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# Morphological aspects of wounds in patients with purulent inflammation of soft tissues in diabetes mellitus and under the influence of granulocyte-colony-stimulating factor

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## Abstract

**Background.** Purulent-inflammatory diseases of soft tissues in patients with diabetes mellitus proceeds quite aggressively and, according to the standards, characteristic of acute infections of soft tissues without diabetes mellitus, can inconsistently affect the fate of patients with concomitant diabetes mellitus. The aim of the study was to study the features of the cytological picture of wounds in patients with purulent-inflammatory diseases of soft tissues on the background of diabetes mellitus after the use of granulocyte-colony-stimulating factor.

**Methods.** In 132 patients with purulent-inflammatory diseases of soft tissues on the background of diabetes mellitus, cytological studies of the wound were performed. The background materials of the cytological preparation were detritus, small protein grains, fat drops, crystals, hematoidin, cholesterol, etc. The cytological material was stained with azure-eosin mixtures.

**Conclusion.** The results obtained indicate that along with cellular elements in the morphology of the wound in patients with purulent-inflammatory diseases of soft tissues against the background of diabetes mellitus, other representatives of the cytological picture also play a significant role. The results of a cytological study of smears-imprints of a purulent-inflammatory wound of soft tissues in patients with diabetes mellitus showed that the microscopic picture of the smear was characterized, first, by the presence of a microbial factor in combination with background elements. Under the influence of the preparation of granulocyte-colony-stimulating factor in patients with purulent-inflammatory diseases of soft tissues against the background of diabetes mellitus, it increases the number of neutrophils and lymphocytes, and increases their phagocytic activity in the wound.

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**Keywords:** purulent-inflammatory wound process, diabetes mellitus, wound cytology, granulocyte-colony-stimulating factor

## INTRODUCTION

Surgical soft tissue infections are the most common reason patients seek surgical care. This indicates that the diagnosis and treatment of surgical infections remains one of the urgent problems of modern surgery.[1] The proof of this judgment is the high level of mortality in severe forms of purulent-

inflammatory diseases of soft tissues, which ranges from 28–56%, and with the development of sepsis - over 90%.

The main pathogenetic mechanism for the development of purulent-inflammatory diseases of soft tissues against the background of diabetes mellitus is the insufficiency of cellular and humoral immunity, intracellular metabolic acidosis, metabolic disorders, imbalance of macro- and microelements, blood protein composition, and others.[2] With severe hyperglycemia, chemotaxis and phagocytic function of leukocytes are inhibited. The features of changes in hematopoietic processes, in particular leukocyte cells, in patients with diabetes mellitus are their non-maturing in hematopoietic organs, with entry into the bloodstream in the form of immature formed elements. All this determines the pathological essence of the features of the course of diabetes mellitus. At the same time, it is known that an increase above normal values of young forms of neutrophils may indicate a generalization of the purulent-inflammatory process.

All the above indicates the need to search for new agents in the treatment of patients with purulent -inflammatory diseases of soft tissues on the background of diabetes mellitus, which could directly affect the pathogenetic factors of stimulating the production of granulocytes, instead of young forms of neutrophils, in order to prevent the development of septic complications.[3,4]

In recent years, in the literature more and more information appears on the effectiveness of the use of drugs with a colony-stimulating factor on blood granulocytes.[5-8] It is known that the human granulocyte-colony-stimulating factor is a glycoprotein that regulates the formation of functionally active neutrophils and their release into the blood from the bone marrow. Filgrastim, containing recombinant granulocyte-colony stimulating factor, markedly increases the number of neutrophils in the peripheral blood already within the first 24 hours after its administration, with a slight increase in the number of monocytes. Filgrastim is a highly purified non-glycosylated protein consisting of 175 amino acids. It is produced by a laboratory strain of the bacterium Escherichia coli, into which the human granulocyte colony-stimulating factor gene has been introduced by genetic engineering. The effectiveness of this drug in the treatment of several diseases has been proven. However, most of the information presented regarding the effectiveness of Filgrastim is devoted to blood diseases, in particular oncological etiology.[9-15]

In this regard, the purpose of our study was to study the features and conduct a comparative assessment of changes in the cytological picture of the wound surface against the background of the use of granulocyte-colony-stimulating factor.

## MATERIALS AND METHODS

The work is based on the analysis of the results of treatment of 132 patients with purulent-inflammatory diseases of soft tissues against the background of diabetes mellitus, who were hospitalized at the Surgical Infection Center of the Republic of Uzbekistan, for the period from 2011 to 2021. All patients were divided into 2 groups depending on the nature of the therapeutic measures:

1 - (control) group, represented by 73 (55.3%) patients with purulent-inflammatory diseases of soft tissues on the background of diabetes mellitus. Being on inpatient treatment, this group of patients received a traditional complex of diagnostic and treatment measures.

2 (main) group, represented by 59 (44.7%) patients with purulent-inflammatory diseases of soft tissues on the background of diabetes mellitus. A feature of this group of patients is that granulocyte-colony stimulating factor (Filgrastim) was added subcutaneously at a dose of 5 μg/kg of the patient's body weight to the complex of therapeutic measures.

The distribution of patients depending on age and gender showed that, in general, elderly patients were dominant. The average age in the control group of patients was 68.1±3.8 years, and in the main group - 67.2±5.9 years. In the control group, patients aged 41 and older accounted for 93.1%, and in the main group - 86.5%. Among the examined were 65 men (49.2%) and 67 women (50.8%). At the same time, female patients prevailed in the control group of patients, and male patients prevailed in the main group.

An analysis of the prevalence of the purulent-inflammatory process in patients with purulent-inflammatory diseases of soft tissues on the background of diabetes mellitus revealed that its localization was mostly noted in the trunk region (59%), almost the same number were in the region of the lower limb (17.5%) and perineum (15.1%).

Most patients were diagnosed with type II diabetes mellitus (94.7%). Type I diabetes mellitus was diagnosed only in 7 out of 132 patients, which accounted for 5.3%.

An analysis of the timing of admission of patients from the moment of the onset of purulent-inflammatory diseases of the soft tissues showed that 5 (6.8%) patients of the control and 4 (6.8%) of the main groups were admitted within 3 days from the onset of the inflammatory process. Up to 5 days after the onset of purulent-necrotic lesions, 15 (20.5%) and 11 (18.6%) patients, respectively, were hospitalized. Late hospitalization (more than 7 days from the onset of the purulent-inflammatory process) was associated with unsuccessful treatment of patients in other surgical hospitals and the development of septic complications.

The main principle of treatment was a short-term preoperative preparation, including the taking of clinical and biochemical blood tests and the beginning of empirical antibiotic therapy. The patients underwent a mandatory examination by an endocrinologist, with correction of the level of glycemia, while high blood sugar levels were not a contraindication to surgical intervention. Patients were prescribed insulin to maintain the sugar level not higher than 9.0 mmol/l. Insulin doses varied depending on the type of diabetes mellitus and the severity of the condition.

The essence of the surgical intervention was based on an adequate wide opening of the purulent focus with the sanitation of all existing streaks and pockets. Opening of phlegmons and necrectomy were performed according to generally accepted

standards. Depending on the length and depth of the pathological process, the wounds either remained open, which we preferred, or were sutured tightly leaving 2 lumen drains, followed by VAC-therapy.

The complex of conservative therapy included:

1) Antibacterial therapy (metronidazole, III–IV generation cephalosporins, III–IV generation fluoroquinolones, aminoglycosides) depending on the results of wound culture under aerobic and anaerobic conditions. Systemic antifungal therapy.

2) Detoxification therapy (infezol, native plasma, protein preparations, if necessary, blood preparations).

3) Measures to normalize all types of metabolism disturbed by diabetes, including switching to insulin therapy and glycemic control. Anticoagulants and disaggregation drugs (heparin, clexane) under the control of clotting time and coagulogram. Drugs that improve the microcirculation of the tissues of the affected area (vazaprostan, rheopolyglucin, rheosorbilact, refortan, no-shpa, trental).

4) Treatment of concomitant diseases.

As noted above, a feature of the main group was the addition of granulocyte-colony stimulating factor (Filgrastim) to the complex of therapeutic measures, which was administered subcutaneously at a dose of 300 μg for patients weighing less than 80 kg and 480 μg for patients weighing more than 80.

Morphological studies included an assessment of the cytological picture of the imprints of the wound surface in the dynamics of the treatment. Microscopic examination of the preparations considered the morphological features of cellular and tissue elements. In addition, attention was paid to the functional signs present in the cytological material (the presence of mucus, protein mass, erythrocytes, inflammatory leukocyte cells, microorganisms, signs of a therapeutic effect in the form of pathomorphosis of the structural elements of smears). The background materials of the cytological preparation were detritus, small protein grains, fat drops, crystals, hematoidin, cholesterol, etc. The cytological material was stained with azure-eosin mixtures.

## RESULTS AND DISCUSSION

The conducted cytological studies of the wound surface in the dynamics of the ongoing traditional treatment in patients with purulent-inflammatory diseases of soft tissues on the background of diabetes mellitus showed a picture with background fatty-protein detritus, which proceeded with dystrophic and necrobiotic changes in tissue elements (Fig. 1). Combinations of this type of change with the presence of inflammatory cells were often noted. Tissue elements were subjected to destructive and necrobiotic changes under the action of microorganisms and the inflammatory process. These changes are known as vacuolization, loosening, and homogenization of nuclear cytoplasmic structures.[16]

On the part of histiocytic cells, cell activation was detected in the form of an expansion of the volume of the cytoplasm and hyperchromasia of the nuclei (Fig. 2). In the early stages of the course of the purulent-inflammatory process, polynuclear leukocytes predominated in the cytological material (Fig. 3), and in later periods - leukocyte infiltration of histiocytic and lymphoid cells (Fig. 4).

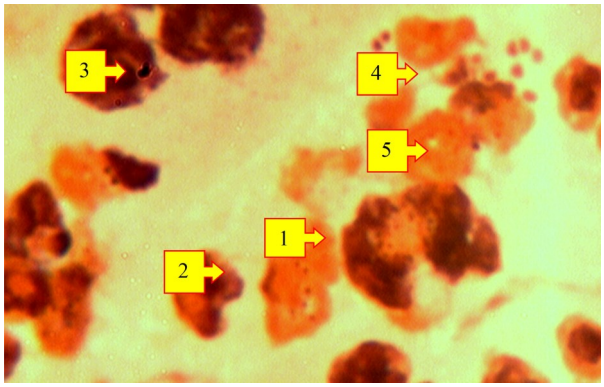


Fig. 1. Imperssion smear of the wound of the patient on the 1st day of treatment. Neutrophilic leukocytes (1), destroyed leukocytes (2), lymphocytes (3), microorganisms (4), proteins (5) are visible. Stain: Romanowsky. Magnification 10x40.

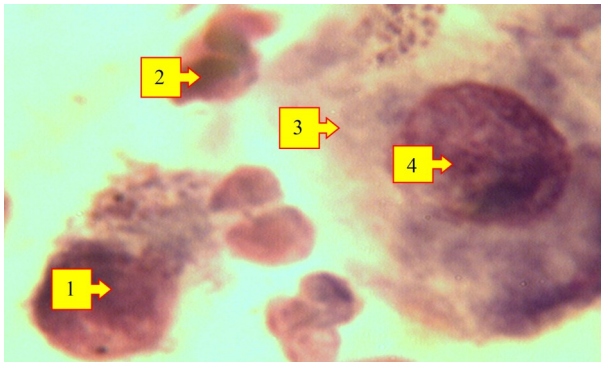


Fig. 2. Imperssion smear of the patient's wound on the 3rd day of treatment. Neutrophilic leukocytes (1), destroyed leukocytes (2), histiocytes (3) with nuclear activation (4) in the form of hyperchromasia with cytoplasm expansion are determined. Stain: Romanowsky. Magnification 10x40.

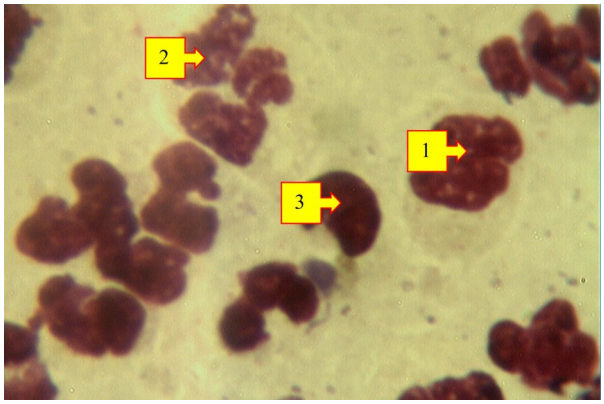


Fig. 3. Imperssion smear of the patient's wound on the 3rd day of treatment. Segmented neutrophils (1) are determined in the form of hyperchromasia, destroyed leukocytes (2) and lymphocytes (3). Stain: Romanowsky. Magnification 10x40.

In the case when weakly colored suspension protein substances were found among the cellular elements, this fact indicated the presence of necrosis.17 Also, extracellular granules and clumps of structureless masses of detritus of various sizes

were visible in the preparations. Detritus had a grayish tint due to its protein origin. A yellowish hue indicated the presence of a necrotic substance of a fatty-lipoid nature. The nature of the detritus and protein mass in the composition of the cytological preparation determined the type of bacteria. In the presence of structureless masses of lipid nature, the infection was caused by gram-positive cocci, which were coated on the outside with a liposaccharide membrane.

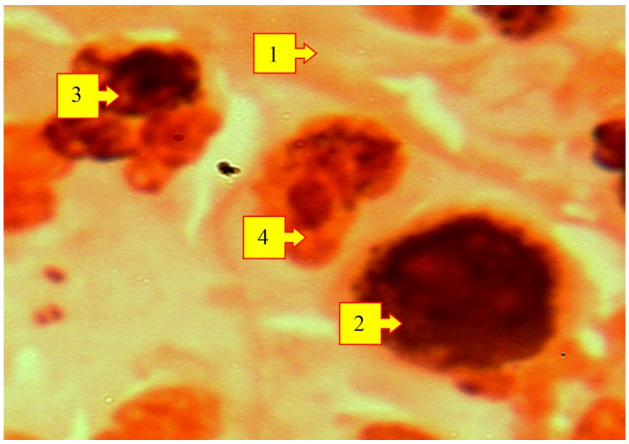


Fig. 4. Against the background of compaction of the protein substance (1), the presence of lymphoid cells (2), as well as neutrophilic leukocytes (3) and histiocytes (4). Stain: Romanowsky. Magnification 10x40.

In a number of cases, the protein mass predominated in the cytological imprint in the composition of the detritus. This variant of the lesion was caused by gram-negative microorganisms. They had a glycoprotein outer shell. The results of microscopic examination showed that the nature of the purulent-inflammatory wound of soft tissues determined the cellular composition of the inflammatory infiltration.18

In the early stages of the disease, polynuclear granular leukocytes were found on cytological preparations. Moreover, they had different shapes and sizes, their nuclear structures were often stained with a hyperchromic color, swollen with a thickening of the nuclear bridges between the chromatin segments. There were granulocytes with karyolytic and karyorectic changes in nuclear structures. Sometimes the chromatin substance of the nuclei was in a state of dispersion and decay (Fig. 5).

The cytoplasm of polynuclear leukocytes was also swollen, expanded in volume, the granular material was often in a state of activation in the form of rupture and dissolution or outflow into the surrounding space. In the cytoplasm of neutrophilic leukocytes, phagocytosed bodies were found (Fig. 6).

In some cases, when the presence of mixed flora was found in cytological preparations, and the presence of single eosinophilic leukocytes among granular leukocytes, in this case it was stated that autoimmune processes were associated with inflammatory diseases.

The results of microscopic studies of cytological preparations of smears-prints from purulent-inflammatory wounds of soft tissues in the dynamics of treatment of patients in the main group showed that, compared with the control group of patients, the degree of dystrophic-destructive and necrobiotic changes in histiocytic and inflammatory cells significantly decreases in the smear. On the part of histiocytic cells, some activation is noted in the form of an expansion of the volume of the cytoplasm and hyperchromasia of the nuclei. In such cases, the morphological state of inflammatory cells changes depending on the treatment.[19]

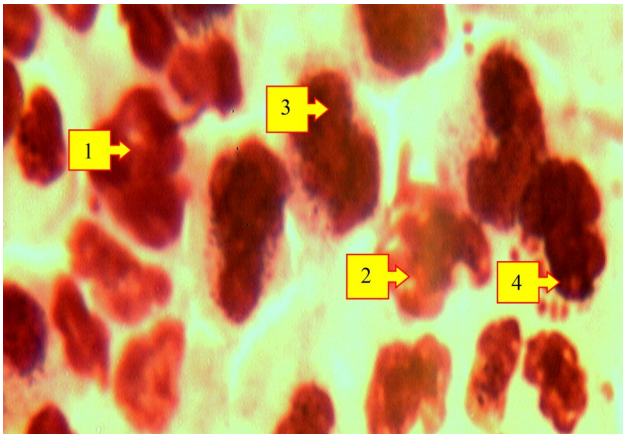


Fig. 5. Neutrophilic leukocytes (1) in a state of karyolysis and karyopyknosis of nuclear structures, destroyed leukocytes (2), lymphocytes (3), microorganisms (4). Stain: Romanowsky. Magnification: 10x40.

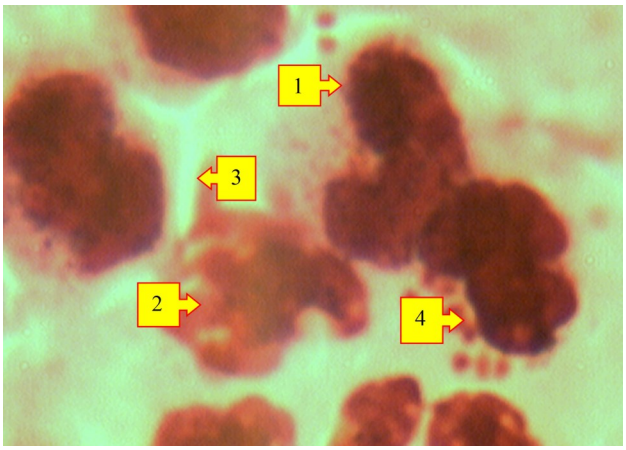


Fig. 6. Neutrophilic leukocytes (1) with signs of phagocytosis (destroyed leukocytes (2), lymphocytes (3), microorganisms (4)). Stain: Romanowsky. Magnification 10x40.

On the 1st day of treatment in the cytological material there was a decrease in the number of polynuclear leukocytes (Fig. 7). Among the cellular elements, weakly colored suspension protein substances were sometimes found, which indicates a decrease in dystrophic and destructive changes on the part of tissue-cellular structures and inflammatory cells. The presence of a grayish hue often predominated in the composition of the detritus, which proved an increase in the release of substances of a protein nature; if the detritus has a yellowish tint, then the necrotic substance is of a fatty-lipoid nature.20

As part of the leukocyte infiltration, monocyte-macrophage cells appeared, in some of them phag-

ocytic activity was noted in the form of the presence of relatively small phagocytosed dark particles in the cytoplasm of macrophages (Fig. 8).

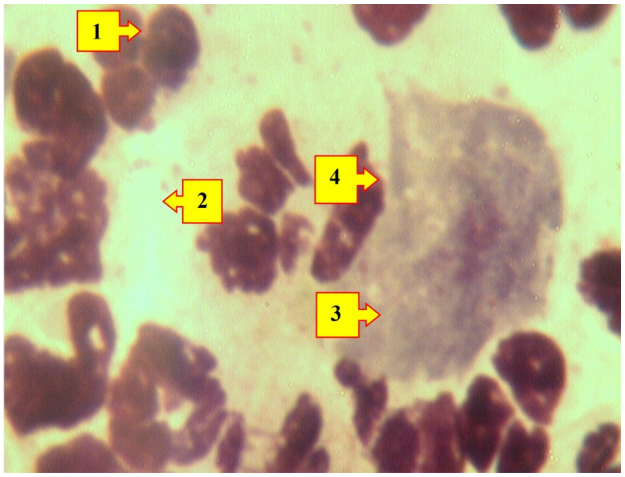


Fig. 7. Imperssion smear of the patient's wound on the 1st day of treatment. Neutrophilic leukocytes (1), lymphocytes (2), macrophages (3), phagocytosed inclusions by macrophages (4) are determined. Stain: Romanowsky. Magnification 10x40.

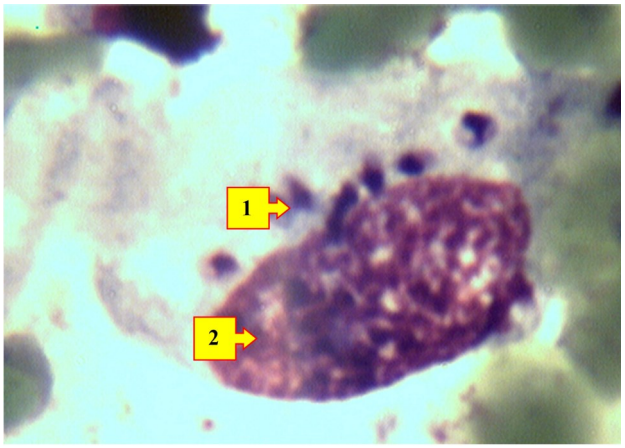


Fig 8. Imperssion smear of the patient's wound on the 1st day of treatment. Increased phagocytic activity of macrophages (1,2). Stain: Romanowsky. Magnification 10x40.

On the 3rd day, a decrease in the activity of the processes of alteration and exudation of inflammation was noted (Fig. 9). Morphologically, this was manifested by a decrease in the amount of inflammatory mucosa and necrotic-fibrinous mass, existing leukocytes in a state of destruction and disintegration, which morphologically looked like an irregularly shaped destructive mass stained with eosin.

By the 3rd day of treatment in cytological preparations, the disappearance of phenomena characteristic of the alterative and exudative phases of inflammation was noted. A significant increase in the number of neutrophilic leukocytes, which are in active form, was determined in the form of hyperchromasia and polysegmentation of nuclear structures (Fig. 10).

Among them, the appearance of lymphoid and monocytic cells was noted (Fig. 11). Polynuclear granular leukocytes had different shapes and sizes, their nuclear structures were often stained hyperchromically, swollen, with thickening of the nuclear bridges between the chromatin segments. Granulocytes with karyolytic and karyorectic changes in nuclear structures were almost not detected.

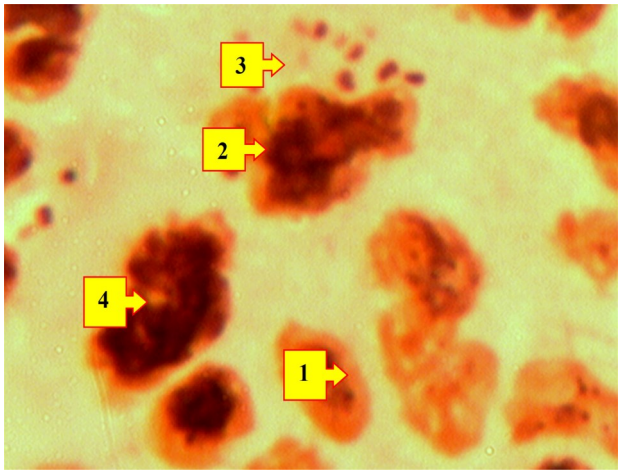


Fig. 9. Fragments of destructive particles of tissue structures, that is, protein substances (1) on the 3rd day after the application of the granulocyte-colony-stimulating factor. In the smear, an increase in the phagocytic activity of macrophages (2.3), lymphocytes (4) is determined. Stain: Romanowsky. Magnification 10x40.

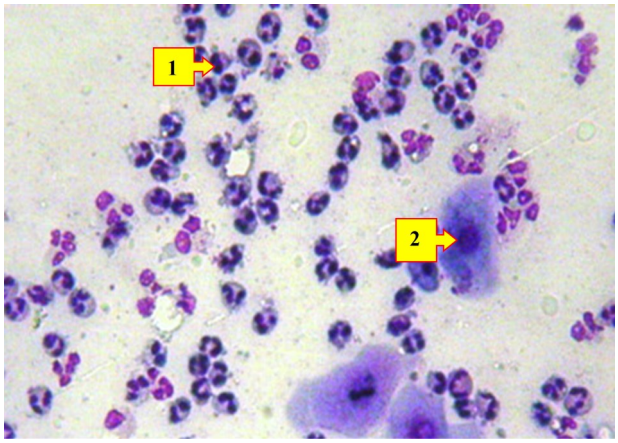


Fig. 10. Increased activity of neutrophilic leukocytes (1) in the form of hyperplasia and hypersegmentation of nuclei (histiocyte - 2). Stain: Romanowsky. Magnification 10x40.

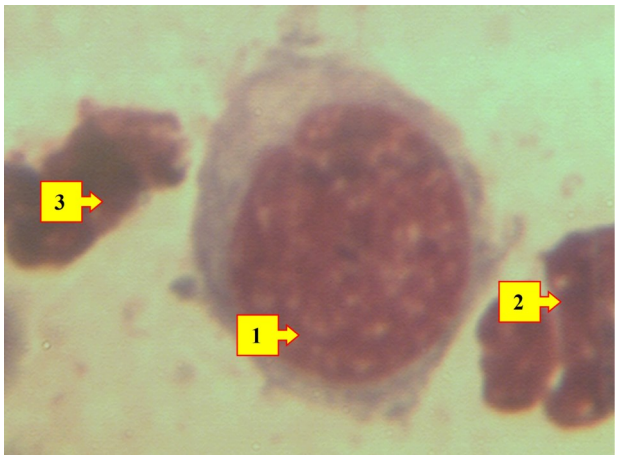


Fig. 11. The appearance of a lymphocyte (1), neutrophilic leukocytes (2) and destroyed leukocytes (3)

in the leukocyte infiltration. Stain: Romanowsky. Magnification 10x40.

The cytoplasm of polynuclear leukocytes was swollen, expanded in volume, and often contained a large number of phagocytosed microorganisms. Often, lymphoid, and histiocytic cells are found in the inflammatory infiltration.21 In such cases, we can say that the course of the wound process has a favorable picture. This was associated with the final proliferative stage. In such cases, in cytological preparations, the number of microorganisms was sharply reduced, and they were located mainly in the vicinity of macrophages and lymphohistiocytic cells. The above cytological changes were also accompanied by an increase in the number of macrophages with increased signs of phagocytosis in the composition of the cellular infiltration (Fig. 12).

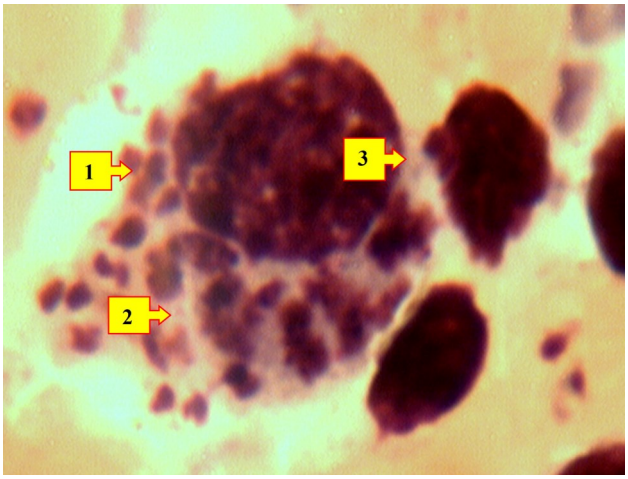


Fig. 12. Increased macrophage activity (1,2) as part of leukocyte infiltration, lymphocytes (3). Stain: Romanowsky. Magnification 10x40.

On the 7th day of treatment, smears were dominated by the number of polynuclear leukocytes, in particular neutrophilic leukocytes, which, being in an active state, densely surrounded layers of tissue detritus with microorganisms. Eosinophilic staining of the cytoplasm of most of the neutrophilic leukocytes indicated their enzymatic and phagocytic activity (Fig. 13).

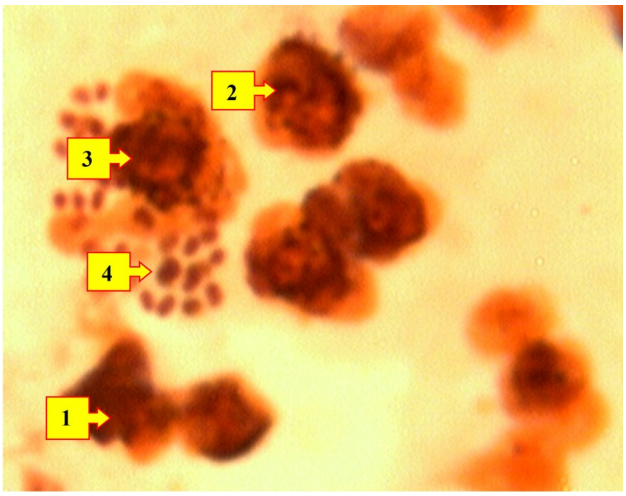


Fig. 13. Increased eosinophilicity of the cytoplasm of neutrophilic leukocytes. Destroyed leukites (1), lymphocytes (2), macrophages (3) and phagocytosed inclusions by macrophages (4). Stain: Romanowsky. Magnification 10x40.

During this period of the study, there were also single eosinophils and basophils among leukocytes. Histiocytic cells were even more enlarged due to the increase in dystrophic changes in the cytoplasm. Most of them had large, flattened dimensions with an increase in eosinophilic staining of both the nucleus and the cytoplasm.

## CONCLUSIONS

The results of a cytological study of smears-imprints of a purulent-inflammatory wound of soft tissues against the background of diabetes mellitus showed that the microscopic picture of the smear was characterized, first, by the presence of a microbial factor in combination with background elements. The main signs of an infectious purulent inflammatory process were the presence of various forms of microorganisms in the smear. In the early stages of the disease, coccal infection and polynuclear leukocyte infiltration predominated, and at later stages, a small number of lymphohistiocytic cells were found in the leukocyte infiltration. All this testified to the presence of a close relationship between the course of the wound process and the role of specific cells of the leukocyte series.

Already in the early stages of the course of the wound process, the nature of both the cellular and microbiological composition of cytological preparations was associated with the effectiveness of the use of granulocyte-colony-stimulating factor (Filgrastim), which contributed to the predominance of the number of inflammatory cells over microorganisms.

The use of the drug granulocyte-colony-stimulating factor (Filgrastim) in a complex of therapeutic measures in patients with purulent-inflammatory diseases of soft tissues against the background of diabetes mellitus contributes to a change during the wound process with an increase in the number of neutrophils and lymphocytes with their phagocytic activity in the wound with a decrease in microbial contamination, which has been proven us cytological studies.

**Ethics approval and consent to participate** - All patients gave written informed consent to participate in the study.

**Consent for publication** - The study is valid, and recognition by the organization is not required. The author agrees to open publication

**Availability of data and material** - Available

**Competing interests** - No

**Financing** – No

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## МОРФОЛОГИЧЕСКИЕ АСПЕКТЫ РАН У БОЛЬНЫХ С ГНОЙНЫМ ВОСПАЛЕНИЕМ МЯГКИХ ТКАНЕЙ ПРИ САХАРНОМ ДИАБЕТЕ И ПОД ВЛИЯНИЕМ ГРАНУЛОЦИТАРНО-КОЛОНИЕСТИМУЛИРУЮЩЕГО ФАКТОРА

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**Абстракт.**

**Актуальность.** Гнойно-воспалительные заболевания мягких тканей у больных сахарным диабетом протекает достаточно агрессивно и соответственно стандарты свойственные острым инфекциям мягких тканей без сахарного диабета, могут противоречиво влиять на судьбу больных с наличием сопутствующего сахарного диабета.

**Цель исследования.** Изучить особенности цитологической картины ран у больных с гнойно-воспалительными заболеваниями мягких тканей на фоне сахарного диабета после применения гранулоцитарно-колониестимулирующего фактора.

**Полученные результаты** свидетельствуют, что наравне с клеточными элементами в морфологии раны у больных с гнойно-воспалительными заболеваниями мягких тканей на фоне сахарного диабета значительную роль играют и другие представители цитологической картины. Результаты цитологического исследования мазков-отпечатков гнойно-воспалительной раны мягких тканей у больных сахарным диабетом показали, что микроскопическая картина мазка характеризовалась, прежде всего, наличием микробного фактора в сочетании с фоновыми элементами.

**Заключение.** Под воздействием препарата гранулоцитарно-колониестимулирующего фактора у больных с гнойно-воспалительных заболеваний мягких тканей на фоне сахарного диабета увеличивает количество нейтрофилов и лимфоцитов, а также повышает их фагоцитарную активность в ране.

**Ключевые слова:** гнойно-воспалительный раневой процесс, сахарный диабет, цитология ран, гранулоцитарно-колониестимулирующий фактор

**QANDLI DIABET BILAN YUMSHOQ TO‘QIMALARNING YIRINGLI YALLIG‘LANISHI BILAN OG‘RIGAN VA GRANULOTSIT-KOLONIYALARNI QO‘ZG‘ATUVCHI OMIL TA’SIRIDA BO‘LGAN BEMORLARDA YARALARNING MORFOLOGIK JIHATLARI.**

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**Abstrakt.**

**Dolzarbligi.** Qandli diabet bilan og'rigan bemorlarda yumshoq to'qimalarning yiringli-yallig'lanish kasalliklari juda agressiv tarzda davom etadi va shunga ko'ra, qandli diabetsiz yumshoq to'qimalarning o'tkir infektsiyalariga xos bo'lgan standartlar, qandli diabet bilan kasallangan bemorlarning taqdiriga qarama-qarshi ta'sir ko'rsatishi mumkin.

**Tadqiqot maqsadi.** Qandli diabet fonida yumshoq to'qimalarning yiringli-yallig'lanish kasalliklari bilan og'rigan bemorlarda granulotsit-koloniyani stimulyator omilni qo'llashdan keyin yaralarning tsitologik rasmining xususiyatlarini o'rganish.

**Olingan natijalar** shuni ko'rsatadiki, qandli diabet fonida yumshoq to'qimalarning yiringli-yallig'lanish kasalliklari bilan og'rigan bemorlarda yara morfologiyasida hujayrali elementlar bilan bir qatorda tsitologik rasmning boshqa vakillari ham muhim rol o'ynaydi. Qandli diabet bilan og'rigan bemorlarda yumshoq to'qimalarning yiringli-yallig'lanish yarasining smear izlarini tsitologik o'rganish natijalari shuni ko'rsatdiki, smearning mikroskopik ko'rinishi, birinchi navbatda, fon elementlari bilan birgalikda mikrobial omil mavjudligi bilan tavsiflanadi.

**Xulosa.** Qandli diabet fonida yumshoq to'qimalarning yiringli-yallig'lanish kasalliklari bilan og'rigan bemorlarda granulotsit-koloniyani stimulyatsiyalovchi omilni tayyorlash ta'siri ostida neytrofillar va limfotsitlar sonini ko'paytiradi, shuningdek yaradagi fagotsitlar faolligini oshiradi.

**Kalit so'zlar:** yaraning yiringli-yallig'lanish jarayoni, qandli diabet, yara tsitologiyasi, granulotsit-koloniyani stimulyatsiya qiluvchi omil