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RESEARCH ARTICLE

CHANGES IN THE CYTOLOGICAL PICTURE IN LONG-TERM NON-HEALING WOUNDS

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Abstract: The physiological process of wound healing includes four stages: hemostasis, inflammation, proliferation and maturation, the correct and coordinated work of which ensures a strict staged regenerative process. However, when wounds do not go through this organized process, their healing is delayed, and this eventually leads to chronic or long-term non-healing wounds. Common signs of long-term non-healing wounds are exudation, reinfection, tissue necrosis, defective re-epithelialization, decreased angiogenesis and excessive production of reactive oxygen species. In general, chronic wounds can be divided into three main categories: diabetic foot ulcers, vascular ulcers, and pressure ulcers. They are usually observed in elderly people suffering from pathological conditions such as diabetes mellitus, vascular diseases and obesity.

Key words: Long-term non-healing wounds, morphological picture of the wound process, diagnosis, pathogenesis.

INTRODUCTION

Impaired regeneration of long-term non-healing wounds associated with many factors, including constant exudation, reinfection, tissue necrosis, defective re-epithelialization, and decreased angiogenesis [1, 2]. All this served as prerequisites for conducting more in-depth studies of the mechanisms of the body's immune response as a guarantee of improving the results of treatment of patients with long-term non-healing wounds.

In order to identify the real scale of the impact on health of long-term non-healing wounds, we analyzed the systematic literature published over the past 10–15 years in the most popular databases. The results showed that health-related quality of life was lowest among patients with physical pathologies, including the presence of long-term non-healing wounds. The same number of patients was noted by us after amputation of limbs as a result of the progression of long-term non-healing wounds.

According to Olsson M. et al. [3] The burden of costs was mainly related to amputations in patients with concomitant type 2 diabetes mellitus, where the cost of hospitalization ranged from $12,851 to $16,267 for this patient population.

Patients with long-term non-healing wounds have a poor quality of life related to overall health. Accordingly, the costs associated with the treatment of long-term non-healing wounds remain significant. This dictates the need to develop and implement treatment strategies for long-term non-healing wounds aimed at improving health-related quality of life and effectively reducing costs for this group of patients.

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Rodrigues M. et al. [4] have estimated that in developed countries, the costs associated with the treatment of long-term non-healing wounds account for up to 3% of total health care costs. In the United States, for example, the total cost of long-term non-healing wounds is estimated to be around US$50 billion per year. The situation is likely to be exacerbated by low healing rates.

Fife C.E. et al. [5] In their scientific article, they reported that the publicly available rates of soft tissue wound healing are significantly overestimated. In particular, data from randomized controlled trials give an average recovery rate of 40%, while the reported rate is usually above 90%.

Bioactive molecules that stimulate neovascularization and re-epithelialization have shown positive results in preclinical studies. In addition, they can be combined with biomaterials, which can improve their half-life and promote controlled release. On the other hand, biomaterials can be used on their own to provide physical protection to damaged soft tissues.

Another therapeutic strategy for wound healing is cell technologies using mesenchymal stem cells derived from bone marrow, fat cells, epidermal cells, and others. Numerous studies have shown that cell therapy improves wound healing by enhancing angiogenesis and re-epithelialization.

The aim of the study was to study the cytological picture of long-term non-healing wounds in various nosological forms.

MATERIAL AND METHODS

The study involved 84 patients with long-term non-healing wounds. They were represented by bedsores in 29 (34.5%) patients, ulcerative formations in patients with diabetic foot syndrome in 28 (33.3%) patients, and trophic ulcers due to complications of chronic venous insufficiency of the lower extremities in 27 (32.1%) patients.

The nature of the necrobiotic process in the wound was diverse. Only in 3.6% of cases (3 patients) the inflammatory process in the long-term non-healing wound was visually absent. They were presented to 2 (66.7%) patients with trophic ulcers of the lower extremities and 1 (33.3%) patients with bedsores.

In 20 (23.8%) patients, the inflammatory process in a long-term non-healing wound was present, but it proceeded without the presence of tissue necrosis. At the same time, this variant of the course of the inflammatory process in a long-term non-healing wound was presented in 65% of cases (13 patients) with trophic ulcers of the lower extremities, in 25% of cases (5 patients) with bedsores, and in 10% of cases (2 patients) with neurotrophic ulcers of diabetic foot syndrome.

In contrast, in 72.6% of cases, the wound was characterized not only by the presence of an inflammatory process, but also by tissue necrosis. Thus, in 36.9% of cases (31 patients) the tissues of long-term non-healing wounds were subjected to dry necrosis, in 11.9% of cases (10 patients) to wet necrosis, and in 23.8% of cases (20 patients) to mixed necrosis.

Patients with dry necrosis in long-term non-healing wounds were mainly represented by cases of ulcerative-necrotic ulcers in diabetic foot syndrome (15 patients – 48.4%) and bedsores (10 patients – 32.3%). Only in 6 (19.4%) patients, dry necrosis was noted in trophic ulcers with chronic venous insufficiency of the lower extremities.

Damage to long-term non-healing wounds by wet necrosis was noted among 10 (11.9%) patients. In half of the cases (5 patients) they were represented by bedsores, in 40% of cases (4 patients) – ulcers in diabetic foot

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syndrome, and in 10% of cases (1 patient) – trophic ulcers of venous etiology.

The development of wet necrosis in long-term non-healing wounds against the background of non-rejected dry necrosis (mixed necrosis) was revealed by us among 20 (23.8%) patients, who were represented by 8 (40%) patients with bedsores, 7 (35%) patients with neurotrophic ulcers of diabetic foot syndrome, and 5 (25%) patients with trophic ulcers with chronic venous insufficiency of the lower extremities.

For an objective assessment of the severity of reparative processes in the wound, we carried out a cytological study of the cellular composition of the wound surface. Both qualitative (morphological) and quantitative (morphometric) research methods were used.

Wound impressions stained according to Romanovsky-Giemsa were examined with a light microscope with a 10x40 magnification objective.

RESULTS AND DISCUSSION

The general morphological picture for all wounds in the studied groups was the presence of a chronic inflammatory process, which included all three phases of the wound process. Long-term non-healing wounds were characterized by the fact that their bottom, as usual, was covered with both fibrin and granulation tissue. In some places, the presence of necrotic tissue changes of the "necrotic islets" type and purulent discharge under them was noted. Granulation tissue in patients with long-term non-healing wounds was usually sluggish in growth and pale in color. The marginal surfaces of long-term non-healing wounds were compacted like craters with epithelialization, and sometimes even with hyperkerotization.

The cytological characteristics of the wound surface of patients with long-term non-healing wounds at all periods of treatment were determined by an inflammatory background consisting of detritus of mainly fatty and protein nature. All of them formed the basis of the existing dystrophic and necrotic changes in the tissue structures of long-term non-healing wounds. Against the background of such transformations of the cytological picture, we identified mainly cells of the inflammatory series, especially among patients with exacerbation of the course of the chronic wound process.

The cytological picture of a long-term non-healing wound on the 1st day of treatment had a feature characterized by the fact that the tissue elements of the surface of long-term non-healing wounds, covered with protein background structures, were under the influence of microorganisms and thereby supported the inflammatory reaction in the tissues. Such changes were determined in the form of necrobiotic manifestations and cell destruction. At the same time, the microorganisms present in the wound formed colonies, which clearly indicated an increasing bacterial load on the tissues.

The presence of vacuolization and loosening of nuclear and cytoplasmic structures was characteristic. In some places, they had the character of loosening and homogenization. As shown in Figure 1, against the background of detectable neutrophilic leukocytes, destroyed white blood cells consisting of lymphocytes are identified, as well as the presence of various bacterial particles in places. In the background space, protein elements are revealed.

For the cytological imprint of the wound at this time of the study, the presence of damaged collagen threads in the peripheral areas on the border with the skin, which clearly did not reach their strength, was typical.

A certain kind of increased activity was revealed in relation to histiocytic cells, which were characterized by some activation in the

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form of an increase in the size of the cytoplasm and the acquisition of their nuclei of hyperchromatic properties.

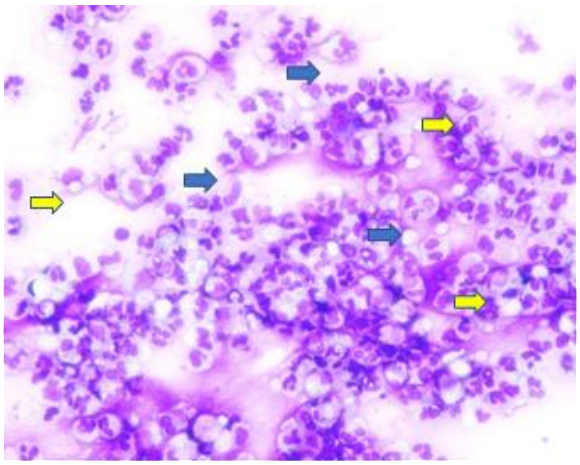

Figure 1. Smear, wound impressions, material sampling on the 1st day of treatment. Staining according to the Ramonovsky-Giems method. Magnification: approx. 10, rev. 40.

Figure 2 shows the cytological picture of the patient's long-term non-healing wound on the 7th day of treatment, where neutrophils and histiocytes are identified against the background of protein substances and destroyed leukocyte cells. At the same time, the cytoplasm of cells is expanded due to hyperchromization of their nuclei.

On the 7th day of the purulent-inflammatory process, polynuclear leukocytes prevailed in the cytological material of long-term non-healing wounds. Neutrophils, mainly segmented cell types, are still preserved in the wound. All of them are in a hyperchromatic state. Around them, destroyed leukocytes and lymphocytes are determined, which are located randomly, sometimes crowded, sometimes diffusely.

The growth of capillaries was significantly reduced, which was apparently due to the ongoing remodeling process.

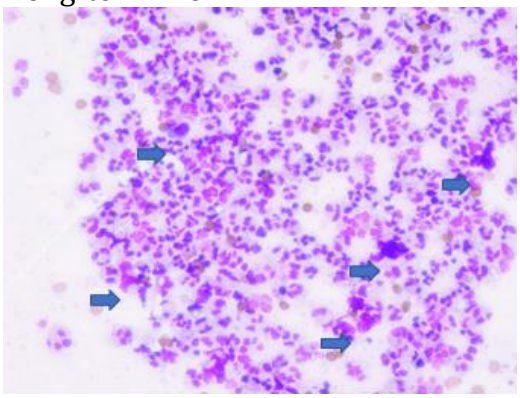

Figure 2. Smear, wound impressions, material sampling on the 7th day of the treatment. Staining according to the Romonowski-Giems method. Magnification: approx. 10, rev. 40.

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In the later stages of the treatment of cytological impressions of the wound, the presence of leukocyte infiltration of histiocytic and lymphoid cells could be noted. Thus, on the 14th day of the treatment, in the cytological picture of imprints of long-term non-healing wounds, we revealed a certain background, which was formed due to protein substances in a compacted state. The cells detected in the wound are characterized by the presence of an advantage of neutrophils, lymphocytes and histiocytes (Figure 3).

In the case when weakly colored protein suspension substances were found among the cellular elements, this fact indicated the presence of necrosis.

Extracellular granules and clumps of structureless detritus masses of various sizes were also visible in the preparations. The detritus had a grayish tint due to its white origin. A yellowish tint indicated the presence of a necrotic substance of a girolipoid nature. The nature of the detritus and protein mass in the composition of the cytological preparation was determined by the type of bacteria. In the presence of structureless masses of a fat-lipid nature, the infection was caused by gram-positive cocci, which, naturally, are covered with a liposaccharide coating on the outside.

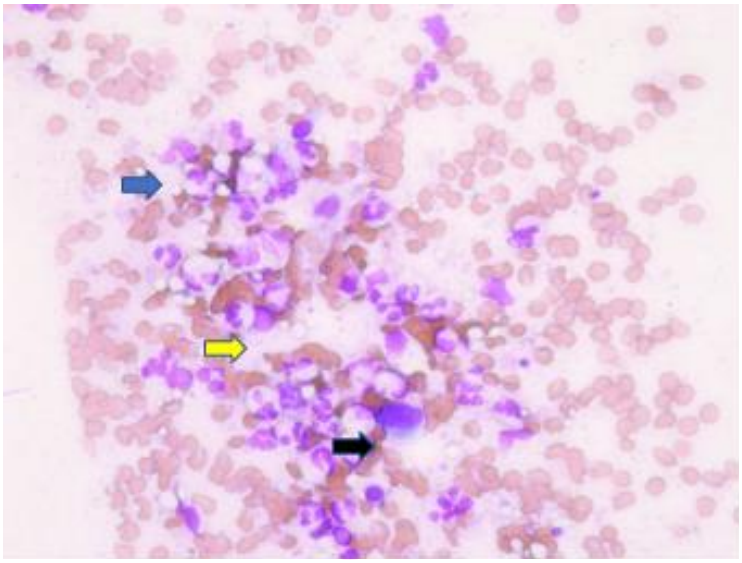

Figure 3. Smear, wound impressions, sampling of material on the 14th day of treatment. Coloring according to the method: Romonovsky-Giems. Magnification: approx. 10, rev. 40.

Figure 4 shows that against the background of detected blood cells, such as leukocytes and lymphocytes, which are in a destroyed state, elements of the destroyed structure of microorganisms can be traced.

Neutrophils are found with an altered structure in the form of karyolysis and karyopyknosis of their nuclei.

The predominant mass in the studied cytological prints was the mass consisting mainly of their protein. This variant of the manifestation of the morphological picture of the wound is often due to the activity of gram-negative microorganisms. As is known, such microorganisms have a mixed glycoprotein coat covering them from the outside.

Cytological studies once again confirmed the variant of the course of long-term non-

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healing wounds in the form of infiltration of a cellular inflammatory nature.

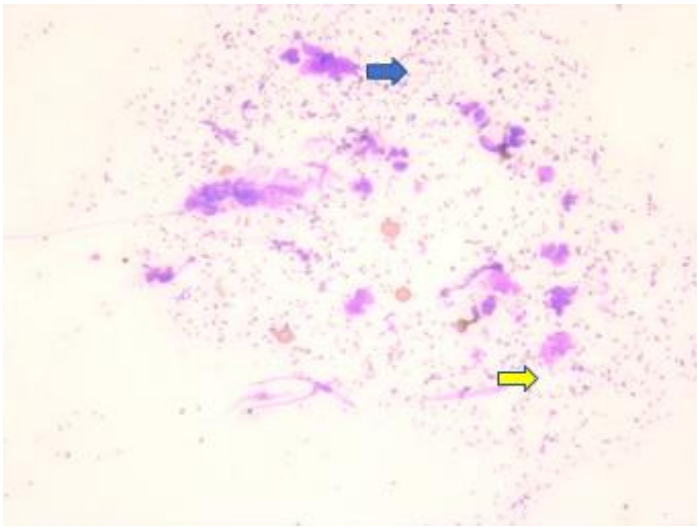

Figure 4. Smear, wound impressions, sampling of material on the 14th day of treatment. Coloring according to the method: Romonovsky-Giems. Magnification: approx. 10, rev. 40.

On the 28th day of treatment, the wound was covered with a scab, which had a yellow color and consisted of fibrous tissue. In the structure of such an eschar, fibrin, pus, and protein-like material were found (Figure 5).

The background of the imprint is formed by destroyed leukocytes, lymphocytes and microorganisms. Against this background, neutrophils with signs of incomplete phagocytosis are detected. At the same time, phagocytized bodies were found in the cytoplasm of neutrophil leukocytes. Fibroblasts had the peculiarity of acquiring low proliferative capacity.

A large number of monocyte-macrophage cells and plasma cells are determined.

In general, morphological changes in the early stages of treatment of long-term non-healing wounds were characterized by the presence of granulocytes, which appeared to be a polynuclear variant. This definition is due to the fact that in the process of staining cells, their nuclear structures often had increased color receptivity (hyperchromia). The bridges connecting the chromatin segments of such nuclei were swollen and thickened. But not all granulocytes had such a morphological structure. Along with them, we also identified granulocytes with karyolytic, and sometimes even karyoretic changes in their nuclei and their structures. In such cases, the chromatin substance of the nuclei was atomized and in a disintegrating state. The same picture was noted in relation to polynuclear leukocytes. Their cytoplasm was increased in volume due to swelling. At the same time, the granular composition acquired an active form, which was manifested by rupture and dissolution, and in some places also by the outpouring of the contents into the pericellular environment.

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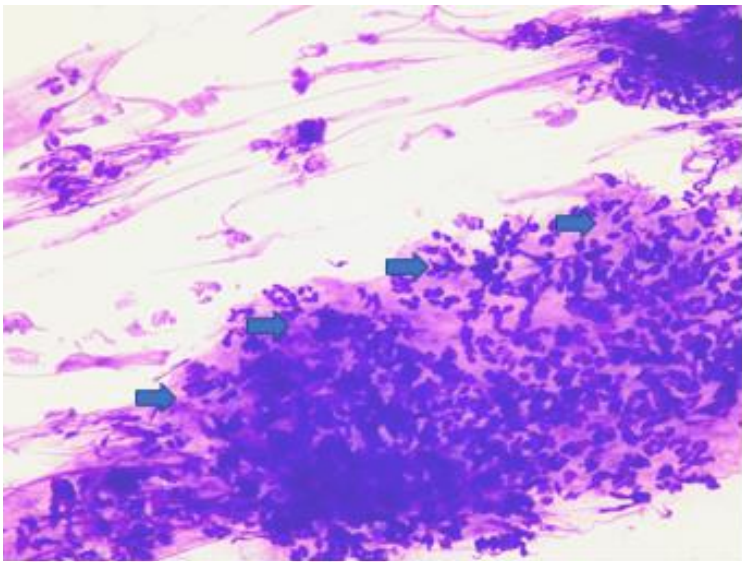

Figure 5. Smear, wound impressions, material sampling on the 28th day of treatment. Coloring according to the method: Romonovsky-Giems. Magnification: approx. 10, rev. 40.

In some cases, when the presence of mixed flora was found in cytological preparations, and the presence of single eosinophilic leukocytes among granular leukocytes, then in this case the addition of autoimmune processes to inflammatory diseases was stated.

CONCLUSION

On the basis of the morphological study of the imprints of long-term non-healing wounds, it is possible to conclude that the presence of a protein background, the basis of which is made up of inflammatory cells and microorganisms, plays a leading role. All this determined the cellular-microbial factor as one of the main components leading the entire process of formation of long-term non-healing wounds. The basis for such a judgment may be the presence of microorganisms of various shapes, but with a predominant gram-negative structural character against the background of multinucleated leukocyte infiltration. And although in later terms leukocyte infiltration decreased, nevertheless, lymphocytes and histiocyte cells still prevailed in long-term non-healing wounds. Such a nature of damage to long-term non-healing wounds determined the role of leukocyte cells as the main factor in organizing the course of the wound process.

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 financial support has been provided for this work

Conflict of interests-The authors declare that there is no conflict of interest.

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