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ESTUDO CITOLÓGICO DA DINÂMICA DO PROCESSO DE FERIDAS EM LESÕES PURULENTES DA SÍNDROME DO PÉ DIABÉTICO COM O USO DE TECNOLOGIAS DE REABILITAÇÃO PROGRAMÁVEL

CYTOLOGICAL STUDY OF THE DYNAMICS OF THE WOUND PROCESS IN PURULENT LESIONS OF THE DIABETIC FOOT SYNDROME WITH THE USE OF PROGRAMMABLE REHABILITATION TECHNOLOGIES

ЦИТОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ ДИНАМИКИ РАНЕВОГО ПРОЦЕССА ПРИ ГНОЙНЫХ ПОРАЖЕНИЯХ СИНДРОМА ДИАБЕТИЧЕСКОЙ СТОПЫ С ПРИМЕНЕНИЕМ ПРОГРАММИРУЕМЫХ САНАЦИОННЫХ ТЕХНОЛОГИЙ

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RESUMO

Introdução: Lesões purulentas dos pés no diabetes mellitus trazem sofrimento excruciante ao paciente, reduzem a qualidade de vida e muitas vezes levam à amputação do membro e possível morte. Os resultados decepcionantes do tratamento de complicações purulentas do diabetes encorajam a busca por novas abordagens de tratamento e métodos para avaliar o potencial reparador de defeitos de feridas. **Objetivo:** O estudo teve como objetivo melhorar os resultados do tratamento das complicações purulento-necróticas do pé diabético, estudar a avaliação morfológica dos processos de cicatrização ao usar métodos de tratamento promissores, como tecnologias de higinização programáveis. **Metodos:** Nos últimos 10 anos, foram analisados os resultados do tratamento de 106 pacientes com complicações necróticas purulentas da síndrome do pé diabético (SLD) sem isquemia crítica. Os pacientes foram randomizados em dois grupos. No grupo experimental (n = 55), após o tratamento cirúrgico, a ferida foi suturada firmemente e, no pós-operatório, foi realizado o desbridamento programado com o aparelho AMP-01 original. No grupo controle de pacientes (n = 51), a ferida purulenta não foi suturada no pós-operatório, o tratamento local foi realizado com soluções de iodóforos, pomadas a base de polietilenoglicol. Para avaliar a dinâmica dos processos reparadores em feridas purulentas, foi utilizado um método citológico, que permite avaliar de forma rápida e confiável o estágio do processo da ferida e a eficácia do tratamento. A simplicidade e disponibilidade do método permitem que seja recomendado a todos os especialistas praticantes. **Resultados e Discussões:** No grupo experimental, no dia 9 após a cirurgia, o número de formas degenerativas de neutrófilos em esfregaços citológicos foi 2,9 vezes menor do que no grupo controle - 12,3 ± 0,3% versus 36,4 ± 0,4% (p <0,001), o indicador RDI no grupo experimental foi 3,4 vezes maior em comparação com o grupo controle - 2,6 ± 0,1 e 0,9 ± 0,1, respectivamente (p <0,001). Isso indicava fagocitose mais ativa, limpeza mais rápida da cavidade purulenta. Um aparecimento mais precoce de células de tecido conjuntivo jovem foi observado no grupo experimental, o número de fibroblastos no dia 9 após a cirurgia foi 4,6 vezes maior (6,4 ± 0,4%) do que no grupo controle - 1,4 ± 0,1% (p <0,001), que confirmou a presença de processos regenerativos ativos na ferida. **Conclusões:** O uso de tecnologias de higienização programáveis no tratamento de complicações purulentas do pé diabético leva a uma redução mais significativa na duração da fase de inflamação e aceleração dos processos reparadores.

Palavras-chave: *síndrome do pé diabético, complicações necróticas purulentas, tecnologias de sanação programáveis, processo de reparo citológico.*

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ABSTRACT

Background: Purulent lesions of the feet in diabetes mellitus bring excruciating suffering to the patient, reduce the quality of life and often lead to limb amputation and possible death. The disappointing results of the treatment of purulent complications of diabetes encourage the search for both new approaches to treatment and methods for assessing the reparative potential of wound defects. **Aim:** This study aimed to improve the treatment of purulent-necrotic complications of the diabetic foot by studying the morphological assessment of healing processes when using promising treatment methods such as then programmed debridement. **Methods:** Over the past 10 years, the results of treatment of 106 patients with purulent-necrotic complications of diabetic foot syndrome (DFS) without critical ischemia have been analyzed. The patients were randomized into two groups. In the experimental group (n = 55), after surgical treatment, the wound was sutured tightly, and in the postoperative period, programmed debridement was carried out using the original AMP-01 device. In the control group of patients (n = 51), the purulent wound was not sutured after the operation, and local treatment was carried out with solutions of iodophors, ointments based on polyethylene glycol. To assess the dynamics of reparative processes in purulent wounds, a cytological method was used, which makes it possible to quickly and reliably assess the stage of the wound process and the effectiveness of the treatment. The simplicity and availability of the method allows it to be recommended to all practicing specialists. **Results and Discussion:** In the experimental group, by day 9 after surgery, the number of degenerative forms of neutrophils in cytological smears was 2.9 times lower than in the control group - 12.3 ± 0.3% versus 36.4 ± 0.4% ($p < 0.001$) - and the RDI indicator in experimental group was 3.4 times higher compared with the control group - 2.6 ± 0.1 and 0.9 ± 0.1, respectively ($p < 0.001$). This indicated more active phagocytosis, more rapid cleansing of the purulent cavity. An earlier appearance of cells of young connective tissue was observed in the experimental group. The number of fibroblasts by day 9 after surgery was 4.6 times higher (6.4 ± 0.4%) than in the control group - 1.4 ± 0.1% ($p < 0.001$), which confirmed the presence of active regenerative processes in the wound. **Conclusions:** The use of programmable sanitation technologies in treating purulent complications of a diabetic foot leads to a more significant reduction in the duration of the inflammation phase and acceleration of reparative processes.

Keywords: *Diabetic foot syndrome, purulent-necrotic complications, Programmable sanitation technologies, cytological reparative process.*

АННОТАЦИЯ

Актуальность: Гнойные поражения стоп при сахарном диабете – это источник мучительных страданий пациента, снижающих качество жизни и нередко приводящих к ампутации конечности и возможному летальному исходу. Неутешительные результаты лечения гнойных осложнений диабета побуждают заниматься поиском как новых подходов к лечению, так и методов оценки репаративного потенциала раневых дефектов. **Цель:** Исследование было направлено на улучшение результатов лечения гнойно-некротических осложнений диабетической стопы, на изучение морфологической оценки процессов заживления при применении перспективных методов лечения -программируемых санационных технологий. **Методы:** За последние 10 лет проанализированы результаты лечения 106 пациентов с гнойно-некротическими осложнениями синдрома диабетической стопы (СДС) без явлений критической ишемии. Пациенты рандомизированы на две группы. В группе 1(n=55) после хирургической обработки рана ушивалась наглухо, а в послеоперационном периоде осуществлялись программируемые санации с использованием оригинального устройства АМП-01. В группе 2 (n=51) после операции пациенты получали традиционное местное лечение. Для оценки динамики репаративных процессов в гнойных ранах использовали цитологический метод, позволяющий быстро и достоверно оценить стадию течения раневого процесса и эффективность проводимого лечения. Простота и доступность метода позволяет его рекомендовать всем практикующим специалистам. **Результаты и обсуждение:** В группе 1 к 9 суткам количество дегенеративных форм нейтрофилов в цитологических мазках было в 2.9 раза ниже, чем в группе 2 -12.3±0.3% против 36.4±0.4% ($p<0,001$), показатель РДИ в группе 1 был в 3.4 раза больше по сравнению с группой 2 - 2.6±0.1 и 0,9±0,1соответственно ($p<0,001$). Это свидетельствовало о более активном фагоцитозе, более быстром очищении гнойной полости. В группе 1 наблюдалось более раннее появление клеток молодой соединительной ткани, количество фибробластов к 9 суткам было в 4.6 раза больше (6.4±0.4%), чем в группе 2 - 1.4±0.1% ($p<0,001$), что подтверждало наличие активных регенераторных процессов в ране. **Выводы:** Применение программируемых санационных технологий при лечении гнойных осложнений диабетической стопы приводит к более значимому сокращению сроков фазы воспаления и ускорению репаративных процессов.

Ключевые слова: *Синдром диабетической стопы, гнойно-некротические осложнения, Программируемые санационные технологии, цитологическое репаративный процесс.*

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1. INTRODUCTION:

At the present stage, the problem of diabetes mellitus (DM) is acquiring medical and social significance, which is explained by the high progressive prevalence, significant disability, and increased mortality in patients with diabetes (Galstyan, Vikulova, *et al.*., 2018; Dedov, Shestakova, *et al.*., 2017; Mitish, Paskhalova, *et al.*., 2014; Bakker, Apelqvist, *et al.*., 2016).

According to the World Health Organization (WHO), diabetes currently affects approximately 285 million people, and experts predict an increase in the number of patients with diabetes to 600 million by 2035 (Galstyan, Vikulova, *et al.*., 2018; Dedov, Shestakova, *et al.*, 2017; Stupin, Silina, *et al.*, 2019). In Russia, the mortality rate from type 2 diabetes is 60.3 per 100 thousand population (Galstyan, Vikulova, *et al.*, 2018). In the United States, diabetes affects approximately 9.4% of the population over 18 (Guariguata, Whiting, *et al.*, 2011). In 2013, diabetes-related health care costs cost US$ 548 billion, or 11% of total adult health spending, and are projected to exceed US$ 627 billion by 2035 (Bordianu, Bobircă, *et al.*, 2018).

Every year in the world, complications of diabetes become the cause of death in 1.6 million people, and, according to scientists, this figure will double in 10 years (Ray, Valentine, *et al.*, 2005). Approximately 25% of diabetics are at risk of developing diabetic foot ulcer (DFU), which is the main reason for their hospitalization, which costs US $ 40,000 per event (Armstrong, Boulton, *et al.*, 2017; Jang, Hong, *et al.*, 2015; Nickerson, 2018).

It is possible to achieve healing of diabetic ulcers by conservative measures only in 63–81% of cases; amputation of various limb levels is required in 14–24% of patients, while mortality rates reach 13%. These figures emphasize the need to search for innovative approaches to the problem of treating purulent-necrotic complications of diabetes mellitus (Ivanov, Serebryakova, *et al.*, 2018; Yusupova, Nabiev, *et al.*, 2017). Most experts consider the conservative approach to their treatment to be the main and decisive one; however, the duration of such therapy is many months. At the same time, invasive methods of treatment are expensive, and standard schemes of local drug therapy for wound and ulcerative defects are ineffective (Mitish, Paskhalova, *et al.*, 2014; Ray, Valentine, *et al.*, 2005); Ivanov, Serebryakova, *et al.*, 2018; Yusupova, Nabiev, *et al.*, 2017).

Slowing down of the healing process in purulent lesions of diabetes is due to polyneuropathy, angiopathy, osteoarthropathy, which are interrelated factors and lead to the development of formidable complications. These complications often lead to the loss of a limb, and sometimes pose a real threat to the patient's life (Braun, L.R., Fisk, W.A. *et al.*, 2014; Zaytseva, E.L., Doronina, L.P. *et al.*, 2014).

Over the past decade, fundamental research in molecular cell biology has made possible a better understanding of the underlying mechanisms of wound healing in diabetes. In this case, a violation of the synthesis of growth factors is observed due to a decrease in the activity of macrophages. The role of inflammatory cytokines and matrix metalloproteases in reducing the rate of extracellular matrix remodeling and the rate of collagen accumulation has been proven (Baltzis, Eleftheriadou, *et al.*, 2014; Liu, Min, *et al.*, 2009); Li, Z., Guo, *et al.*, 2013; Dinh, Tecilazich, *et al.*, 2012; Brancato, Albina, 2011; Mingyuan, Yiwen, *et al.*, 2012). These and other pathophysiological mechanisms lead to a chronic protracted course of wound defects in diabetes with a delay in their healing.

Today, it is becoming more and more urgent to solve the problems of predicting the course of reparative processes that underlie the structural and functional restoration of altered tissues. In this regard, there is an urgent interest in the development of both new approaches to treatment and methods for assessing the dynamics of healing of wound defects in DFS (Zemskov, Chorochilov, *et al.*, 2011; Lacci, Dardik, 2010; Han, Wang, *et al.*, 2012; Larichev, Shishlo, *et al.*, 2011; Kuzin, Kostyuchenok, 1990).

This research aimed to study the results of a cytological study of healing processes in purulent lesions of diabetic foot syndrome (DFS) using programmable sanitation technologies.

2. MATERIALS AND METHODS:

2.1. Sample

A total of 106 patients with purulent-necrotic complications (PNC) of Diabetic Foot Syndrome (DFS) without critical ischemia were treated at the Oryol Regional Clinical Hospital for the period 2008-2018.

2.2 Ethics

This study was carried out under the requirements of the Declaration of Helsinki on

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World Medical Practice (revised in 2013). The study was approved by the Ethics Committee of the Oryol State University named after I. S. Turgenev, Protocol No. 16 of 20.11.2020

2.3. Inclusion and Exclusion criteria

Inclusion criteria: patients with type 1 and type 2 diabetes, no critical ischemia with TcPO2 values not lower than 27 mm Hg, degree of foot tissue damage according to F.W. Wagner (1979) Grade II-IV, signed informed consent. The study did not include patients under 18 years of age, with Wagner grade I and V of DFS, with TcPO2 in the foot skin below 27 mm Hg, with extensive skin defects in the surgical treatment area. The presence of malignant tumors, concomitant diseases in the stage of decompensation, circulatory and respiratory insufficiency of the III degree, endocrine-metabolic and cerebral obesity, drug addiction, pregnancy and lactation were also exclusion criteria.

2.4. Groups of study

The patients included in the study were randomized into two groups depending on the methods of rehabilitation of purulent foci in the postoperative period. The experimental group consisted of 55 (51.9 %) patients (25 men and 30 women), after surgical treatment of the purulent focus, tubular drains were installed in the wound cavity, removed out through separate incisions, and the wound was sutured tightly. Drains were connected to the original AMP-01 device (patent for invention No. 2539165 dated 27.11.2014), which was used for programmed sanitation in the postoperative period. The control unit of the device was equipped with an individually selected program of cyclic irrigation processes, antiseptic aspiration and constant vacuuming, carried out in an Autonomous mode. The set vacuum level in the purulent cavity was maintained using a built-in pressure sensor.

The control group included 51 (48.1 %) patients (23 men and 28 women); after surgical treatment of a purulent focus, they received traditional local treatment using solutions of iodophores, ointments based on polyethylene glycol, and after the inflammation was stopped, plastic foot reconstruction (PRS) was performed or the wound was healed by secondary tension

Table 1 presents a comparative description of the clinical parameters of the study groups. The groups of patients were comparable in age, gender, type of DM, clinical form of SDS and indicators of transcutaneous oximetry in the skin of the foot.

The volume of PNC of the diabetic foot in patients of the study groups was diverse. In 75.5% of cases, the bone and joint apparatus of the foot was involved in the purulent-destructive process. In 27 (25.5%) cases, there were purulent-necrotic and purulent-granulating wounds after finger amputations or foot resection performed in other medical institutions. Tissue damage in the form of dry gangrene was found in 21 (19.8%) patients, and in the form of phlegmon, wet gangrene – in 15 (14.2%) patients. There was no correlation between the distribution of patients according to the nosological form of PNC of DFS and the lesion volume in the study groups (χ^2- Pearson criterion p=0,989). The distribution of patients in the study groups depending on the nosological form of PNC of DFS and the volume of lesion in accordance with the classification of F.W. Wagner (1979) is shown in table 2.

In the experimental group of patients with neuropathic form, radical surgical treatment and plastic reconstruction of the foot (PRS) were performed in one stage. Stage-by-stage sanation of the purulent focus was performed in the neuroishemic form, and after the inflammation was stopped, PRS was performed. Programmable sanation was used in all cases in the postoperative period after wound suturing.

Patients of the study groups received complex therapy, which included complete foot relief, insulin therapy with fractional administration of adequate doses of the drug under the control of glycemia, etiotropic antibiotic therapy, and anticoagulants and immunomodulators were prescribed according to indications. Patients with neuro-ischemic DFS were prescribed perfusion vascular therapy. Primary surgical operations in patients in the study groups are presented in table 3. According to Pearson's χ^2-test, there are no differences between the groups (p=0.72).

2.5. Research methods

Patients underwent the following research methods: a collection of complaints, anamnesis of the disease, assessment of comorbital conditions, clinical examination of organs and systems. All patients underwent laboratory tests, radiography of the feet in two projections, assessment of macro - and microhemodynamics using ultrasound duplex scanning of the arteries, Doppler examination of the lower extremity arteries, transcutaneous oximetry on the TSM 400 device of Radiometer medical (Denmark), and

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bacteriological examination of the wound discharge in dynamics.

2.6. Cytological examination

To access the healing of purulent foci of the diabetic foot, a cytological research method was used which makes it possible to quickly and reliably assess the stage of the wound process and the effectiveness of the treatment (Ivanusa, S.Y., Risman, B.V. *et al.*, 2016; Kamaev, M.F., 1954).

In the experimental group, material was taken from the depth of the sutured wound using the "puncture biopsy" method (Kaem and Karlov, 1977; Sergel and Goncharova, 1990). According to this technique, the wound exudate and cellular tissue elements were aspirated from the depth of the wound and its walls with a syringe through a sterile injection needle, which was attached to it and inserted into the depth of the wound. The resulting material was applied to a glass slide, evenly spreading and drying at room temperature. In the control group, the method of surface biopsy was used according to M.P. Pokrovskaya and M.S. Makarov (1942) modified by M.F.Kamaev (1954) (Kamaev M.F., 1954). With this material sampling technique, the surface layer of the wound is scraped with a special spatula or the handle of a surgical scalpel. The resulting material was applied to a glass slide, evenly distributed in a thin layer, and dried at room temperature. The smears thus obtained were immersed in methanol for 5 minutes for fixation and then stained according to the Romanovsky-Giemsa method using a solution (1 ml of standard liquid paint + 2 ml of basic buffer solution at pH 6.4-6.5 + 47 ml distilled water). Staining was carried out for 40–120 min, and the duration of staining was selected empirically. The swabs were then rinsed in distilled water, dried, and examined by immersion.

Cytological examination of smears from the surface of wounds was carried out in dynamics: on the first day at the time of randomization, and then on the 3rd, 5th, 7th, and 9th days. Smears were studied by microscopy with a magnification of 630 times with the calculation of shaped elements and the output of the average for 10 fields of view. The resulting value was expressed as a percentage per 100 counted cells. Used an Axio A1 light microscope (Zeiss, Germany) with a set of accessories. Photographs were taken using the AxioCam 105 color camera (Zeiss, Germany) using the ZEN 2 blue edition software (Zeiss, Germany), which was calibrated using an object micrometer of transmitted light with a division price of 0.005 mm.

The study followed the characteristics of cytograms with the definition of one of the 6 types of cytological picture according to V. F. Kamaev (1954), corresponding to different stages of the wound process: degenerative-necrotic type, degenerative-inflammatory type, inflammatory type, inflammatory-regenerative type, regenerative-inflammatory type, regenerative type (Davydov, Larichev, Abramov, 1990).

To fully assess the wound healing picture, the regenerative-degenerative index (RDI) was calculated, which was determined by the formula RDI = (STAB+SEGS)/DN, where STAB is the number of rod – shaped neutrophils in the field of vision; SEGS is the number of segmented neutrophils; DFN is the number of degenerative forms of neutrophils (Davydov *et al.*., 1990; (Davydov, Larichev, Abramov, 1990). If the RDI was less than one, it indicated a pronounced inflammatory process in the wound, if the value of this indicator became more than one, it meant the transition of the wound process to the regeneration phase.

2.7. Statistics

The work was performed in the design of a simple randomized comparative controlled study in parallel groups. The SPSS Statistics 25 (IBM) program was used for statistical processing of the obtained data. To study the relationship between qualitative features, conjugacy tables and calculated Pearson's χ^2-criterion or the Fisher's exact criterion were made. If there is a relationship between qualitative features, the shares of features were compared using the z-criterion of equal shares. The significance of differences between groups by quantitative characteristics was evaluated using the student's parametric t-test and the nonparametric Mann-Whitney test for unrelated groups. For related groups, the parametric t-test for dependent samples and the nonparametric Wilcoxon sign test were used. To assess changes in the dynamics of quantitative indicators, an analysis of variance with repeated measurements with the setting of a time factor and a group was used. As a result of the analysis of variance with repeated measurements, hypotheses were tested: whether the change in indicators over time is significant, whether there is a difference between groups, and if there is a statistically significant interaction between the time factor and the group. The latter hypothesis allowed us to prove the difference in the change of controlled indicators over time depending on the

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group. Differences were considered statistically significant when the probability value was less than 0.05 for a two-way critical region.

3. RESULTS AND DISCUSSION:

In cytological smears at the time of randomization in both groups of the study, patients with PNC of DFS had a pronounced predominance of degenerative neutrophils (DN) (82.6±3.2%), and there were very few preserved forms of white blood cells. The regenerative-degenerative index (RDI) was significantly lower than one (0.2±0.1). A degenerative-necrotic type of cell reaction characterized the cytological picture. Microflora was present in large numbers, mainly extracellularly, but sometimes it could be found intracellularly in a state of incomplete or perverted phagocytosis. The preparations contained accumulations of necrotic masses and amorphous gelatinous interstitial substance. The cellular composition of cytological smears in patients with purulent complications of DFS in the study groups at the time of randomization is presented in table 4.

All parameters of the statistical assessment of the cellular composition of cytological smears on the first day of observation in both study groups are close in values ($p > 0.05$), which made it possible to judge the homogeneity of the groups.

On the 5th day after randomization in the experimental group, the cytological picture corresponded to the inflammatory or inflammatory-regenerative cellular reaction type. There was a statistically significant decrease in the number of degenerative neutrophils (DN) (37.34 ± 2.33%), an increase in the number of preserved forms of neutrophils: segmented neutrophils amounted to 38.69 ± 1.33%, stab - 5.55 ± 0.39% ($p < 0.001$).

There was a statistically significant increase in the regenerative-degenerative index (RDI) - 1.19 ± 0.09 ($p < 0.001$). Plasma cells, histiocytes appeared. There was a statistically significant increase in the number of active macrophages - 3.59 ± 0.25%, lymphocytes - 4.79 ± 0.24%, fibroblasts - 3.21 ± 0.31% ($p < 0.001$). Groups of cells of young connective tissue in the form of fibrocytes, fibroblasts, fibrous fibers were found. Microflora was determined in small amounts at the stage of complete phagocytosis.

In the control group, on the 5th day of treatment, the cytological picture in smears changed little, the number of DNs significantly decreased - 72.5 ± 1.37% ($p < 0.05$), the number of segmented and stab neutrophils slightly increased, the degree of their preservation slightly increased. The RDI value decreased statistically significantly - 0.49 ± 0.04 ($p < 0.05$). Microflora was found in moderate quantities; cases of completed phagocytosis were more often observed. The appearance of low-differentiated mononuclear cells was noted. There were single actively phagocytic leukocytes, lymphocytes, single elements of granulation tissue (table 5). The cytological picture corresponded to the degenerative-inflammatory type of cell reaction, reflecting the weak course of inflammatory reactions in the wound. Figures 1 and 2 show fragments of cytological smears from the wound surface on the 5th day in the study groups.

When assessing the dynamics of the cellular composition of cytological smears on the 5th day of the postoperative period, a significant difference was obtained between the study groups. According to the results of analysis of variance with repeated measurements, it was proved that there are statistically significant changes in the cellular composition of cytograms in both groups on the 5th day after surgery ($p < 0.001$). There was a statistically significant presence of interaction between the time factor and the group ($p < 0.001$).

On the 9th day of the postoperative period, a statistically significant decrease in degenerative neutrophils (12.28 ± 0.29%) was observed in the experimental group, preserved forms of neutrophils prevailed - segmented - 28.21 ± 0.94%, stab - 3.63 ± 0,18% ($p < 0.001$). There was a statistically significant increase in RDI values - 2.61 ± 0.09, the number of macrophages - 12.17 ± 0.68%, fibroblasts - 6.44 ± 0.38% ($p < 0.001$). Young elements of connective tissue, polyblasts prevailed over neutrophils and were located among the fibrous structures of the intermediate substance. The epithelium was presented in smears in the form of sheets of cells. Microflora was absent. The cytological picture corresponded to the regenerative-inflammatory or regenerative type of cellular reactions.

By the 9-10th day, the cytological picture began to acquire features characteristic of regenerative processes in the control group. The number of degenerative neutrophils decreased statistically significantly - 36.42 ± 0.36%, there were more preserved forms: HF - 24.31 ± 0.88%, PN - 8.09 ± 0.84% ($p < 0.05$). There was a statistically significant increase in the RDI value, which approached one - 0.90 ± 0.03. Microbes in the wound were detected in small numbers and

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were intracellularly in the phase of complete phagocytosis. The number of mononuclear cells significantly decreased, and the detection of their derivative forms in the process of differentiation became natural: the number of polyblasts increased, fibroblasts appeared - 1.39 ± 0.13%, the number of macrophages increased (2.21 ± 0.19%). Along with cellular elements, delicate fibers of the intermediate substance were observed. The cytological picture in smears corresponded to the inflammatory regenerative type of cellular reactions (Table 5). Figures 3 and 4 show fragments of cytological smears from the wound surface on the 9th day in the study groups.

For both groups, statistically significant changes in the cellular composition in cytograms on the 1st and 9th days of the postoperative period were proved: a decrease in the number of degenerative neutrophils in the experimental group (M ± σ) from 82.5 ± 1.9% to 12.3 ± 0.3%, in the control group from 84.6 ± 3.7% to 36.4 ± 0.4% ($p < 0.001$); an increase in the preserved forms of neutrophils (segmented neutrophils) - in the experimental group (M ± σ) from 14.2 ± 0.2% to 28.2 ± 0.9%, in the control group from 16.2 ± 0.3% to 24 .3 ± 0.9% ($p < 0.001$); increase in RDI value - in the experimental group (M ± σ) from 0.2 ± 0.1% to 2.6 ± 0.1%, in the control group from 0.2 ± 0.1% to 0.9 ± 0, 03% ($p < 0.001$).

In the experimental group, where programmable sanitation technologies were used, there was a faster decrease in the number of degenerative neutrophils, an increase in their preserved forms, a faster increase in RDI, and the differences between the groups were statistically significant in general over the entire observation period ($p < 0.001$). The presence of a significant interaction between the time factor and the group ($p < 0.001$) was also found, in the experimental group by the 9th day the number of degenerative forms was significantly lower - 12.28 ± 0.29%, in contrast to the control group (36.42 ± 0.36 %), the number of intact forms of neutrophils was higher - 28.21 ± 0.94% and 24.31 ± 0.88%, respectively. There was a more rapid increase in RDI in the experimental group on day 9 (2.61 ± 0.09) compared with the control group (0.90 ± 0.03). This indicated a more active phagocytosis, rapid cleansing of the purulent cavity. The dynamics of the average values of degenerative neutrophils and RDI in the study groups is shown in Figures 5 and 6.

To assess the reparative potential of purulent wounds, a cytological assessment of the quantitative composition of lymphocytes, macrophages and fibroblasts in the study groups on the 1st and 9th days of the postoperative period was carried out. For both groups, a statistically significant increase in the number of lymphocytes was proved - in the experimental group (M ± σ) from 0.4 ± 0.1% to 7.5 ± 0.6%, in the control group from 0.2 ± 0.1% up to 2.5 ± 0.6% ($p < 0.001$); statistically significant increase in the number of active macrophages - in the experimental group (M ± σ) to 12.2 ± 0.7%, in the control group to 2.2 ± 0.2% ($p < 0.001$). Also, on day 9, a statistically significant increase in fibroblasts was observed - in the experimental group (M ± σ) up to 6.4 ± 0.4%, in the control group up to 1.4 ± 0.1% ($p < 0.001$).

In the experimental group, where programmable sanitation technologies were used, there was a faster increase in the number of lymphocytes, earlier appearance of macrophages and cells of young connective tissue in the form of fibrocytes, fibroblasts, and the differences between the groups were statistically significant in general over the entire observation period ($p < 0.001$).

The presence of a significant interaction between the time factor and the group ($p < 0.001$) was also found, in the experimental group by the 9th day the number of lymphocytes was significantly higher - 7.5 ± 0.6%, in contrast to the control group (2.5 ± 0.6%), the number of macrophages was higher - 12.17 ± 0.68% and 2.21 ± 0.19%, respectively. In the experimental group, there was an earlier appearance of young connective tissue cells in the form of fibrocytes, fibroblasts, and their increase by day 9 to 6.44 ± 0.38%, in contrast to the control group - 1.39 ± 0.13%. This indicated active regenerative processes in the wound in the experimental group of patients. A regenerative type characterized the structure of cytograms. The dynamics of the average values of macrophages and fibroblasts in the cytograms of both study groups is shown in Figures 7 and 8.

Thus, analyzing the dynamics of the cytological picture in patients with purulent-necrotic complications of DFS in the study groups, it was noted that traditional treatment revealed a low intensity of cellular reactions in the wound, an elongation of the inflammatory phase, and the inflammatory type of cytograms was noted only by the 9th day after surgery. Also, the control group observed sluggishness of reparative processes in the wound, causing a significant duration of the regeneration phase, and later onset of the scar reorganization phase. This led to a lengthening of the timing healing.

The use of programmable sanitation

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technologies allowed to create conditions for better sanitation of the purulent focus, led to a reduction in all phases of the wound process. Surgical treatment of the purulent focus, long-term washing of the wound cavity in the postoperative period, and software for the drainage process made it possible to clear the wound of non-viable tissues, toxins, and proteolytic enzymes in a short time, reducing microbial contamination in the wound. As a result, the stage of rejection of necrotic tissues was extremely reduced. Early closure of the wound with sutures using active drainage under conditions of minimal inflammatory reaction in the wound significantly accelerated the reparative processes, creating conditions for developing and completing the regeneration phase. Already on the 3rd day after the operation in the experimental group, the smears showed the appearance of a large number of mononuclear cells, the rapid maturation of which later led to the appearance of fibroblasts and the predominance of non-cellular elements of connective tissue. The regeneration phase and the rumen reorganization phase were reduced.

4. CONCLUSIONS:

Cytological examination of smears in patients with purulent lesions of diabetic foot syndrome using programmed sanitation technologies revealed a higher rate of cellular reactions in the wound. There was a statistically significant faster decrease in degenerative forms of neutrophils, a positive redistribution of rod and segmentonuclear neutrophils in combination with an increase in the regenerative-degenerative index, indicating an acceleration of the relief of the inflammatory process. The appearance of macrophages and young connective tissue cells in the form of fibrocytes, fibroblasts, and fibrous fibers was also reliably observed at an earlier time, which indicated active regenerative processes in the wound.

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Table 1. *Comparative characteristics of the clinical parameters of the studied groups. Source: the author*

Parameter		**Experimental group (*n*=55)**	**Control group (*n*=51)**	**p-value of significance of differences between groups**
Average age *Me* (M±σ)***		56(59±8)	59(60±9)	0,26*
Gender	male (*n*, %)	25 (45,5%)	23 (45,1%)	1,00**
	female (*n*, %)	30 (54,5%)	28 (54,9%)	
Diabetes mellitus type	Type 1	5 (9,1%)	4 (7,8%)	1,00**
	Type 2	50 (90,9%)	47 (92,2%)	
DFS form	neuropathic	32 (58,2%)	31 (60,8%)	0,66**
	neuroischemic	23 (41,8%)	20 (39,2%)	
TcPO2 (*Me* [Q1;Q3])*** mm Hg	neuropathic	32,8 [32,3; 34,8]	33,2 [32,2; 34,8]	0,462*
	neuroischemic	28,8 [27,8; 29,4]	28,6 [27,8; 29,4]	0,536*

Notes: * t-test for independent samples; ** Fisher's exact test *** *Me*-median, M±σ - mean and standard deviation, Q1-lower quartile, Q3-upper quartile

Table 2. *Distribution of patients in the study groups depending on the nosological form of purulent-necrotic complications of DFS and the extent of the lesion in accordance with the classification of F.W. Wagner (1979). Source: the author*

Nosological form of purulent-necrotic process on the foot	**The volume of the lesion under the classification of F.W. Wagner (1979)**	**Experimental group (*n*=55)**		**Control group (*n*=51)**		**Total**
		n	**%**	***n***	**%**	
Deep ulcer	2	5	9,1	4	7,8	9
Deep ulcer + Chronic osteomyelitis of the finger	3	3	5,5	3	5,9	6
Phlegmon of the toe + phlegmon of the foot	2	5	9,1	6	11,8	11
Osteomyelitis of the toe + phlegmon of the foot	3	8	14,5	9	17,6	17
Purulent wound after amputation of toes or resection of the foot, previously performed in other medical institutions	3	14	25,5	13	25,5	27
Dry gangrene of one or more fingers	4	12	21,8	9	17,6	21
Wet gangrene of the toe + phlegmon of the foot	4	8	14,5	7	13,7	15
Total:		55	100	51	100	106

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Table 3. *Primary operations in patients of the study groups*

Primary operations	Experimental group (*n*=55)		Control group (*n*=51)		Total
	n	**%**	***n***	**%**	
Surgical treatment	9	16,4	9	17,6	18
Opening phlegmon	13	23,6	18	35,3	31
Finger amputation	9	16,4	6	11,8	15
Amputation of the distal foot	12	21,8	8	15,7	20
Transmetatarsal amputation of the foot	12	21,8	10	19,6	22
Total:	55	100	51	100	106

Table 4. *Cell composition of cytological smears in patients with purulent complications of DFS in the study groups at the time of randomization, in% per 100 cells*

Cell types	Experimental group		Control group		p-value of t-test	p-value of the Mann-Whitney test
	M±σ*, in% per 100 cells	Me[Q1;Q3]*	M±σ*, in% per 100 cells	Me[Q1;Q3]*		
STAB	1,8±0,2	1.8 [1.7;1.9]	1,8±0,2	1,7 [1,6;1,8]	0,706	528,500
SEGS	14,2±0,4	14,2 [14,0;14,4]	16,2±0,4	16,2 [16,0;16,2]	0,647	502,500
DN	82,6±3,2	64,5 [64,5;72,4]	84,2±6,2	68,2 [68,2;72,4]	0,005	317,500
RDI	0,2±0,1	0,2 [0,1;0,2]	0,2±0,1	0,2 [0,1;0,2]	0,976	558,000
L	0,4±0,2	0,4 [0,3;0,5]	0,2±0,1	0,2 [0,1;0,2]	0,000	69,000

Notes: stab - stab neutrophils, segs - segmented neutrophils, DN - degenerative neutrophils, RDI - regenerative-degenerative index, L – lymphocytes**Me*-median, M±σ - mean and standard deviation, Q1-lower quartile, Q3-upper quartile

Table 5. *Cell composition of cytological smears in patients with purulent complications of DFS in the study groups (M ± σ), % per 100 cells*

Cell types	Experimental group		Control group	
	5 days after surgery	9 days after surgery	5 days after surgery	9 days after surgery
DN	37,34±2,33	12,28±0,29	72,5±1,37	36,42±0,36
STAB	5,55±0,39	3,63±0,18	6,2±0,24	8,09±0,84
SEGS	38,69±1,33	28,21±0,94	28,96±2,25	24,31±0,88
RDI	1,19±0,09	2,61±0,09	0,49±0,04	0,90±0,03
L	4,79±0,24	6,81±0,48	0,77±0,23	1,84±0,43
M	3,59±0,25	12,17±0,68	0,17±0,06	2,21±0,19
F	3,21±0,31	6,44±0,38	0,12±0,02	1,39±0,13

Notes: DN - degenerative neutrophils, STAB - stab neutrophils, SEGS- segmented neutrophils, RDI - regenerative-degenerative index, L - lymphocytes, M - macrophages, F - fibroblasts

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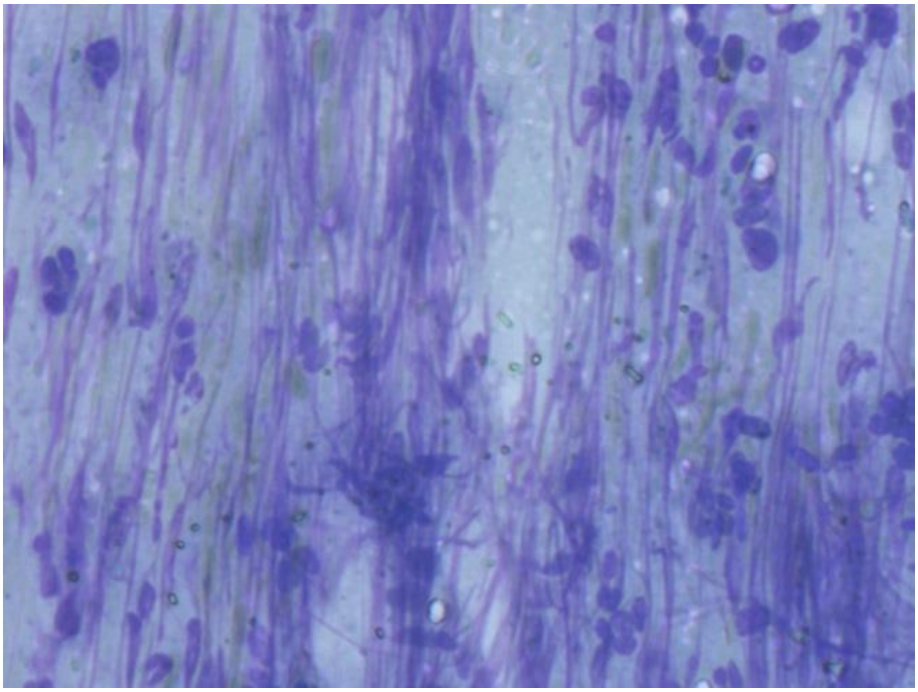

Figure 1. *Fragment of a cytogram of a smear from the wound surface on the 5th day, the in the experimental group. Among neutrophils and polyblasts, fibrocytes, fibroblasts, fibrous fibers were found. Romanovsky-Giemsa staining. Magnification × 63*

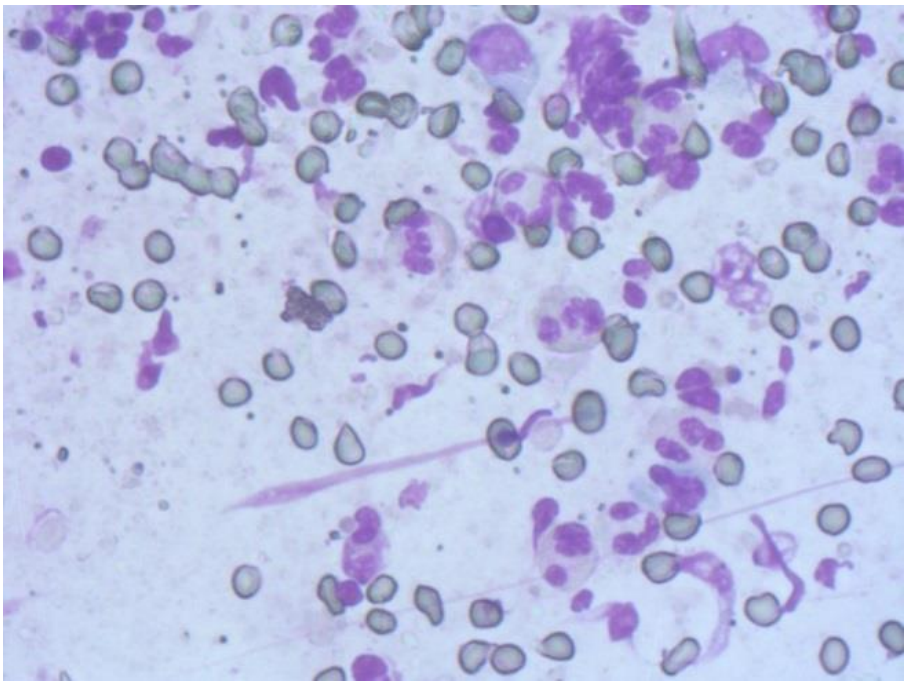

Figure 2. *Fragment of the cytogram of a smear from the wound surface on the 5th day, the control group. There are single actively phagocytic leukocytes, macrophages, lymphocytes. Elements of granulation tissue are rare. Romanovsky-Giemsa staining. Magnification × 63*

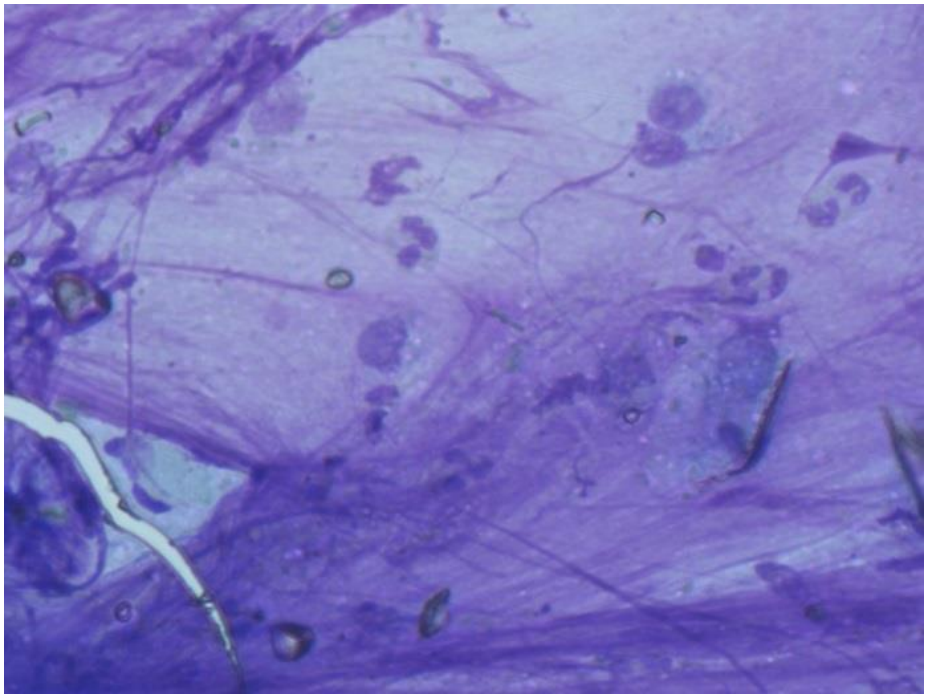

Figure 3. *A cytogram fragment for a smear from the wound surface, Day 9, Intervention group. New elements of connective tissue, fibroblasts, polyblasts, macrophages are located among the fibrous structures of the intercellular substance. The epithelium is presented in the form of layers of cells. Romanovsky-Giemsa staining. × 63 lens*

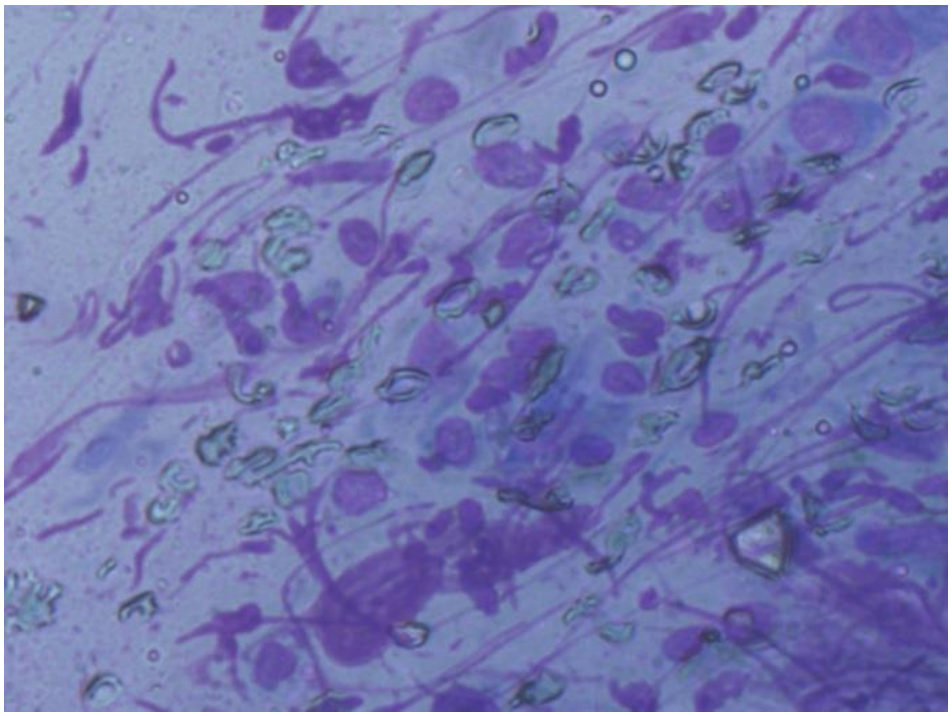

Figure 4. *Fragment of the cytogram of a smear from the wound surface on the 9th day, the control group. The number of mononuclear cells has decreased, the number of polyblasts, fibroblasts, and macrophages has increased. Delicate fibrous structures of the intermediate were observed. Romanovsky-Giemsa staining. Magnification × 63*

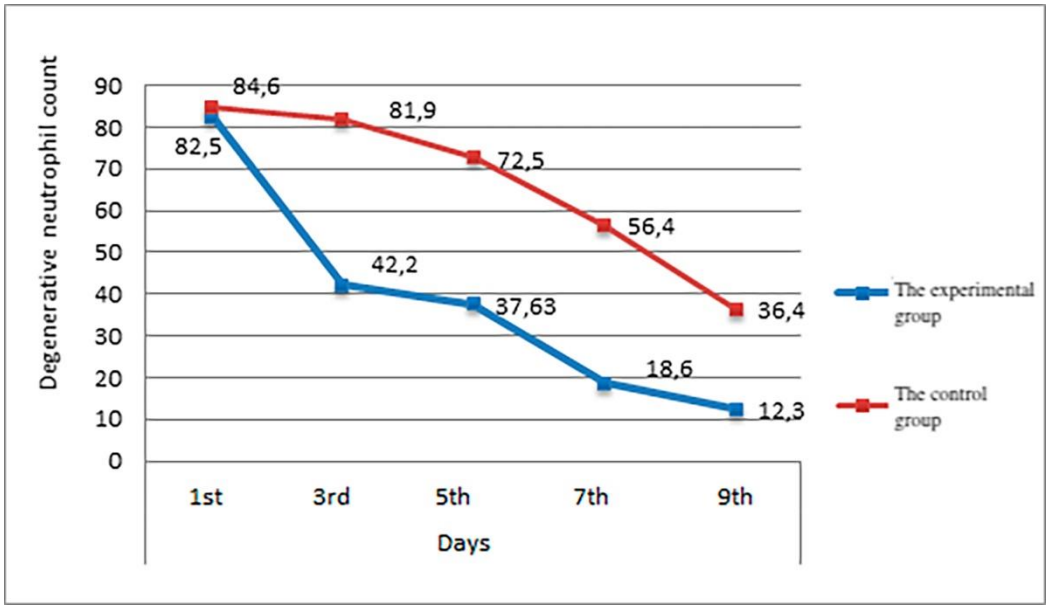

Figure 5. *Dynamics of mean values of degenerative neutrophils in cytograms of both study groups. Source: the author*

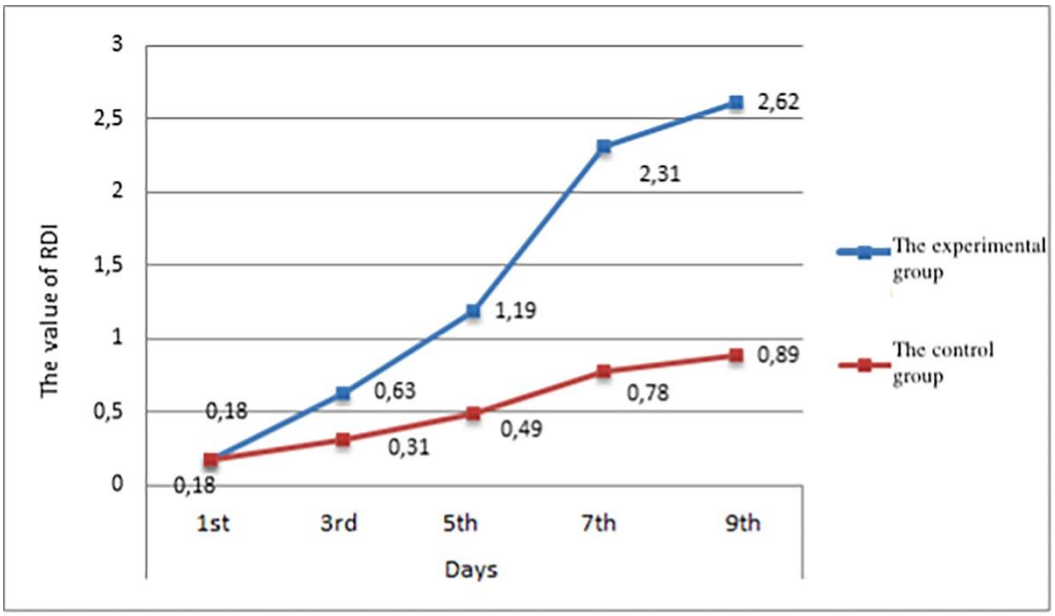

Figure 6. *Dynamics of average RDI values in cytograms of both study groups. Source: the author*

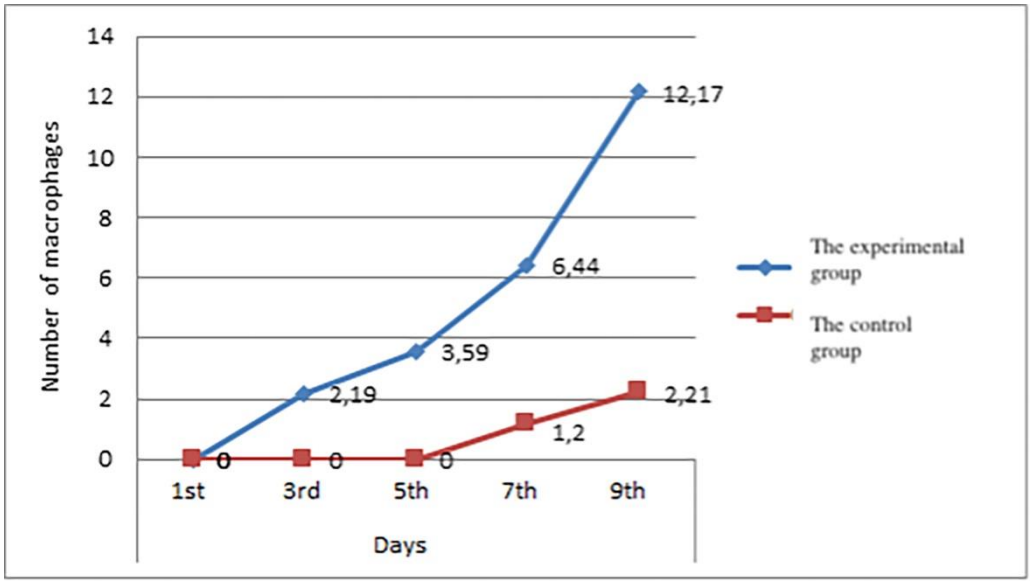

Figure 7. *Dynamics of mean values of macrophages in cytograms of both study groups. Source: the author*

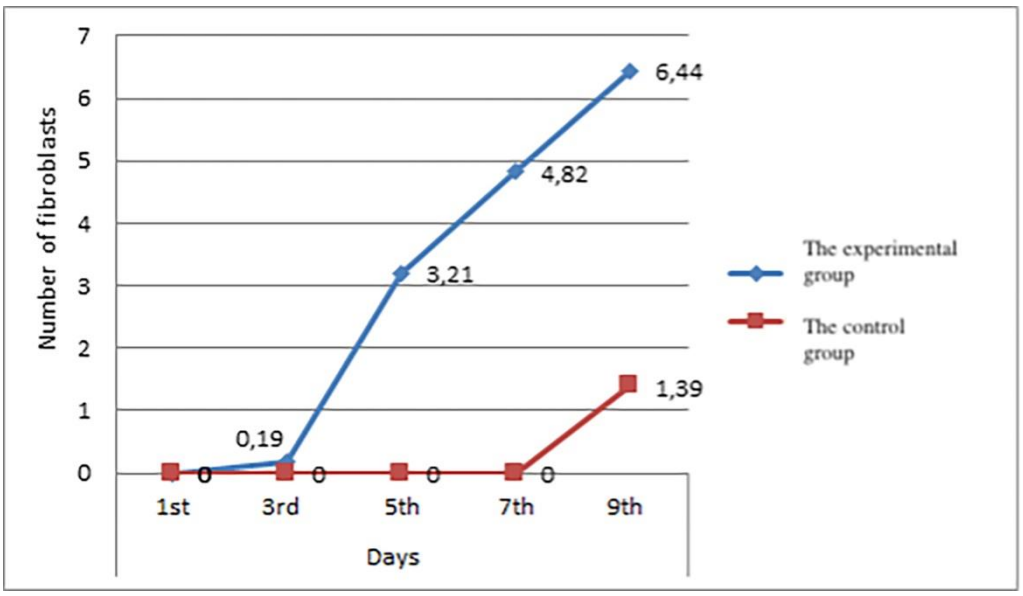

Figure 8. *Dynamics of average fibroblast values in cytograms of both study groups. Source: the author*

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