ORIGINAL ARTICLE

CONTENTS

# Treatment of chronic wounds of the lower limb with the use of VAC-therapy. Impact on immunity and bacterial films

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## ABSTRACT

**Aim:** To improve the results of treatment of patients with chronic wounds of the lower extremities by using complex treatment, including surgical interventions, VAC- therapy, as well as studying the effect of negative pressure on bacterial films of wounds, based on microbiological examination and immune-histochemical data.

**Materials and Methods:** During the period from 2019 to 2023 at the department's clinic, 68 patients with chronic wounds of the lower extremities were examined and treated. These are mainly women (n=63) aged from 35 to 80 years.

**Results:** Complete wound healing was achieved in all patients. In 79% of patients, the transplanted skin graft took root completely. Partial lysis was observed in 17%, requiring repeated operations. In 4%, the wounds healed without surgery. The VAC- therapy we used prevented the restoration of the biofilm and prevented further infection of the wound. After complex treatment, we observed positive dynamics of clinical and immunological parameters. Indicators of T-cell immunity (CD3+, CD4+, CD19+) were restored, the level of CIC significantly decreased and the level of IgM increased. The level of interleukins on day 21 showed a tendency towards normalization.

**Conclusions:** A modern integrated approach gives the chance to radically solve the problems of healing chronic wounds. VAC- therapy in combination with surgical treatment and antibacterial therapy prevented the restoration of biofilm and further infection of wounds, which led to the wound healing in 100% cases.

**KEY WORDS:** Chronic wounds, negative pressure treatment, biofilms, immunity

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## INTRODUCTION

Bacterial films often complicate the course of inflammatory processes, causing antibiotic resistance, relapses, and chronicity [1]. According to modern literature, the incidence of infections caused by biofilm is about 80% of all infectious and inflammatory diseases [2]. An important reason for the complicated course of biofilm infections is the increased resistance of biofilm bacteria to immune effectors. Today, the statement that bacteria in biofilms are inaccessible to various parts of the immune system is very relevant. Some authors argue that biofilms can block the initial stages of inflammation, leading to "stupor and inactivity of the innate immune system" [3,4]. Research over the past two decades has established the pervasive role of biofilm in the formation of chronic wounds [5]. Analysis of published data indicates a prevalence of biofilms of 78.2% in chronic wounds, which poses a significant threat to their healing [6]. Our knowledge of biofilm and its impact on wound healing, especially non-healing wounds, is still evolving. In patients with chronic wounds, a long-lasting inflammatory phase, inflammatory mediators and proteases delay the onset of proliferation and disrupt the normal epithelialization process. The inability of the host immune system to suppress hyperinflammation induces the conversion of the innate immune response into an adaptive immune response, which leads to arrest of wound healing in the inflammatory phase and inhibition of the proliferative phase and epithelialization [7]. One of the promising methods for treating wounds of various etiologies is the vacuum method, which is used to improve the course of the wound process [8-11]. In recent years, significant progress has been made in the study and use of VAC- therapy, however, the mechanisms of influence of this method on various parts of the wound process require further research.

## AIM

To improve the results of treatment of patients with chronic wounds of the lower extremities by using complex treatment, including surgical interventions, VAC- therapy, as well as studying the effect of negative pressure on bacterial films of wounds, based on microbiological examination and immune-histochemical data.

## MATERIALS AND METHODS

### STUDY DESIGN, SETTING, AND POPULATION

During the period from 2020 to 2024 at the department's clinic, 68 patients with chronic wounds of the lower extremities were examined and treated. These are mainly women (n=63) aged from 35 to 80 years. The effectiveness of treatment was assessed based on the study of clinical indicators of wound healing and microbiological examination and immunohistochemical data. The studies were carried out when patients were admitted to the hospital on the 14th and 21st days after the start of treatment. Identification of microorganisms was carried out using MALDI-TOF spectrometry (Bruker, Germany). Quantitative assessment of the ability of microorganisms to form biofilms was carried out using the photometric method in 96-well plates for enzyme immunoassay [12]. To study the ability to form biofilms, one-day cultures of Staphylococcus aureus and Escherichia coli isolated from patients were used. The quantitative expression of the degree of biofilm formation was the optical density values measured on a StatFax2100 photometer. We studied various components of the immune system, in particular its cellular immunity (total number of T- and B-lymphocytes, subpopulations of T-lymphocytes: CD3, CD19, CD4, CD8, CD16) using flow cytometry, the humoral link of immunity – changes in the content of immunoglobulins of classes: IgA, IgM, IgG in blood serum were determined by the radial immunodiffusion method according to Mancini (1965) [13]. Mononuclear cells were isolated from the peripheral venous blood of patients in a density gradient of 1.077 g/cm. The functional activity of granulocytes was assessed in the NBT test using the nitroblue tetrazolium reduction reaction. Circulating immune complexes were determined by the method of V. Haskova (1977).

The C-300 TS VAC- system was used to treat wounds with negative pressure. During the first installation of the system, the device was set to a constant aspiration mode with a negative pressure in the wound cavity of 80-110 mm Hg. Art. Vacuum dressings were used (hydrophilic polyurethane sponges with a pore size of 400–2000 μm with a transparent adhesive coating, connected by a drainage tube to a vacuum source apparatus). The first period of system operation lasted from 24 to 48 hours, the second up to 3 days, the subsequent ones – on average up to 5 days. The system was remounted for the purpose of wound revision and during surgical treatment in the operating room.

### ETHICS

Ethical approval for this study was obtained from the ethical authority committee of the Dnipro State Medical University, Dnipro, Ukraine. This study was conducted in accordance with the principles Declaration of Helsinki. Written informed consent for participation in this study was not required in accordance with national legislation and institutional requirements.

### STATISTICAL ANALYSIS

Statistical processing of the data was carried out by determining the arithmetic mean (M) and its error (m). The significance of the difference in mean values was determined using the Student's t test. Statistical data processing was carried out using a personal computer using STATISTICA 6.1 software (StatSoftInc., serial AGAR909E415822FA) and Microsoft Excel (Microsoft Office 2016 Professional Plus, Open License 67528927) using descriptive and analytical biostatistics methods and multivariate statistical analysis methods.

## RESULTS

Treatment of the patient has begun with VAC – therapy, which was used both as an independent method and to prepare wounds for surgery. VAC- therapy was also used after skin graft surgery (Fig. 1).

The use of VAC- therapy contributed to the rapid cleansing of wounds, reducing their area and depth, accelerated formation of granulations and epithelization of edges, and reduced costs for wound care products. This is confirmed by positive changes in the local status on days 4–5 of treatment: a decrease in hyperemia, tissue edema, as well as the amount of wound exudate, a change in its nature from purulent to serous. Signs of epithelization of the wound edges appeared, the tissue defect was gradually filled with granulations and decreased in size (Fig.2).

Complete wound healing was achieved in all patients. In 79% of patients, the transplanted skin graft took root completely. Partial lysis was observed in 15%, requiring repeated operations. In 4%, the wounds healed without surgery. In 2%, severe pain was observed during exposure to negative pressure, which forced the abandonment of VAC- therapy and plastic surgery using a


**Fig.1.** The C-300TS VAC system was used to treat wounds with negative pressure.

perforator propeller flap. Relapses were observed in 3 patients after 1 year and in 4 patients 2 years after treatment.

Before starting antimicrobial therapy and during the course of antibiotic treatment, microbiological monitoring was carried out. As a result of the study, 58 strains of pathogens were isolated (Fig. 3). The main representatives among the isolated microorganisms were Staphylococcus aureus (18 strains), coagulase-negative staphylococci Staphylococcus haemolyticus (6 strains). Enterobacter cloaceae (5 strains) and Escherichia coli (9 strains) were found among enterobacteria; P.aeruginosa (16 strains), and Acinetobacter spp. (4 strains). The contamination of the wound in the examined patients at the time of the initial examination averaged 2.8±0.03x104 CFU/ml.

To examine the ability of isolated microorganisms to participate in the formation of biofilm conglomerates consisting of bacteria and extracellular matrix, a photometric method was used. The intensity of biofilm formation was assessed 24 hours after introducing microbes into plastic plates with Mueller-Hinton broth. Biofilm formation was studied before the start of antimicrobial therapy and 10-14 days after the start of treatment. Targeted antibacterial therapy was carried out against the background of VAC- therapy and complex surgical treatment. The course of treatment was carried out in accordance with the results of bacteriological cultures and the choice of drugs to which the greatest sensitivity was identified.

A slight predominance of P. aeruginosa activity in the formation of biofilms was noted at 1,8 – 2,1 cu of optical

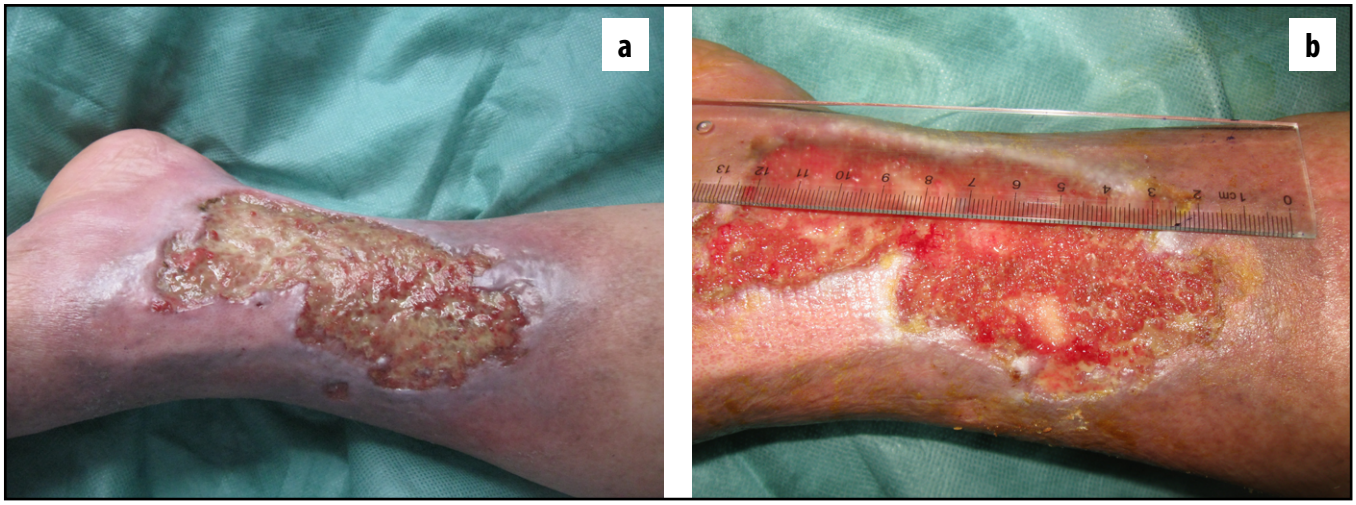


**Fig. 2.** a) Chronic wound of the left leg. b) On the 10th day after VAC- therapy.

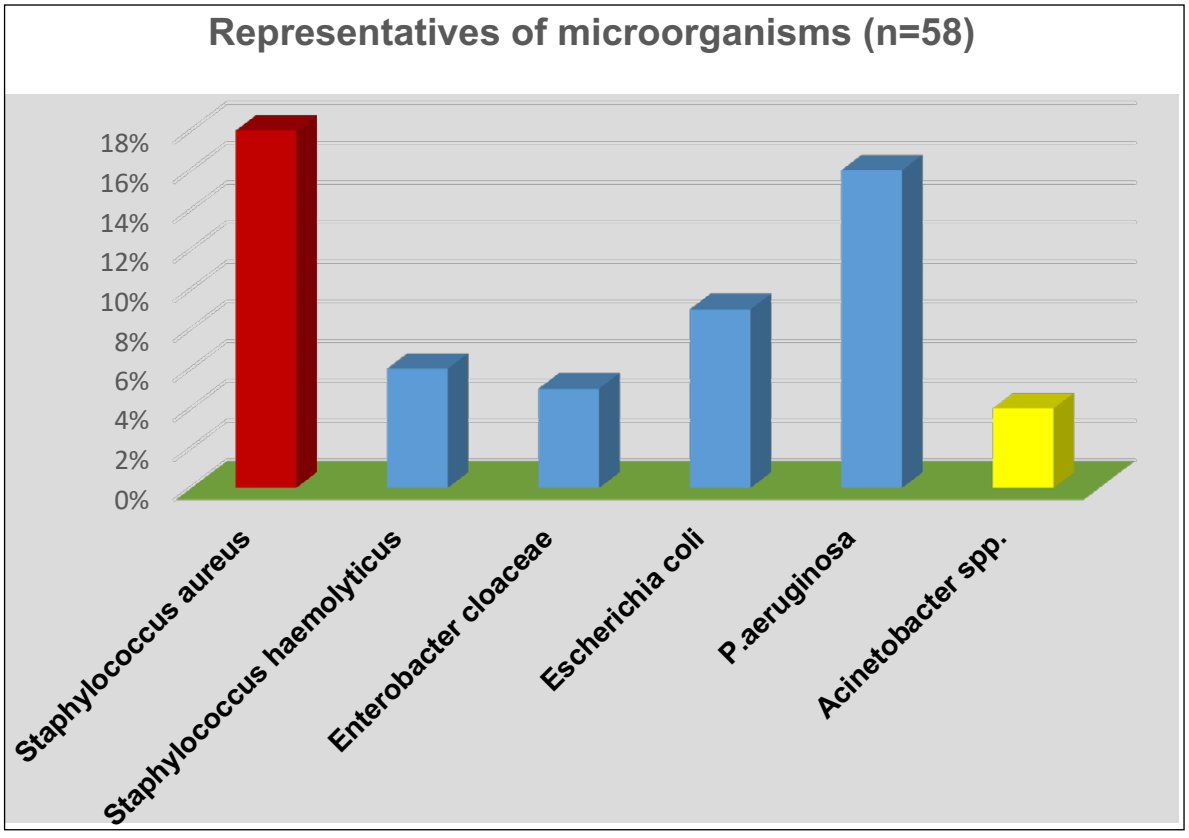


**Fig. 3.** Representatives of microorganisms in chronic wounds of the lower extremities.

plane compared to S. aureus – 1,5-1,9 cu of optical plane and with E. coli – 1,2-1,4 cu of optical plane before the start of treatment. After a certain period of time after the start of the use of antimicrobial agents, a tendency to a decrease in the intensity of biofilm formation was found: in P. aeruginosa – 1,3 -1,6 cu of optical plane for S. aureus – 0,8-0,9 cu of optical plane and in E. coli – 0.7-0.9 cu of optical plane respectively.

Studies have shown that all patients before treatment had deep disturbances in the T- and B-cell components of immunity. An analysis of the results of studies of the T-cell and humoral immunity in patients with chronic wounds of the lower extremities is given in Table 1.

In the patients studied, the number of phagocytic cells and their functional activity drops sharply. The number of B- cells increases, an inversion of immune-regulatory subpopulations is observed, and the balance between the T- and B – cell components of immunity is disrupted. The data we obtained indicate significantly pronounced immune disorders and we did not observe a full-fledged immune response.

**Table 1.** The state of cellular and humoral immunity in the examined patients before and after treatment

| Indicators | Tests values (n=25) | Before treatment (n=68) | After treatment (n=68) |
|---|---|---|---|
| Leukocytes | 5,35±0,21 | 7,92±0,76* | 5,4±0,73 |
| Lymphocytes %<br>a.n. | 28,71±0,81<br>1,61±0,07 | 19,72±1,56*<br>1,31±0,19 | 29,61±2,54<br>1,52±0,17 |
| T- lymphocytes %<br>CDZ+ a.n. x$10^9$ cel/l | 50,88±0,68<br>0,76±0,04 | 32,12±2,68*<br>0,43±0,15* | 50,37±2,58**<br>0,69±0,05 |
| T- helpers %<br>CD4+ a.n. x$10^9$ cel/l | 38,71±0,52<br>0,53±0,03 | 21,42±1,58*<br>0,24±0,08* | 33,4±2,24 **<br>0,45±0,03 |
| T- suppressors % CD8+ a.n. x$10^9$<br>cel/l | 18,39±0,57<br>0,30±0,02 | 26,17±2,1<br>0,38±0,04 | 19,35±3,89<br>0,29±0,06 |
| B- lymphocytes % CD19+a.n x$10^9$<br>cel/l | 14,78±0,48<br>0,25±0,01 | 25,16±2,13*<br>0,42±0,08 | 14,26±1,39**<br>0,32±0,07 |
| Th/Ts | 1,97±0,07 | 1,13±0,13* | 1,63±0,21 |
| CIC cu | 3,42±0,23 | 9,89±1,35*, | 3,69±1,68** |
| Ig A g/l | 2,25±0,26 | 2,85±0,36 | 2,2±1,93 |
| IgM g/l | 1,53±0,1 | 1,07±0,18 | 2,14±0,36** |
| IgG g/l | 12,72±0,42 | 18,07±1,33 | 13,81±0,87 |

Notes:
1. * – p<0.05 compared to control
2. ** – p<0.05 between indicators before and after treatment.

**Table 2.** Level of interleukins in examined patients before and after treatment

| Indicators | Control values (n=15 ) | Before treatment (n=68) | After treatment for 21 days (n=68) |
|---|---|---|---|
| **IL –8** pg/ml | 26,0±2,62 | 219,63±15,87* | 127,42±17,98* |
| **I**L- 4 pg/ml | 18,5±1,67 | 82,62±12,49* | 65,43±10,74* |
| **I**L1Ra pg/ml | 750±186 | 1596,93±582,88* | 1053,74±497,68* |
| **I**L – 2 pg/ml | 14,50±0,93 | 658,39±48,49 * | 455,54±47,38 * |

Note: * – p<0.001 compared to control.

The range of immune disorders includes a deficiency of T- cells, T- helper cells in all patients. The suppressor effect is due to insufficient production of T- cells and is accompanied by leukocytosis, lymphopenia, and suppression of the phagocytic system. Changes in the subpopulation ratios of T-lymphocytes in patients lead to a statistically probable inversion of immunoregulatory indices. A decrease in T-helper cells with an increase in the CIC leads to a decrease in the immunoregulatory index, which indicates an immunological imbalance and an important pathogenetic role. The response characteristics of the immune system indicate the possible specificity of changes in the immune system in patients with chronic wounds of the lower extremities. We found an increase in the level of the studied interleukins in all patients Table 2.

A significant increase in IL-2 concentration occurred in all patients. Influencing T- and B- cells, IL-2 is a central regulator of the immune response. Long-term stimulation by bacterial antigens leads to chronicity of the inflammatory process and indicates an insufficient voltage of the immune response in the studied patients.

Indicators of T-cell immunity (CD3+, CD4+, CD19+) were restored, the level of CIC significantly decreased and the level of IgM increased. The level of the studied interleukins on day 21, despite the short period of time, showed a tendency towards normalization, which indicates the positive dynamics of treatment using VAC – therapy.

## DISCUSSION

Clinically, a wound with red, loose granulation tissue covered with a mucous layer of exudate that returns after debridement, with increased production of discharge, and signs of recession of the epithelial edge, most likely has a biofilm [14]. Features of the immune system response indicate the specificity of changes in immunity in patients with chronic wounds. Long-term stimulation with bacterial antigens modified by altered autologous

tissue cells leads to chronicity of the inflammatory process and indicates an insufficient tension of the immune response. The profile of the T-cell response to the Th2 type changes, probably ensuring the persistence of bacteria, the pathological process turns out to be chronic. IL-4 acts as a growth factor for Staphylococcus aureus, leading to systemic infection. It inhibits the synthesis of the pro-inflammatory cytokine IL-8 by macrophages and promotes the formation of highly active oxygen and nitrogen metabolites. It was found that in the studied patients, an increase in the blood level of anti-inflammatory cytokines leads to an increase in wound processes. Also, the increase in the pro-inflammatory cytokine IL-8 is maximally expressed. The increase in IL-8 in the blood serum is associated with the activity of the inflammatory process, since it, by activating neutrophils, leads to their degranulation, the release of lysosomal enzymes and reactive oxygen metabolites, which have a damaging effect on the mucous membranes, increasing the damaging effect on the wound surface. Increased production macrophages of soluble mediators IL-2, IL-8, oxygen radicals and other biologically active substances leads to tissue damage, impaired penetration and the formation of a chronic focus of inflammation. Inflammatory reactions are accompanied by a complex systemic response, mediated through IL-1. It should be noted that for the progression of the inflammatory process, the imbalance in the production of IL-1 antagonists by phagocytes: the antagonist of its receptors IL-1Ra, which we observed in patients with chronic wounds of the lower extremities, is of great importance. All this indicates that the formation of a biofilm is associated with an immunodeficiency state which ultimately leads to the formation of long-term non-healing chronic wounds.

## CONCLUSIONS

1. A modern integrated approach gives the chance to radically solve the problems of healing chronic wounds. VAC- therapy in combination with surgical treatment and antibacterial therapy prevented the restoration of biofilm and further infection of wounds, which led to the wound healing in 100% cases.
2. Microbial biofilms were found in 72% of patients with chronic wounds. The causative agents of chronic infection were representatives of gram-positive and gram-negative flora with a tendency to predominance of representatives of the genus Staphylococcus.
3. Imbalance of immune-regulatory mechanisms plays an important role in the wound process in patients with chronic wounds of the lower extremities, as evidenced by a decrease in B-cell component of immunity against an adequate increase in T-helper cells and the restoration of the balance between the T- and B-cell links with the normalization of the CIC index on 21th days after treatment.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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