction, type I diabetes may be represented by in different clinical forms [2]. According to modern concepts, the nonspecific factors of protection, as the first stage of development of the defensive reaction of an organism, play a crucial role in the pathogenesis of purulent infections. Both quantitative and qualitative insufficiency of a neutrophilic link is considered as an infection development risk factor [3]. Antibiotic treatment frequently does not render the necessary effect in the treatment of purulent infections because of the increasing antibiotic resistance of pathogens, a combination of several antibiotics, necessary in the identification of microbial associations, might have an adverse influence on an organism [4.5]. For example, chronic foot ulcers for patients with diabetes are polymicrobial and have a high probability of becoming infected by multidrug-resistant organisms [1]. Therefore, a combination of antibiotic therapy with a local treatment of purulent focus with antiseptics is considered as the optimal treatment of purulent infections. The oxidative effect of ozone on the organic compounds in an aqueous environment can take place in three ways. 1. A direct oxidation with the loss of an oxygen atom. 2. The accession of the ozone molecule to the oxidized substance. 3. The catalytic effect, increasing the oxidative role of oxygen. All three mechanisms render bactericidal action on the aerobic and anaerobic microflora. The purpose of the present study is the characteristic of the microbial picture of purulent focus and phagocytic activity of neutrophils during the treatment with antibiotics in conjunction with local treatment by ozonated physiological salt solution.

**Materials and techniques.** The study involved 25 patients (13 men and 12 women) with a purulent infection of different nature aged from 29 to 70 years. All patients were bacteriologically examined 4-5 times, on the 1st, 3rd, 5th and 7th days of treatment. In 20 patients, within the same time frame was examined the phagocytic activity of neutrophils of peripheral blood. For bacteriological examination, patients provided the exsudate and pieces of tissue from the necrotic sections of the wound. Quantitative determination of the microflora was performed according to the method of J.C. Gould (1965) and U. M. Feldman with co-author. (1991). The virulence of staphylococci was studied according to five criteria (haemolytic properties, plasma-coagulation, formation of lecithinase, pigment formation, splitting of mannitol) of the Christy-Chapman method in the modification of Smorodinzhev (1987). The phagocytic activity of neutrophils was determined by the method of I. J. Serebriyskiy and M. M. Antonova (1990). The test microorganisms - *Staphylococcus aureus* 209P, phagocytic number (PN) and phagocytic index (PI) were determined. The treatment of patients included treatment of wounds with ozonated physiological salt solution and antibioticotherapy taking into account the susceptibility of microorganisms. The control group consisted of 12 patients with a purulent infection treated by conventional techniques.

**Results and discussion.** Under the bacteriological examination of the material from patients with purulent infections, the growth of facultative anaerobic microorganisms was observed in 85% of cases; within 49.5% of cases Gram-positive bacteria were isolated, in 30.3% of cases – Gram-

negative. In 5.2% of patients *Candida* type fungi were seeded. Among Gram-negative bacteria, dominated *Escherichia coli*, representatives of the *Proteus* and *Citobacter* genus. Among Gram-positive bacteria predominated *Staphylococcus aureus* and *Staphylococcus epidermidis*, the virulent strains predominated, reaching 77% from the general number of isolated cultures. In the quantitative bacteriological examination, conducted before the local treatment, a high dissemination of tissues has been established within purulent focus (109 - 1010 CFU). After twice-daily treatment of the focus with a fresh ozonated physiological salt solution, on the 3rd day, there was a reduction of bacterial contamination, while on the 5th day was observed the clearance of pyogenic microorganisms. In the control group who received only antibiotic treatment, bacterial contamination decreased on the 5th day for 40-50%, while a complete reorganization was observed on the 7th day. In patients whose purulent focus microflora was represented by monocultures, complete purification of tissues was observed on 6th-7th day, while in case of association of various types of gram-positive and gram-negative bacteria, the clearance proceeded more slowly and completed on the 10th day. In the control group, the degree of bacterial contamination of tissues on all the stages of the research was significantly higher (1012-1016 CFU,  $P \leq 0.05$ ). It should be noted that in 50% of patients in this group was observed combined semination of tissues both by Gram-positive and Gram-negative bacteria, and in these cases, the persistence of bacteria continued for a longer time – 10 days and more. Thus, on the 10th day, the clearance of microorganisms was observed in only 20.7% of patients. The study of the phagocytic activity of neutrophils showed that with repeated treatment of wounds with ozonated physiological salt solution, the PN, significantly reduced before beginning treatment (norm is  $-4.7 \pm 0.3$ , before treatment  $-2.7 \pm 0.2$ ,  $P \leq 0.001$ ), increased after the treatment cycle to normal parameters to  $-4.6 \pm 0.2$ ,  $P \leq 0.001$ . In the control group, similar dynamics was not observed and in spite of the significant increase in value in PN, still, the values of this parameter were significantly different from the normal values (before treatment  $2.8 \pm 0.2$ , after treatment  $-3.5 \pm 0.1$ ,  $P \leq 0.01$ ). The latter value was significantly different from the normal values of PN ( $P \leq 0.05$ ). The positive dynamics of the FI had a similar nature in patients of the experimental group: at norm rate of  $67.3 \pm 6.9$ , this indicator before the treatment amounted  $50.4 \pm 5.7$ , and after treatment  $61.5 \pm 7.5$ , i.e. being significantly different from the norm ( $P \leq 0.05$ ) before treatment, this indicator reached the normal value of  $P \leq 0.05$ . In the control group, the tendency was to the increase of this indicator, but it did not reach normal values: before treatment  $50.4 \pm 5.7$ , after treatment  $-53.2 \pm 3.5$ ,  $P \leq 0.05$ . Thus, the effective combined therapy, removing the microbial load and thereby reducing the toxic effects on the organism, contributes to the normalization of the phagocytic activity of neutrophils.

**Conclusion.** 1. The microflora extracted from the purulent necrotic focus in patients with diabetes mellitus is different from that of people not suffering from this disease. A major role of Gram-negative microflora as causative agents is typical (most frequently encountered bacteria of the genus are *Proteus* and *Klebsiella*). 2. Application of ozonated physiological salt solution in the treatment of surgical patients with purulent-inflammatory processes is an effective method, which in a short time leads to a complete purification of a purulent focus from bacteria and reduction of healing time of wounds. 3. A local application of ozonated physiological salt solution has a stimulating effect on phagocytic activity of neutrophils of peripheral blood.

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