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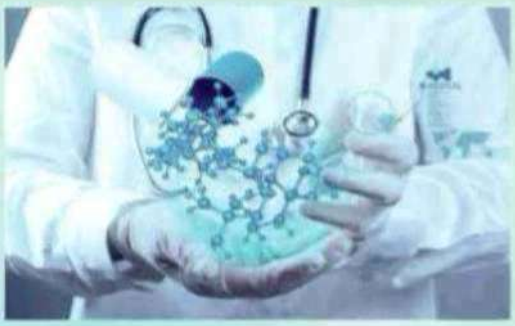

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www.bsmi.uz
https://newdaymedicine.com E:
ndmuz@mail.ru
Тел: +99890 8061882

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CHARACTERISTICS OF LONG-TERM NON-HEALING WOUNDS

Umarov Bakhtiyorjon Yatgarovich https://orcid.org/0000-0001-9173-2028
E-mail: bakhtiyorumarov@mail.ru
Khamdamov Bakhtiyor Zarifovich https://orcid.org/0000-0003-3569-6688
E-mail: dr.hamdamov@mail.ru

Bukhara State Medical Institute named after Abu Ali ibn Sina, Uzbekistan, Bukhara, st. A. Navoi. 1 Tel: +998 (65) 223-00-50 e-mail: info@bsmi.uz

✓ ***Resume***

The concentration of lysozyme in long-term non-healing wounds is characterized by a change in dynamics depending on the presence of generalization of infection in the form of an increase in production above the initial value. Such changes in lysozyme concentration are directly related to the presence of generalization of infection and can be used in predicting the course of the inflammatory process in patients with long-term non-healing wounds.

Key words: long-term non-healing wound, immunity, generalization of infection.

ХАРАКТЕРИСТИКА ДЛИТЕЛЬНО НЕЗАЖИВАЮЩИХ РАН

Умаров Бахтиёржон Ятгарович https://orcid.org/0000-0001-9173-2028
E-mail: bakhtiyorumarov@mail.ru
Хамдамов Бахтиёр Зарифович https://orcid.org/0000-0003-3569-6688
E-mail: dr.hamdamov@mail.ru

Бухарский государственный медицинский институт имени Абу Али ибн Сины, Узбекистан, г. Бухара, ул. А. Навои. 1 Тел: +998 (65) 223-00-50 e-mail: info@bsmi.uz

✓ ***Резюме***

Концентрация лизоцима в длительно незаживающих ранах характеризуется изменением динамики в зависимости от наличия генерализации инфекции в виде повышения продукции более чем исходного значения. Такие изменения в концентрации лизоцима напрямую связаны с наличием генерализации инфекции и могут быть использованы при прогнозировании течения воспалительного процесса у больных с длительно незаживающими ранами.

Ключевые слова: длительно незаживающая рана, иммунитет, генерализация инфекции.

УЗОҚ МУДДАТ БИТМАЙДИГАН ЯРАЛАРНИНГ ХАРАКТЕРИСТИКАСИ

Умаров Бахтиёржон Ятгарович https://orcid.org/0000-0001-9173-2028
E-mail: bakhtiyorumarov@mail.ru
Хамдамов Бахтиёр Зарифович https://orcid.org/0000-0003-3569-6688
E-mail: dr.hamdamov@mail.ru

Абу али ибн Сино номидаги Бухоро давлат тиббиёт институти Ўзбекистон, Бухоро ш., А.Навоий кўчаси. 1 Тел: +998 (65) 223-00-50 e-mail: info@bsmi.uz

✓ ***Резюме***

Узоқ муддат битмайдиган ярларда лизоцим концентрациясининг бошлангич миқдордан кўп ишлаб чиқарилиши инфекция генерализациясининг мавжудлиги билан тавсифланади. Лизоцим миқдоридаги бундай ўзгаришлар инфекциянинг генерализацияланиши билан бевосита боғлиқ бўлиб, узоқ муддат битмайдиган яралари мавжуд бўлган беморларда яллиғланиш жараёнининг кечишини баҳоратлаш учун ишлатилиши мумкин.

Калит сўзлар: узоқ муддат битмайдиган яра, иммунитет, инфекциянинг генерализацияси.

Relevance

The long term of wound healing contributes to the accumulation of the number of such patients, which reached more than 40 million people worldwide 10 years ago. This type of DPR spread was noted by P. Driscoll [1,3,5,17] as a "silent epidemic". However, after 5 years, there were reports that]the number of patients with DPR had reached 500 million [2,4,18]. Such an impressive figure leads to an increase in financial costs in the healthcare system [6,15,16]. In particular, M. Olsson et al. [1,7,19] carried out calculations and showed that in developed countries such expenses account for up to 3% of total healthcare costs.

Prolongation of the last stage of the inflammatory process is typical for long-term non-healing wounds (DNR). Accordingly, cytological studies of wound imprints and assessment of the immunological cell population in patients with DNDD revealed the long-term presence of myeloid cells such as macrophages, neutrophils and monocytes.

Immune cells actively interact with non-hematopoietic cells, such as keratinocytes, through the secretion of various signaling molecules [8,9,20]. Keratinocytes make a significant contribution to the formation of chronic wounds, but the exact mechanism is not fully understood. It is only known that dysregulation of certain microRNAs, such as miR-34a/c, miR-203, miR-19a/b, and miR-20a, in keratinocytes affects immune functions and leads to DNP [10, 21].

Immune and structural cells actively express and regulate cytokines, chemokines, and growth factors during wound healing [11]. For example, increased levels of INF-γ, VEGF, and soluble VCAM-1 observed in patients with DNDD promote their healing (4,12).

However, in non-healing wounds, certain factors are disrupted, which is partly responsible for the pathogenesis of the injury. Mice deficient in IL-36 receptor antagonists showed delayed wound healing due to overproduction of IL-36y, TGF-β, and CXCL1, excessive infiltration of neutrophils and macrophages, and excessive formation of granulation tissue [5,9]. In addition, the CCR4 chemokine receptor negatively affects chronic wounds caused by diabetes mellitus. Diabetic mice depleted of CCR4 showed decreased expression of cytokines that promote wound healing, such as IL-6, IL-12, IL-1b, TNF-α, and IL-10 [3,5,22].

During normal wound healing, cells in the damaged area, such as fibroblasts, keratinocytes, and immune cells, are induced by local mediators to secrete matrix metalloproteinase. Such mediators include various cytokines and growth factors involved in wound healing, such as TGF-β, VEGF, EGF, interleukins, and interferons [1,9]. Matrix metalloproteinase is usually required in small amounts and is responsible for proper epithelialization and proliferation. However, their dysregulation leads to impaired epithelialization and is closely related to DNPR [4,6,8].

Thus, the process of full-fledged regeneration of DNR does not occur when the immune system is unable to continue the normal recovery process, which leads to the prolonged presence of neutrophils and pro-inflammatory macrophages in the damaged skin, which contributes to inflammation, tissue fibrosis and poor vascularization. Research in this area continues, however, today it is necessary to clarify the causes of the development of the generalization of the inflammatory process when using well-known methods of treating DNDD and to determine the role of changes in immune status. This would make it possible to develop effective methods of immunodiagnostics, as well as prediction and prevention of generalization of infection, which ultimately, in our opinion, can improve the results of treatment of patients with DNDD.

The purpose of the study. Development of methods for determining changes in the morphological and local immunological picture of long-term non-healing wounds.

Material and methods

Local clinical methods of wound research were based on an assessment of the nature of the necrobiotic process in the DNR. The presence/absence of a local inflammatory process and the type of tissue necrosis (dry, wet or mixed) were visually assessed.

The type of tissue in the DNR bed was determined, which could be in the form of dense and red granulation, brittle and pale granulation, fibrous film or tissue, as well as in the form of scab formation.

The nature of the wound exudate (serous, hemorrhagic, purulent), its color (without color, from pink to red, white, creamy and green), consistency (transparent, watery, bloody, watery and thick) and the smell of the exudate (available/not available) were evaluated.

The depth of the wound was estimated by us according to the classification of Knighton (2000). With this classification, six degrees of wound depth were distinguished. The first degree was characterized by the presence of a superficial wound between the epidermal and dermal layers of the skin. The second degree was characterized by a lesion at the level of subcutaneous fat. When the fascial space was affected, the depth of the wound acquired a third level. At the fourth degree, the wound process reached the muscular layer of tissues, and with the lesion of bone-tendon formations, the depth of the wound acquired the fifth degree of lesion. The sixth degree of development of the wound process was characterized by damage to the body cavities and internal organs.

To assess the course of the wound process, qualitative and quantitative characteristics of the microbial contamination of the wound are important. In this regard, we determined the species spectrum of the microflora isolated from the wound.

To objectively assess the severity of wound repair processes, we conducted a cytological study of the cellular composition of the wound surface. At the same time, both qualitative (morphological) and quantitative (morphometric) research methods were used.

The Romanovsky-Giemse stained wound prints were examined with a light microscope with a 10x40 magnification lens. Granulocytes (%), macrophages (%), fibroblasts (%) and lymphocytes (%) were counted. Along with the count of leukocytes, the degree of their degeneration, the number of mononuclear and mast cells, cellular and extracellular tissue elements were assessed.

The severity of degenerative and regenerative processes in the wound was assessed by calculating the regenerative-degenerative index, which was calculated using the formula RDI=(PYANG+XIANG)/DPN, where PIAN is the number of rod–shaped neutrophils, XIANG is the number of segmented neutrophils, and DPN is the number of degenerative forms of neutrophils in the field of vision.

Pathohistological studies were performed on days 1, 7, 14, 28 and for the final period of treatment before performing wound closure with skin plasty.

Special research methods were more related to immunological ones and consisted of determining sample parameters of cellular and humoral immunity in blood serum and wound.

For immunological studies, blood was taken from the ulnar vein into a centrifuge tube treated with 5.0 ml heparin. We selected 10 μl for counting leukocytes and lymphocytes on the Goryaev chamber using Zadorozhny S.I. and Dozmorov I.M. paint (1987). Mononuclear cells from peripheral blood were obtained by isolating ficoll verografin with a density of 1,077g/l according to Boyum (1968) on a density gradient. The number of cells was counted in the Goryaev chamber using the conventional method under a microscope and the concentration of lymphocytes was adjusted to 2x106 in 1 ml, the viability of lymphocytes was determined in a test with 0.1% trypan blue.

The assessment of the state of the immune system of the patients was carried out by the expression of CD-differentiation and activation antigens. The following markers of immunocompetent cells were determined: CD3+-, CD4+, CD8+-, CD16+-, CD20+-, CD23+-, CD38+-, as well as CD25+-, CD95+-lymphocytes. CD receptor expression was carried out in the rosette formation reaction using LT series monoclonal antibodies manufactured by Sorbent LLC (RF) according to the method of Gharib F.Yu. et al. (1995).

The serum concentrations of the examined immunoglobulins of the main three classes M, A and G g/l were determined by radial immunodiffusion according to Mancani (1963).

Interleukins (cytokines) were determined in the blood serum of the examined by solid-phase enzyme immunoassay. To implement this option, two monoclonal antibodies with different etiotropic specificity for interleukins IL-1b, TNF-α and TGF-β were used. The concentrations of MIP-1a, MIP-2b and PDGF in the blood serum were also determined using special enzyme immunoassay kits according to the standard procedure.

Immunological studies of blood serum were carried out by us on the 1st, 7th, 14th, 28th day of treatment of patients.

Studies of the local immunological reaction of DNR included the determination of immunoglobulins G, A and M in wound washes by radical immunodiffusion in gel using monospecific sera according to the method of B. Manchini (1968). The lysozyme concentration in the wound washes was determined by the nephelometric method according to the method of G.D. Dorofeichuk (1968).

We conducted local immunological studies on days 1, 7, 14, 28 and for the final period of treatment before performing wound closure with skin plasty.

The results and their discussion

The common morphological pattern for all wounds in the studied groups was the presence of a chronic inflammatory process, which included all three phases of the wound process. DNZRS 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 islands" type and purulent discharge under them was noted. Granulation tissue in patients with DNDD was usually of sluggish growth and pale in color. The marginal surfaces of long-term non-healing wounds were compacted like craters with epithelialization, and sometimes even with hyperkerization.

The cytological characteristics of the wound surface of patients with DNDD during all periods of treatment were determined by the inflammatory background, consisting of detritus of a predominantly 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 inflammatory cells, especially among patients with exacerbation of the course of the chronic wound process.

The cytological picture of DNZR on day 1 of the treatment had a feature characterized by the fact that the tissue elements of the DNZR surface, covered with protein background structures, were under the influence of microorganisms and thereby supported the inflammatory response in the tissues. Such changes were defined as necrobiotic manifestations and cell destruction. At the same time, the microorganisms present in the wound formed colonies, which clearly indicated a growing bacterial load on the tissues.

The presence of vacuolization and loosening of nuclear and cytoplasmic structures was characteristic. In places, they had the character of loosening and homogenization. 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. Protein elements are revealed in the background space.

The cytological impression of the wound during this period of the study was characterized by the presence of damaged collagen filaments in the peripheral areas on the border with the skin, which clearly did not reach their strength.

A certain kind of increased activity was revealed in relation to histiocytic cells, which were characterized by some activation in the form of an increase in the size of the cytoplasm and the acquisition of hyperchromatic properties by their nuclei.

Cytological picture of the patient's DPR 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 hyperchromasia of their nuclei.

On the 7th day of the course of the purulent-inflammatory process, polynuclear leukocytes prevailed in the cytological material of the DNR. Neutrophils, mainly segmented cell types, still remain in the wound. All of them are in a hyperchromatic state. Destroyed leukocytes and lymphocytes are detected around them, which are randomly located, sometimes crowded, sometimes diffuse.

Capillary growth was significantly reduced, which was probably due to the ongoing remodeling process.

At a later stage of the treatment of cytological imprints of the wound, the presence of leukocyte infiltration of histiocytic and lymphoid cells could be noted. So, on the 14th day of the treatment, we detected a certain background in the cytological picture of the dna fingerprints, which was formed due to protein substances in a condensed state. The detectable cells in the wound are characterized by the presence of neutrophils, lymphocytes, and histiocytes.

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

Extracellular granules and lumps of structureless detritus masses of various sizes were also visible in the preparations. Detritus had a grayish tinge due to its protein origin. The yellowish tint indicated the presence of a necrotic substance of a lipid nature. The type of bacteria determined the nature of detritus and protein mass in the cytological preparation. 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 shell on the outside.

Figure 4 shows that against the background of detectable 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 a modified structure in the form of karyolysis and karyopycnosis of their nuclei. The predominant mass in the studied cytological prints was the mass consisting mainly of their protein. This type of 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 shell covering them from the outside.

Cytological studies have once again confirmed the variant of the course of DNZR in the form of infiltration of a cellular inflammatory nature.

On the 28th day of the treatment, the wound was covered with a scab, which had a yellow color and consisted of fibrous tissue. Fibrin, pus, and protein-like material were found in the structure of such a scab.

The background of the print 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 neutrophilic leukocytes. Fibroblasts had a feature of acquiring low proliferative capacity.Monocyte-macrophage cells and plasma cells are detected in large numbers.

In general, the morphological changes in the early stages of DNDD treatment were characterized by the presence of granulocytes, which appeared to be a polynuclear variant. This definition is due to the fact that during the coloring of cells, their nuclear structures often had increased color sensitivity (hyperchromia). The bridges connecting the chromatin segments of such nuclei were swollen and thickened. However, not all granulocytes had such a morphological structure. Along with them, we also identified granulocytes with karyolytic, and in some places even karyorectic 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 pattern was observed with respect 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 contents into the near-cellular environment.

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

Thus, based on the morphological study of dna fingerprints, it is possible to conclude that the presence of a protein background, based on inflammatory cells and microorganisms, plays a leading role. All this determined the cellular-microbial factor as one of the main components leading the course of the entire DNZR formation process. The basis for such a judgment may be the presence of microorganisms of various forms that we have identified, but with predominantly gram-negative structural characteristics against the background of multinucleated leukocyte infiltration. Although leukocyte infiltration decreased at a later date, lymphocytes and histiocytic cells still prevailed in the DNR. This type of damage to the DNR determined the role of leukocyte cells as the main factor in organizing the course of the wound process.

Counting of cells in the wound imprint in patients with DNDD showed a predominance of the proportion of granulocytes in all cases. At the same time, the minimum average level for the entire period of treatment was noted in relation to the number of lymphocytes (6.86±1.06%). The average number of macrophages in the wound in patients with a long-term non-healing process prevailed over the number of fibroblasts (17.37±2.62% and 12.97±2.65%, respectively).

The minimum number of granulocytes in DNPR prints [CI: 50.8; 64.8] occurred in the long-term periods of treatment ($p<0.05$). In contrast, the minimum number of fibroblasts [CI: 6; 8.2] and macrophages [CI: 10.6; 13.8] was detected on day 1 of the wound treatment.

A separate analysis of the dynamics of changes in the number of cells in the DNA fingerprint revealed an ambiguous cytomorphometric picture.

In patients of the first subgroup, the average number of fibroblasts in the DNR imprint over the entire period of treatment was 13.82±2.6%. The dynamics of changes in the number of these cells was

manifested by their relative increase in the DNR from 2.9±0.1% on the 1st day of treatment to 11.9±2.1% ($p<0.05$) on the 7th day and to 17.4±2.8% ($p<0.05$) on the 14th day of the study. Starting from day 28 and until the end of the traditional treatment, our studies showed a stabilization of the number of fibroblasts in the DNR imprint at the level of 18.1±3.2% and up to 18.8±4.8% ($p<0.05$, a significant change compared to day 1 of the treatment).

In the case when the patients showed signs of generalization of infection (the second subgroup), the number of fibroblasts in the DNR prints initially, on the 1st day of treatment, exceeded 3.9 times ($p<0.05$) the values of the first subgroup of patients and averaged 11.3 ± 2.1%. At the same time, on the 7th day of treatment, the number of fibroblasts in the DNR prints in patients of the second subgroup remained at this level (11.1±2.6%), characterized by a slight decrease in the number compared with the data of patients of the first subgroup. The number of fibroblasts in the DNA prints of patients of the second subgroup on the 14th-28th day of treatment, compared with previous periods, increased to 12.1± 2.8% and 12.5±3.2%, which was lower than in patients of the first subgroup during this study period. Even in the long-term terms of the treatment in patients of the second subgroup, the number of fibroblasts in the DNA fingerprints did not exceed similar values among patients of the first subgroup and amounted to 13.6 ±2.8%.

Thus, a comparative characteristic of the fibroblast content in the patients' DNR revealed a local increase in their expression, and in the case of generalized infection, this trend was less pronounced and was characterized by relatively low values of this indicator.

The average number of granulocytes in DNR prints for the entire period of treatment among patients of the second subgroup exceeded (65.76±10.66%) similar values than in patients of the first subgroup (59.84±9.2%).

Only on the 1st day of treatment, the number of granulocytes was higher among patients of the first subgroup (72.0±12.9%) than among patients of the second (68.0±12.8%).

In the dynamics of traditional treatment, the number of granulocytes in DNR prints in patients of different subgroups did not change identically (Figure 6). Among the patients of the first subgroup, we revealed a progressive decrease in the number of granulocytes in the DNR prints in the form of a significant decrease on the 7th day of treatment (to 63.8±9.7%; $p<0.05$) and a relatively stable level (67.3±11.3%) among patients of the second subgroup.

The conducted studies showed that the 14th day of the treatment was characterized by a continued marked decrease in the number of granulocytes in the DNR prints in patients of the first subgroup, which reached a value of up to 54.9±8.9%. At the same time, in patients of the second subgroup, this indicator changed slightly compared to the previous study periods and equated to 66.4±9.7%.

It should be noted that in the subsequent periods of the traditional treatment, that is, on the 28th day and before scarring of the DNR, among the patients of the first subgroup, we revealed a relative stabilization of the number of granulocytes in the wound prints (54.3±9.4% and 54.2±5.1%, respectively), whereas among the patients of the second subgroup, we revealed a continuing local decrease in the number of granulocytes (from 65.7±9.6% and up to 61.4±8.9%, respectively).

Thus, the dynamics of changes in the number of granulocytes in DNR prints is characterized by a decrease in these types of cells throughout the entire period of treatment. At the same time, in the absence of generalization of infection, granulocytes in the wound progressively decrease in the early stages of treatment, which is characteristic of a relatively favorable course of the chronic inflammatory process.

The average number of lymphocytes in DNR prints among patients of the first subgroup for the entire period of treatment was higher (8.48±1.12%) than among patients of the second subgroup (5.24±1.0%; $p<0.05$) and, in general, the dynamics of changes was identical to the dynamics of granulocytes.

In patients of the first subgroup, the number of lymphocytes in the DNR throughout the dynamics of traditional treatment progressively decreased from 14.6±2.1% on day 1 and to 10.2±1.7% on day 7, to 9.3±1.2% on day 14, to 4.9±0.4% on day 28 and to 3.4±0.2% in subsequent periods before scarring of the wound. As can be seen from the above, we noted sharp points of decrease in lymphocytes in the DNR on the 7th and 28th days of the traditional treatment. Although the last period of the study had a different prolongation period, nevertheless, the dynamics was relatively pronounced.

In contrast to the above, we did not notice such relatively sharp jumps in the decrease in the number of lymphocytes in the DNR prints among the patients of the second subgroup. The maximum

number of lymphocytes in the dna fingerprints was noted by us on the 1st day of treatment (6.8±1.1%). Subsequently, on the 7th day of treatment, a decrease in the number of lymphocytes in the wound to 5.7 ± 1.2% remained stable on the 14th day of the study (5.6± 1.1%). Starting from the 28th day of treatment, we again detected a sharp decrease in the number of lymphocytes in the DNA samples, which reached 4.9±0.9%, which was 1.4 times less than the initial indicator. In the long-term total period of the study, the decrease in the number of lymphocytes in the DNA fingerprint reached 3.2±0.7%, which was already 2.1 times less than the initial values ($p<0.05$). Thus, DNRs are characterized by the presence of lymphocyte cells in the wound, which progressively decrease as the regenerative process is achieved, which was probably due to active immunological processes. At the same time, in patients with generalized infection, the initial value of lymphocytes becomes not pronounced, although the dynamics of treatment also tend to decrease the expression of these cells in the wound.

Regarding the dispersion dynamics of changes in the number of monocytes and macrophages in the DNR, it should be noted that the average values for the entire period of treatment were almost identical.

So, if, among patients of the first subgroup, the average number of monocytes in the DNR was 17.86±3.72%, then among patients of the second subgroup it was 16.88±1.52%. However, separate dynamic monitoring revealed an ambiguous picture of changes in the number of lymphocytes in long-term non-healing wounds.

In general, among the patients of the first subgroup, we found relative progress in increasing the number of macrophages in the surface prints of long-term non-healing wounds. At the same time, if the initial value of this indicator on the 1st day of treatment was 10.5±2.3%, then over the next 7-28 days it only increased progressively (14.1±3.1% on the 7th day, 18.4±3.9% on the 14th day and 22.7±4.2% on the 28th day, respectively).

Although the number of macrophages increased (23.6±5.1%) in the long-term period of DNR regeneration, it was insignificant and not reliable.

A distinctive feature of the dynamics of changes in the number of macrophages in the surface prints of the DNR of patients in the second subgroup was the relatively stable preservation of the numerical value during the 7-28 days of treatment. So, if, on the 1st day of treatment, the number of macrophages in the imprints of the DNR surface was 13.9±0.9%, then on the 7th day of the study, a slight increase in the number of these cells to 15.9±1.3% remained at this level on the 14th day of the study (15.9±1.2%).

On the 28th day of treatment, the number of macrophages in the imprints of the wound surface increased unreliably to 16.9±1.6%, however, in the long-term period of treatment, this indicator changed significantly (21.8±2.6%; $p<0.05$).

Changes in the dynamics of changes in the forms of wound neutrophils were also very characteristic. In particular, there was an increase in the regenerative-degenerative index at a significant level as early as the 14th-28th day of traditional treatment methods with a relatively stable number of degenerative forms of neutrophils.

Thus, the dynamics of changes in the number of macrophages in the dna surface prints is characterized by the presence of these cells throughout the treatment, while in the absence of generalization of infection, an increase in their local expression is characteristic.

The total value of immunoglobulins in the rinses of the DNR surface on the 1st day of treatment was equal to 50.5±9.8 micrograms/ml. At the same time, in 62% of cases they were represented by IgG, in 23.8% of cases by IgA, and in 14.3% of cases by IgM.

The use of traditional treatment methods in patients of the control group already on the 7th day of dynamics led to an increase in the number of immunoglobulins in the surface flushes of long-term non-healing wounds to 61.25±11.3 micrograms/ml. These changes were expressed by reducing the proportion of IgG to 51.9% ($p<0.05$), while the proportion of IgA increased to 26.5% ($p<0.05$) and IgM to 13.2% ($p<0.05$). This period was characterized by a peak in the level of detectable immunoglobulins in the wound compared to the entire period of the study.

Starting from the 14th day of the traditional treatment, we detected a decrease in the amount of immunoglobulins in the surface flushes of the DNR. The total value of immunoglobulins in the rinses of the DNR surface was 57.55±9.7 micrograms/ml.

However, such changes in the structure of the studied immunoglobulins were not unambiguous. Thus, during the study period, IgG production increased by 20.9% ($p<0.05$) compared to the previous study period and amounted to 2/3 of the total proportion of detected immunoglobulins in DNR surface washes. An increase in the proportion of IgG immunoglobulin was noted due to a decrease in the specific gravity of IgA by 1.9 times ($p<0.05$) and IgM by 1.6 times ($p<0.05$).

Subsequently, on the 28th day of the traditional treatment and before the appearance of signs of scarring of the wound, the total value of immunoglobulins in the rinses of the DNR surface, the amount of immunoglobulins decreased to 34.4±6.1 micrograms/ml and to 31.9±4.9 micrograms/ml, respectively. That is, the decrease in the number of immunoglobulins in the wound in such a long time was relatively stable. During this period, the proportion of detectable immunoglobulins in DNR surface washes decreased due to IgG to 68% and 64.9% with a stable specific gravity of IgM (9.6%, respectively). Regarding IgA, an increase in its percentage on the 28th day of treatment should be noted by 1.6 ($p<0.05$) and 1.8 times ($p<0.05$), respectively.

Thus, an analysis of the dynamics of changes in the level of immunoglobulins in the surface flushes of DNR showed that the critical period during the traditional treatment was 14 days, when there was an increase in the production of immunoglobulins due to IgG and a decrease in the production of IgA and IgM.

The average IgG level in the surface flushes of the DNR in patients of the first and second groups for the entire period of the study had no significant difference and was equal to 30.26±3.86 micrograms/ml and 29.38±5.3 micrograms/ml, respectively.

The dynamics of IgG concentration changes in the surface flushes of the DNR were also distinctive, which among patients of the first subgroup was characterized by a gradual increase from 27.9±8.6 micrograms/ml on day 1 of traditional treatment to 33.4±3.4 micrograms/ml on day 7 and to 56.9±4.6 micrograms/ml on day 14, respectively.

In the long term of treatment, IgG concentration progressively decreased to 17.3±2.3 micrograms/ml on day 28 and to 15.8±0.4 micrograms/ml during the average final sampling period.

Among patients of the second subgroup, the concentration of this immunoglobulin in the surface flushes of the DNR, in contrast to the previous subgroup, progressively decreased from 34.7±4.3 micrograms/ml on day 1 of traditional treatment to 30.2±6.1 micrograms/ml on day 7 and to 26.9±4.7 micrograms/ml on day 14, respectively. In the long term of the treatment, the IgG concentration did not change significantly, ranging from 20.1 micrograms/ml to 35.4 micrograms/ml.

The comparative nature of the dynamics of changes in the concentration of IgA in the surface flushes of the DNR, depending on the presence of generalization of surgical infection, was manifested by an almost identical pattern among both patients of the first and second subgroups. It was manifested by an increase in the concentration of IgA in the surface flushes of the DNR from 15.3±4.2 micrograms/ml on the 1st day of treatment to 17.9±3.7 micrograms/ml on the 7th day of treatment among patients of the first subgroup, as well as from 8.7±1.9 micrograms/ml on the 1st day of treatment to 14.6±4.6 micrograms/mlml on the 7th day of treatment among patients of the second subgroup. At the same time, on the 14th day of traditional treatment, the concentration of IgA in the surface flushes of the DNR in both studied subgroups of patients decreased to 3.7± 1.5 micrograms/ml among patients of the first and to 12.3±2.1 micrograms/ml among patients of the second subgroup. In the long–term terms of the study, we revealed a discrepancy in the dynamics of the IgA concentration curve in the surface flushes of the DNR, which among patients of the first subgroup was characterized by a decrease in values to 1.8±0.6 micrograms/ml and to 1.6±0.3 micrograms/ml, respectively, and among patients of the second subgroup - an increase to 13.6±2.7 micrograms/ml and to 14.7±3.1 micrograms/ml, respectively.

Thus, the obtained data indicate the fact the consequences of chronic sepsis, when even in conditions of elimination of the generalization of infection, IgA production continues with their filling of the wound surface.

The concentration of IgM in the surface flushes of the DNR among patients of the first subgroup was 5.5 times higher than among patients of the second subgroup ($p<0.05$). As in the previous case, on the 7th day of the traditional treatment, the IgM concentration in the surface flushes of the DNR increased by 1.7 times ($p<0.05$) among patients of the first subgroup, and by 2.6 times ($p<0.05$) among

patients of the second subgroup. At the same time, the difference in IgM production among patients of the first and second subgroups was 3.5 times ($p<0.05$), which was less pronounced than at baseline.

On the 14th day of treatment, the concentration of IgM in the wound surface flushes among patients of the first subgroup decreased from 20.6±4.2 micrograms/ml to 10.4±2.8 micrograms/ml, that is, almost 2 times ($p<0.05$). Among the patients of the second subgroup, we also noted a decrease in IgM concentration in the wound surface washes from 5.8±0.7 micrograms/ml to 4.9±0.2 micrograms/ml ($p>0.05$). The difference in the concentration of this immunoglobulin between patients of the first and second subgroups during this period was 2.1 times ($p<0.05$). Thus, the early period of treatment was characterized by a wave-like change in IgM concentration among both patients of the first and second subgroups. At the same time, in the case of generalized infection, the changes were insignificant.

In the long-term terms of traditional treatment, among patients of the first subgroup, the concentration of IgM in the DNR was detected only in isolated cases and very low concentrations compared to previous study periods (from 1.1 micrograms/ml to 1.9 micrograms/ml) averaging 1.5±0.4 micrograms/ml and 1.3±0.2 micrograms/ml, respectively. At the same time, among the patients of the second subgroup, the changes were unreliable and ranged from 4.3 micrograms/ml to 5.9 micrograms/ml, averaging 5.1±0.8 micrograms/ml and 4.8±0.5 micrograms/ml, respectively.

Thus, in the presence of initially low IgM production in the wound surface of patients with generalized infection, the concentration of this immunoglobulin only increases, which, apparently, was due to the peculiarities of the manifestation of the general disease. At the same time, in the absence of generalization of infection and under the condition of a favorable outcome of the disease, IgM production decreases.

The concentration of lysozyme on the surface of the DNZR was insignificant (1.84±0.33 micrograms/ml) and was mainly represented by patients of the first subgroup (2.56±0.58 micrograms/ml) than the second (1.11±0.08 micrograms/ml). In the early stages of wound treatment, the lysozyme concentration in the wound gradually increased to 2.62±0.35 mcg/ml on day 7 and to 2.99±0.34 mcg/ml on day 14 of the study. In both cases, the lion's share of lysozyme concentration in the wound was represented by patients of the first subgroup, exceeding the values of patients by 1.5 ($p<0.05$) and 2.4 times ($p<0.05$), respectively.

In the subsequent, long-term periods of treatment, the concentration of lysozyme in the DNR gradually decreased to 2.23±0.29 mcg/ml on day 28 and to 1.55±0.24 mcg/ml ($p<0.05$) in subsequent periods. However, unlike in the early stages of the study, during this period the main proportion of lysozyme was patients of the second subgroup. Higher concentrations of lysozyme, 1.5-fold ($p<0.05$) and 2.2-fold ($p<0.05$) in DNR flushes in patients of the second subgroup, were associated with a long period of generalization of infection.

Thus, the lysozyme concentration in DNZR is characterized by a change in dynamics depending on the presence of generalization of infection in the form of an increase in production above the initial value. Such changes in lysozyme concentration are directly related to the presence of generalization of infection and can be used in predicting the course of the inflammatory process in patients with DND.

Conclusions

1. The early period of treatment was characterized by a wave-like change in IgM concentration among both patients of the first and second subgroups. At the same time, in the case of generalized infection, the changes were insignificant.
2. In the presence of initially low IgM production in the wound surface of patients with generalized infection, the concentration of this immunoglobulin only increases, which, apparently, was due to the peculiarities of the manifestation of the general disease. At the same time, in the absence of generalization of infection and under the condition of a favorable outcome of the disease, IgM production decreases.
3. The concentration of lysozyme in DNZR is characterized by a change in dynamics depending on the presence of generalization of infection in the form of an increase in production above the initial value. Such changes in lysozyme concentration are directly related to the presence of generalization of infection and can be used in predicting the course of the inflammatory process in patients with DND.

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