Cytological study of the dynamics of the wound process in purulent diseases of soft tissues using programmable sanitation technologies

Sergeev Vladimir Anatolyevich
Candidate of Medical Sciences, Associate Professor
Orel State University named after I.S. Turgenev
Orel, Russia
Glukhov Alexander Anatolyevich
Doctor of Medical Sciences, Full Professor
Voronezh State Medical University named after N. N. Burdenko
Voronezh, Russia
Morozov Yuri Mikhailovich
Doctor of Medical Sciences, Full Professor
Orel State University named after I.S. Turgenev
Orel, Russia

Purpose of research: to study the cytological features of the healing processes in patients with soft tissue phlegmons using programmable sanitation technologies.

Methods: The study involved 245 patients with purulent phlegmon of soft tissues of various localizations. Patients were randomized into two groups. In the comparison group (n=118), patients received traditional local treatment after surgery. In the main group (n=127), in the postoperative period, programmed readjustments were performed using the AMP-01 device. At the same time, the purulent wound was drained with tubular drains, taken out through separate incisions, and a blind suture was applied to the wound. The device operates on a cyclical principle in an autonomous mode, allows you to select the parameters of sanitation (speed, volume of injection or aspiration) and to carry out an individual approach to the treatment of each specific case. The dynamics of reparative processes in purulent wounds was assessed by the cytological picture of the material taken by the method of superficial or puncture biopsy.

Results: In the main group of patients, a higher rate of cellular reactions in purulent wounds was noted. By the 9th day after the operation, the cytological picture corresponded to the regenerative type of cytograms. There was a statistically significant faster decrease in degenerative forms of neutrophils, positive redistribution of stab and segmented neutrophils in combination with high values of the regenerative-degenerative index ($p<0.001$), indicating an acceleration in the relief of the inflammatory process. Also, in the main group, the appearance of macrophages and cells of young connective tissue in the form of fibrocytes, fibroblasts, fibrous fibers ($p<0.001$) was observed at an earlier date, which indicated active regenerative processes in the wound. In the comparison group, a lower intensity of cellular reactions in the wound, a lengthening of the inflammation phase, a significant duration of the regeneration phase, and later periods of the onset of the scar reorganization phase were revealed.

Conclusion: The conducted cytological study proved the effectiveness of the use of programmable sanitation technologies that help to reduce the phase of inflammation, accelerate the reparative processes of purulent foci with phlegmon of soft tissues.

Keywords: Purulent-inflammatory diseases of soft tissues, phlegmon of soft tissues, programmable sanitation technologies, cytological examination, reparative process.

Introduction

Treatment of purulent-inflammatory diseases of soft tissues is one of the most difficult and intractable problems of practical surgery [1,2,3]. According to domestic and foreign experts, the urgency of this problem in modern conditions is associated not only with the widespread incidence of surgical infection, but also with the difficulties of its diagnosis, variability and persistence of the clinical course, the complexity of treatment and unpredictability of prognosis [4,5,8]. According to US national statistics, more than 3 million people visit emergency departments every year for skin and soft tissue infections, of which more than 500,000 patients are hospitalized, and the cost of their treatment is over 10 billion dollars [6,7].

In recent years, fundamental research in molecular cell biology has made it possible to better understand the basic mechanisms of wound healing. It has been proven that the healing process in any wound is genetically determined, initially there is always a phase of inflammation, followed by a phase of regeneration and a phase of scar reorganization and epithelialization [5,9,10,11].

At the present stage, it is becoming increasingly important 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 [12-15]. To obtain quick and objective information about the course of reparative processes in wounds of various origins, it is preferable to use the cytological method [5,16,17,18,19]. A cytological study allows one to characterize various types of the course of the wound process, to reliably assess the effectiveness of the treatment [5,20,21, 24,25]. There are 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 [5,22]. For a complete assessment of the picture of wound healing, the regenerative-degenerative index (RDI) is calculated using the formula [23]. The RDI value less than one indicates a pronounced inflammatory process in the wound, and if the value of this indicator becomes more than one, then this means the transition of the wound process to the

regeneration phase. Assessment of the reparative response in the wound based on cytological verification is one of the objective methods for studying the characteristics of the course of the wound process, which allows to optimize the treatment tactics.

Purpose: to study the results of a cytological study of healing processes in purulent diseases of soft tissues using programmed sanitation technologies.

Material and methods

During the period 2011-2020, under our supervision there were 245 patients with purulent phlegmon of soft tissues of various localizations. Inclusion criteria for this study: the age of the patients over 18 years, the presence of phlegmon of the soft tissues of the limb or neck, the presence of informed voluntary consent. Exclusion criteria for the study: the presence of extensive skin defects in the area of surgical treatment, signs of anaerobic infection, pregnancy, diabetes mellitus, oncological pathology, circulatory insufficiency and respiratory failure of the III degree.

All patients were randomized into two groups depending on the methods of debridement of purulent foci in the postoperative period. In the comparison group, after surgical treatment, patients received traditional local treatment using iodophore solutions, polyethylene glycol-based ointments. In the main group, after surgical treatment, the wound was drained with tubular drains, taken out through separate incisions, and the wound was then sutured tightly. The drains were connected to the original AMP-01 device (patent for invention № 176572 dated January 23, 2018), with the help of which programmed sanitation was carried out in the postoperative period. On the control unit of the device, an individually selected program of cyclically occurring processes of irrigation, aspiration of an antiseptic and constant evacuation, carried out in an autonomous mode, was installed. Programmable sanitation was carried out every 3 hours, alternating with a vacuum period of 1 hour. The preset level of vacuum in the purulent cavity (80-100 mmHg) was maintained using a built-in pressure sensor. Basic therapy was the same in both groups of patients.

In the main group, the average age of patients (M±σ) was 59±13 years, in the comparison group - 60±11 years. In the main group there were 68 men, 59 women, 62 and 56 in the comparison group, respectively. Thus, there are no statistically significant differences between the study groups by sex and age, which made it possible to judge the homogeneity of the groups ($p = 0.845$ and $p = 0.875$, respectively).

The distribution of patients with soft tissue phlegmon in the study groups according to the phlegmon localization is presented in table 1.

Table 1. Distribution of patients by nosological form in the study groups

Nosological form	Main group *n* (%)	Comparison group *n* (%)	Total *n* (%)
Phlegmon of the wrist	10 (7.9)	8 (6.8)	18 (7.3)
Phlegmon of the forearm	33 (25.9)	29 (24.6)	62 (25.3)
Phlegmon of the shoulder	11 (8.7)	13 (11.1)	24 (9.8)
Phlegmon of the foot	13 (10.2)	15 (12.7)	28 (11.4)
Phlegmon of the lower leg	38 (29.9)	29 (24.6)	67 (27.3)
Phlegmon of the thigh	13 (10.2)	13 (11.1)	26 (10.6)
Phlegmon of the neck	9 (7.1)	8 (6.8)	17 (6.9)
Total	127 (100)	118 (100)	245 (100)

According to the independent criterion χ^2-Pearson (p = 0.953) the dependence of the distribution of patients by nosological form in the study groups was not found.

To assess the healing of purulent foci, a cytological research method was used. We used the technique of surface biopsy according to M.P. Pokrovskaya and M.S. Makarov (1942) modified by M.F. Kamaeva (1954). In the comparison group, the material was taken by light scraping of the surface layer of the wound with the handle of a surgical scalpel. The resulting material was applied to glass, fixed, and stained according to the May-Grunwald-Romanovsky-Giemsa method. In the main group of the study, the collection of cellular and tissue elements was performed by the method of "puncture biopsy" (Kayem R.I., Karlov V.A., 1977; Sergel O.S., Goncharova Z.N., 1990). 4-5 smears were sequentially taken from the same area of the wound. Cytological examination of smears from the surface of wounds was carried out on the first day, and then on the 3rd, 5th, 7th, 9th days. The smears were studied under microscopy with a × 40 objective, while the formed elements were counted and the average value was deduced over 10 fields of view. The value obtained was expressed as a percentage per 100 counted cells. We used an Axio A1 light microscope (Zeiss, Germany) with a set of accessories.

At the time of randomization in both study groups, in patients with soft tissue phlegmons, the cytological picture was characteristic of the degenerative-necrotic type of cell reaction. Among the cellular elements, degenerative neutrophils (DN) prevailed (64.5±9.2%), and there were very few preserved forms of leukocytes. The regenerative-degenerative index (RDI) was well below unity (0.2±0.1). Microflora was found in large quantities, mainly extracellular. In the preparations, accumulations of necrotic masses and an amorphous gelatinous interstitial substance were observed. Figure 1 shows a fragment of a cytological smear from the wound surface in patients with soft tissue phlegmon at the time of randomization on the 1st day of the study.

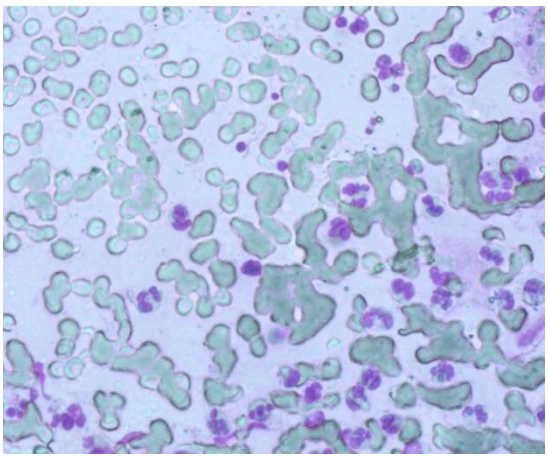

Figure 1. Fragment of the cytogram of a smear from the wound surface in patients with phlegmon of soft tissues on the 1st day. Degeneratively altered polymorphonuclear leukocytes predominate, microflora is found in large numbers, mainly extracellularly, there is an accumulation of necrotic masses and an amorphous gelatinous intermediate substance. Staining according to Romanovsky-Giemsa. Lens × 40

The cellular composition of cytological smears in patients with soft tissue phlegmon in the study groups at the time of randomization is presented in table 3.

Table 3. Cell composition of cytological smears in patients with soft tissue phlegmon in the study groups at the time of randomization, in% per 100 cells

Cell types	Main group		Comparison group		p-value t-criterion
	M±σ, in% per 100 cells	Me[Q1;Q3]	M±σ, in% per 100 cells	Me[Q1;Q3]	
STN	1.8±0.2	1.82 [1.77;1.87]	1.7±0.2	1.74 [1.64;1.81]	0.682
SGN	15.2±0.6	15.19[15.07;15.32]	16.2±0.4	16.16[16.07;16.25]	0.647
DN	64.5±9.2	65.89[63.65;68.13]	62.2±6.2	68.84[67.57;70.09]	0.022
RDI	0.2±0.1	0.18 [0.16;0.21]	0.2±0.1	0.18 [0.16;0.21]	0.976
L	0.4±0.2	0.39 [0.35;0.43]	0.2±0.1	0.17 [0.15;0.19]	0.000

Note: STN – stab neutrophils, SGN - segmented neutrophils, DN - degenerative neutrophils, RDI - regenerative-degenerative index, L - lymphocytes

Most of the parameters of the statistical assessment of the cellular composition of cytological smears on the first day of observation in both study groups were similar in values ($p > 0.05$), which made it possible to judge the homogeneity of the groups. However, the indicators DN and L were not statistically proven to be homogeneous, which can be explained by the small sample of the study.

The work was carried out in the design of a simple, randomized, comparative controlled study in parallel groups. The SPSS Statistics 25 software (IBM) was used for statistical processing of the obtained data. To study the relationship between qualitative characteristics, contingency tables were constructed and the $\chi 2$-Pearson test or Fisher's exact test was calculated. To assess the change in the dynamics of quantitative indicators, analysis of variance with repeated measurements was used with the setting of the time factor and the group. Differences were considered statistically significant if the probability value was less than 0.05 for the two-sided critical region.

Results

The cytological picture of smears in the main group on the 5th day from the moment of randomization corresponded to the inflammatory or inflammatory-regenerative type of cellular reaction. There was a statistically significant decrease in the number of degenerative neutrophils (DN) (16.7±2.2%), an increase in the number of intact forms of neutrophils: segmented (SGN) (41.6±3.8%) and stab neutrophils (STN) – 6.2±0.8% ($p<0.001$). There was a statistically significant increase in RDI - 2.9±0.4 ($p <0.001$). Plasma cells, histiocytes appeared. There was a statistically significant increase in the number of active macrophages - 4.6±0.6%, lymphocytes - 5.8±0.6%, fibroblasts - 4.2±0.5% ($p <0.001$). Found groups of cells of young connective tissue in the form of fibrocytes, fibroblasts, fibrous fibers. Microflora was detected in a small amount at the stage of complete phagocytosis.

In the comparison group, on the 5th day of treatment, the cytological picture in smears was characterized by a neutrophilic reaction - the number of intact forms of neutrophils increased: SGN - 42.6±3.8%, STN - 6.2±0.8%. The number of degenerative forms has decreased - 56.7±2.2%. The RDI was close to unity - 0.9±0.2. The microflora was determined intra- and extracellularly, but cases of complete phagocytosis were more common. There were single actively phagocytic leukocytes, macrophages, lymphocytes. Elements of granulation tissue are rare. The cytological picture corresponded to the inflammatory type of the cellular reaction. Figures 1, 2 show fragments of cytological smears from the wound surface on the 5th day in the study groups.

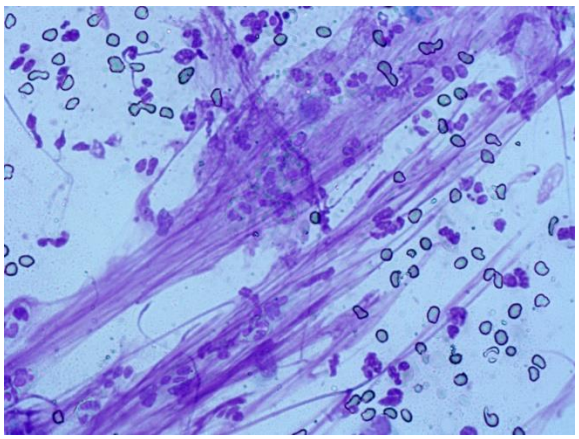

Figure 1. Fragment of the cytogram of a smear from the surface of the wound on the 5th day, the main group. Fibrocytes, fibroblasts, fibrous fibers were found among neutrophils and polyblasts. Staining according to Romanovsky-Giemsa. Mag.× 40

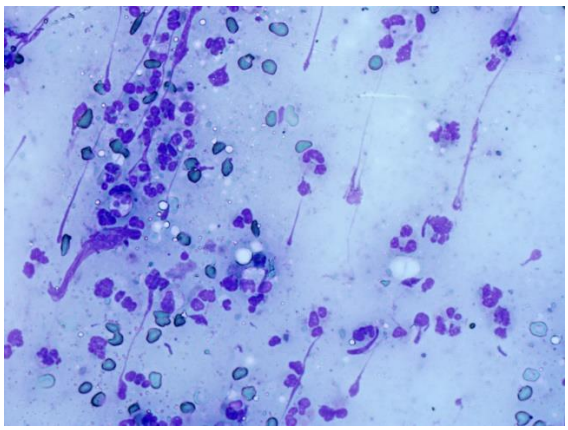

Figure 2. Fragment of the cytogram of a smear from the wound surface on the 5th day, comparison group. There are single actively phagocytic leukocytes, macrophages, lymphocytes. Elements of granulation tissue are rare. Staining according to Romanovsky-Giemsa. Mag.× 40

On the 9th day of the postoperative period in the main group, the cytological picture corresponded to the regenerative-inflammatory or regenerative type of cellular reactions. Figures

3,4 show fragments of cytological smears from the wound surface on the 9th day in the study groups.

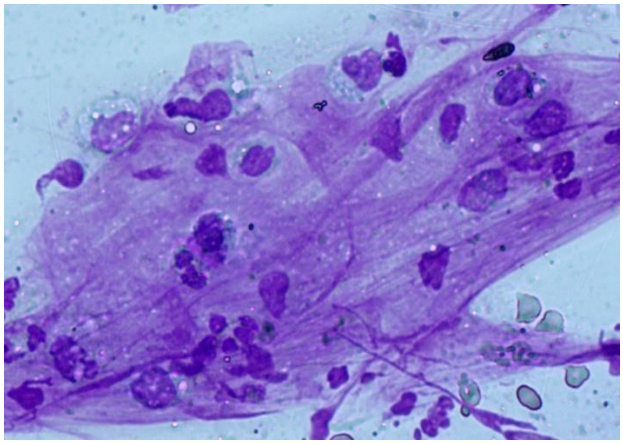

Figure 3. Fragment of the cytogram of a smear from the surface of the wound on the 9th day, the main group. Young elements of connective tissue, fibroblasts, polyblasts, macrophages are located among the fibrous structures of the intermediate substance. The epithelium is presented in the form of layers of cells. Staining according to Romanovsky-Giemsa. Mag. × 40

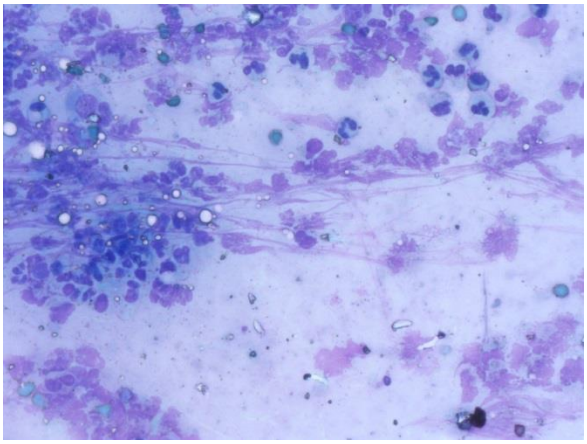

Figure 4. Fragment of the cytogram of a smear from the wound surface on the 9th day, comparison group. Decreased the number of mononuclear cells, increased the number of polyblasts, fibroblasts, macrophages. Delicate fibrous structures of the intermediate were observed. Staining according to Romanovsky-Giemsa. Mag.×40

Based on the results of the analysis of variance with repeated measurements for both groups, statistically significant changes in the cellular composition in cytograms on the 1st and 9th days of the postoperative period were proved. The number of DN in the main group significantly decreased from 64.5±9.2% to 3.8±0.3%, in the comparison group from 68.8±6.2% to 12.5±0.4%; RDI values in the main group increased significantly from 0.2±0.1% to 8.2±0.1%, in the comparison group from 0.2±0.1% to 2.4±0.1%. Statistically significant differences were proved between the groups as a whole for the entire observation period from the first to the 9th day, therefore, we reject the hypothesis about the equality of the means between the groups without taking into account the time ($p < 0.001$). The presence of a significant interaction between the time factor and the group was also proven ($p < 0.001$). In the main group in the postoperative period, there was a faster decrease in the number of DN, which on the 9th day was 3.8±0.3%, in contrast to the comparison group - 12.5±0.4%. There was also a more rapid increase in RDI values in the main group, which on the 9th day increased to 8.2±0.1, in contrast to the comparison group (2.4±0.1). This indicated a more active phagocytosis, more intense cleansing of the purulent cavity of purulent foci in patients of the main group. The dynamics of the mean values of degenerative neutrophils and RDI values in cytological smears in patients with phlegmon of soft tissues of both study groups are shown in figures 5 and 6.

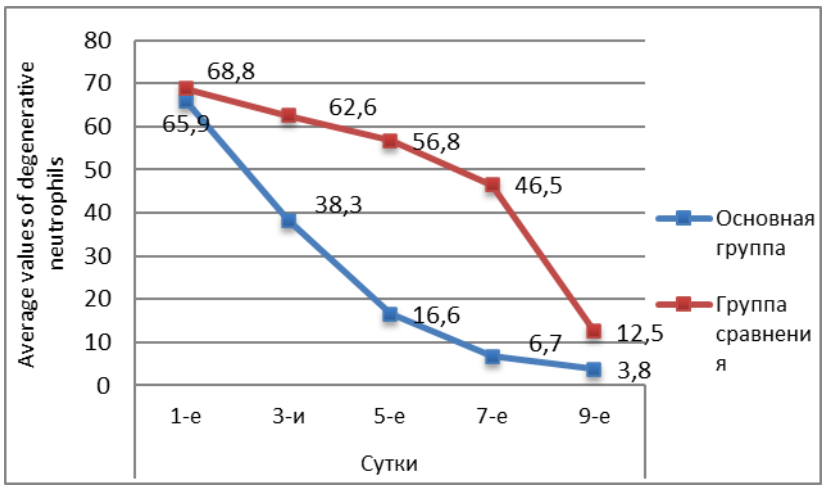

Figure 5. Dynamics of average values of degenerative neutrophils in cytograms of both study groups in patients with soft tissue phlegmon

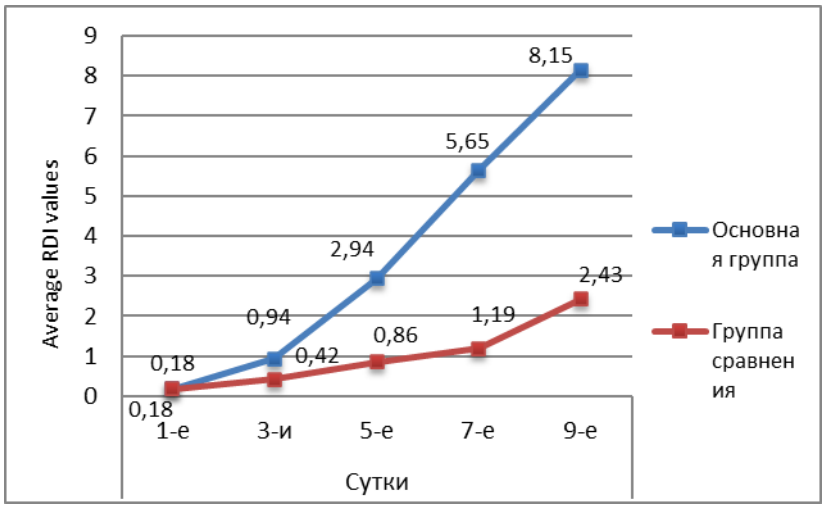

Figure 6. Dynamics of the average values of the regenerative-degenerative index in the cytograms of both study groups in patients with soft tissue phlegmons

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. The results of calculating the analysis of variance with repeated measures showed that for these variables we reject all three null hypotheses, for all three hypotheses $p < 0.05$. The difference between groups for these variables is statistically significant as a whole for the entire observation period. In the main group, where programmable sanitation technologies were used, the number of lymphocytes, macrophages and fibroblasts was always higher. Also, these variables were found to have a significant interaction between the time factor and the group. This indicated active regenerative processes in the wound in the main group of patients, and the structure of cytograms in cytological smears in the main group was characterized by a regenerative type.

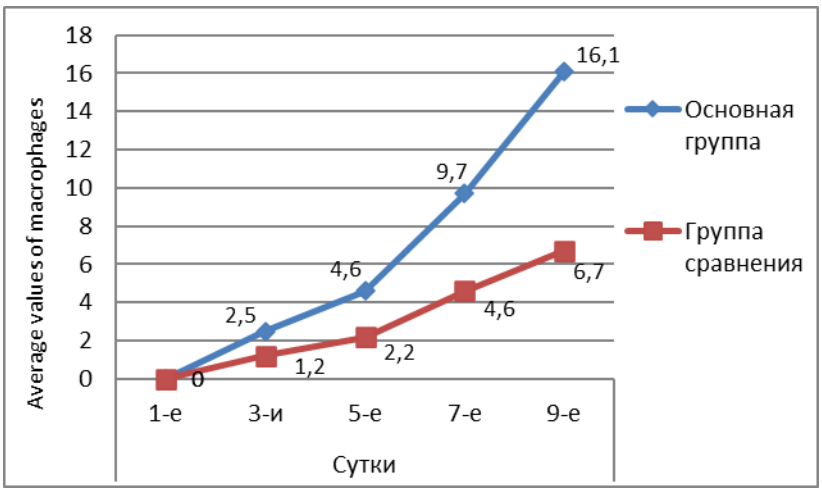

Figure 7. Dynamics of mean values of macrophages in cytograms in study groups in patients with phlegmon of soft tissues

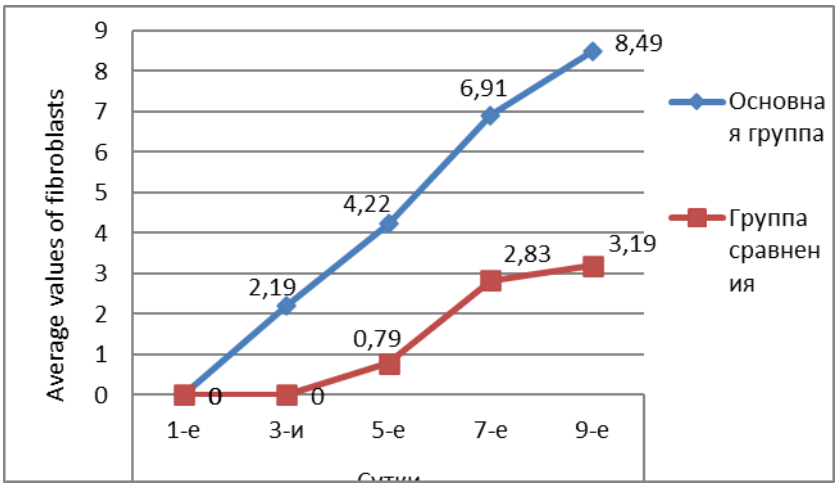

Figure 8. Dynamics of mean values of fibroblasts in cytograms in study groups in patients with phlegmon of soft tissues

Discussion

Analyzing the dynamics of the cytological picture in patients with phlegmon of soft tissues in the study groups, it was noted that in the comparison group with traditional treatment, a low intensity of cellular reactions in the wound, lengthening of the inflammation phase were revealed, and the inflammatory type of cytograms was noted only by 9 days after surgery. Also, in the comparison group, the lethargy of the reparative processes in the wound was observed, causing a significant duration of the regeneration phase, the later timing of the onset of the scar reorganization phase. This led to a lengthening of the healing time.

The use of programmable sanitation technologies made it possible to create conditions for better sanitation of a purulent focus, and led to a reduction in all phases of the wound process. Surgical treatment of a purulent focus, prolonged lavage of the wound cavity in the postoperative period, software of the drainage process made it possible to quickly cleanse the wound from non-viable tissues, toxins and proteolytic enzymes, 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 a minimally pronounced inflammatory reaction in the wound significantly accelerated the reparative processes, creating conditions for the development and completion of the regeneration phase.

Conclusion

Cytological examination of smears in patients with soft tissue phlegmon using programmable sanitation technologies revealed a higher rate of cellular reactions in the wound. At the same time, a statistically significant faster decrease in degenerative forms of neutrophils was noted, a positive redistribution of stab and segmented neutrophils in combination with an increase in the regenerative-degenerative index, indicating a more rapid relief of the inflammatory process. The appearance of macrophages and cells of young connective tissue in the form of fibrocytes, fibroblasts, fibrous fibers was also observed at an earlier date, which indicated active regenerative processes in the wound.

References

1. Sartelli M, Guirao X, Hardcastle TC et al. 2018 WSES/SIS-E consensus conference: recommendations for the management of skin and soft-tissue infections. World J Emerg Surg. 2018;13,58. DOI: 10.1186/s13017-018-0219-9

2. May AK. Skin and soft tissue infections. Surg Clin North Am. 2009;89:403–20. DOI: 10.1016 / j.suc.2008.09.006. [PubMed: 19281891].

3. Ustin JS, Malangoni MA. Necrotizing soft-tissue infections. Crit Care Med. 2011;39:2156–62. DOI: 10.1097 / CCM.0b013e31821cb246. [PubMed: 21532474].

4. Tretyakov AA, Neverov AN, Petrov SV, Shchetinin AF. Treatment of Purulent Wounds. Novosti Khirurgii. 2015;23(6):680-687. DOI: 10.18484/2305-0047.2015.6.680

5. Kuzin MI, Kostyuchenok BM. Wounds and wound infections: A guide for physicians. Moscow: Medicine. 1990; 592 p. Available at: http://med-books.by/hirurgiya/2948-rany-i-ranevaya-infekciya-kuzin-mikostyuchenok-bm-1990-god-592-s.html.

6. May L, Klein EY, Martinez EM, Mojica N, Miller LG. Incidence and factors associated with emergency department visits for recurrent skin and soft tissue infections in

patients in California, 2005–2011. Epidemiology and Infection. 2016;145 (4),746-754. DOI: 10,1017 / S0950268816002855

7. Stevens DL, Bisno AL, Chambers HF, Dellinger EP, Goldstein EJC, Gorbach ShL, Hirschmann JV, Kaplan ShL, Montoya JG, Wade JC. Practice Guidelines for the Diagnosis and Management of Skin and Soft Tissue Infections: 2014 Update by the Infectious Diseases Society of America. Clinical Infectious Diseases. 2014;59(2):e10–e52. DOI: 10.1093/cid/ciu296

8. Statistical collection 2018. Resources and activities of medical health organizations. Key health indicators. Part VI: stat. coll. / M., 2019. – 170 P.

9. Stadelmann, W.K. Physiology and healing dynamics of chronic cutaneous wounds / W.K.Stadelmann // American Journal of Surgery. - 1998. - V. 176. – N 2. - P. 26S–38S.

10. Midwood, K.S. Tissue repair and the dynamics of the extracellular matrix / K.S.Midwood // The International Journal of Biochemistry & Cell Biology. -2004. - V. 36. - N 2. –P. 1031-1037.

11. Deodhar, A.K. Surgical physiology of wound healing: a review / A.K. Deodhar // Journal of Postgraduate Medicine. - 1997. – N 43. – P. 52-56.

12. Eskes AM, Gerbens LA, van der Horst CM, Vermeulen H, Ubbink DT. Is the red-^ellow-black scheme suitable to classify site wounds? An inter-observer analysis. Burns. 2011;37(5):822-826. doi: 10.1016/j.burns.2010.12.019. [PubMed: 21345594].

13. Zemskov MA, Chorochilov AA, Iljina EM, Domnich OA. Peculiarities of changes of immune status in chronic inflammatory diseases. Journal of experimental. and clinical surgery. 2011; 4(3):468-72. doi: 10.18499/2070-478X-2011-4-3-468-472

14. Lacci K, Dardik A. Platelet-rich plasma: support for its use in wound healing. Yale J Biol Med. 2010;83(1):1-9. [PubMed: 20351977].

15. Han T, Wang H, Zang YQ. Combining platelet-rich plasma and extracellular matrix-derived peptides promote impaired cutaneus wound healing in vivo. J Craniofac Surg. 2012;23(2):439-47. doi: 10.1371 / journal.pone.0032146. [PubMed: 22384158].

16. Larichev AB, Shishlo VK, Lisovsky AV, Chistyakov AL, Vasiliev AA. Wound infection prevention and morphological aspects of aseptic wound healing. J of Exp and Clin Surg. 2011;4(4):728-33. doi.org/10.18499/2070-478X-2011-4-4-728-733.

17. Titova MI, Svetukhin AM, Kurochkina AI, Astasheva NG, Doroshina TI, Krylova NN, Agofonov VA. Current methods of morphological and hemostasiological analysis of reparative process in wounds making use of information programs. Klin Lab Diagn. 2000;7(24-36). [PubMed: 10981393].

18. Kocjan Gabrijela. Cytopathology of the Head and Neck: Ultrasound Guided FNAC. 2nd ed. -Wiley-Blackwell. 2017;212 P.

19. Field Andrew S. (ed.) Practical Cytopathology. A Diagnostic Approach to Fine Needle Aspiration Biopsy. Elsevier. 2017;563 P.

20. Ivanusa SY, Risman BV, Ivanov GG. Modern ideas about methods of assessment the course of the wound process in patients with purulo-necrotic complications of the diabetic foot syndrome. Herald of the Russian Military Medical Academy. 2016;54(2):190–194. Internet address: http://elibrary.ru/item.asp?id=26280216

21. Larichev AB, Chistyakov AL, Komlev VL. Comparative assessment of wound healing by using a local flap and full-thickness skin graft in reconstructive head and neck surgery. Wounds and wound infections. The prof. B.M. Kostyuchenok journal. 2016;3(2):37-46. https://doi.org/10.17650/2408-9613-2016-3-2-37-46

22. Kamaev MF. Types of cytograms in a superficial biopsy of the wound. Collection of works of the Odessa medical Institute named after N. I. Pirogov. Kiev. 1954;267-276.

23. Davydov IA, Larichev AB, Abramov AI. Substantiation of Using Forced Early Secondary Suture in the Treatment of Suppurative Wounds by the Method of Vacuum Therapy. Vestn Khir Im I I Grek. 1990;144(3):126-8. [PubMed: 2169094].

24. Sergeev VA, Glukhov AA. Results of operative therapy of diabetic foot purulent complications using programmed sanitation technologies. Zeitschrift für Gefäßmedizin. 2020; 17 (3): 13-17

25. Sergeev VA, Glukhov AA, Sorokin AS. Cytological study of the dynamics of the wound process in purulent lesions of the diabetic foot syndrome with the use of programmable rehabilitation technologies. Periódico Tchê Química. 2021; 18(37): 212-227.