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MORPHOLOGICAL PECULIARITIES OF THE SURROUNDING TISSUES STRUCTURE AND CYTOLOGICAL CHARACTERISTICS OF EXUDATE IN CASE OF DIFFERENT TYPES OF TISSUE DISSECTION

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Abstract

The results on alloherniaplasty of postoperative ventral hernias by the method of onlay are presented in this work. The goal of the research was to improve direct outcomes of surgical treatment of postoperative ventral hernias through development and introduction of methods aimed to prevent wound complications. The material for histological examination was taken perioperatively, after the discharge of hypodermic tissue from aponeurosis, while using either ultrasound harmonic scalpel or high-speed coagulator. After dissection, parts of tissues of subcutaneous fat and aponeurosis were examined. The cytological examination of exudate discharging from the patients' wound was taken on the 2nd, 4th, 6th day and the day when the drainage tubes were removed.

The possibility to use ultrasonic scalpel in dissection of tissues for treatment of postoperative ventral hernias was examined as well. The comparable analysis of the quality of dissection and coagulation by high-frequency coagulator and ultrasonic scalpel was done. Morphological peculiarities of the structure of surrounding tissues and cytological characteristics of exudate in case of different methods of tissues dissection were studied.

The results of research showed that usage of high-frequency coagulator and ultrasonic scalpel caused series of pathological changes in the tissues close to the place of influence, which was displayed by the set of necrotic, dystrophic and exudative reactions. The ultrasonic scalpel made less expressed pathological changes in the adjoined tissues, less necrotic changes in the connective tissue component, as well as preserved the organotypic structure of small blood vessels. Statistically significant zones of necrosis in case of ultrasonic scalpel as compared with high frequency coagulator ($p<0.001$) and preserving the small blood vessels connected to the zones of tissues necrosis created conditions for faster regeneration of tissues in case the ultrasound scalpel was used. Low content of degenerating forms of neutrophilic white cells in case of ultrasonic scalpel usage proved the less damaging influence on connective tissues and immunocompetent cells.

Thus, the results showed higher efficiency of ultrasonic scalpel use as compared with the high frequency coagulator.

Keywords: *postoperative ventral hernia, alloherniaplasty, ultrasonic scalpel, high frequency coagulator, dissection of tissues, morphology, cytology.*

INTRODUCTION

Nowadays, postoperative hernias are among quite urgent challenges and this is explained not only by dissatisfaction of doctors as related to the treatment of the patients with that pathology, but also by the fact that the postoperative hernias exist.

According to the information from different authors, 2-15% percent of all laparotomies result in formation of postoperative hernias, despite the progress of science, up-grading technical possibilities, skills of the surgeons and surgical methods [*Murdge M., Hughes L., 1985; Kupczyk-Joeris D. et al., 1990; Soler N. et al., 1993; Gislason H. et al., 1995; Browne J. et al., 2000*]. Currently the postoperative ventral hernia is viewed not as a local pathological process in the abdominal wall, but as a complex polyetiologic disease resulting in several disorders of the internals [*Zhebrovski V., Elbashir M., 2002*].

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Recently the concept of hernioplasty with the use of polypropylene reticular prosthesis has obtained recognition. The peculiarity of hernioplasty with nadaponeurotic placement of the prosthesis is the necessity of large separation of subcutaneous fat with the skin from aponeurosis. This breaks their blood supply, lymphatic and venous outflow, which is the main reason for unsatisfactory results: extended exudation from the wound, formation of seromas, infiltrates, haematomas, marginal skin necrosis and subcutaneous fat [*Podoluzhnij V. et al., 2006; Slavin L. et al., 2006; Tutov A. et al., 2006; Fedorov I. et al., 2007*]. Optimization of the choice of tissue dissection method is one of the possibilities, which help to improve direct results of surgical treatment.

MATERIAL AND METHODS

The treatment outcomes of 117 patients with postoperative ventral hernia, which underwent hernioplasty with polypropylene net using onlay method, are summarized and presented. With the purpose to conduct research on the influence of the dissection method on connective tissues, two groups of examination were formed: basic and control group.

The basic group involved 67 patients, whose dissection of subcutaneous fat was done by ultrasound method with Ultracision ("Ethicon" Co.) device in the mode of simultaneous cutting and coagulation. There were 12 (17.9%) men and 55 (82.1%) women in this group. According to age, the number of patients made: below 40: 9 (14.7%); from 41 to 50 years: 12 (14.7%); from 51 to 60: 25 (43.9%); above 60: 21 (26.8%). The average age was 54.8±10.8 years. As for the sizes of hernias the patients were presented as follows: small-sized hernias in 9 (13.4%) patients, medium-sized ones: 30 (44.8%) patients, big hernias: 22 (32.9%) patients, giant hernias in 6 (8.9%) patients.

The control group of 50 patients involved 10 (20.0%) men and 40 (80.0%) women, whose dissection of subcutaneous fat was done by classic method with the use of high-speed coagulator of Valleylab or Martin Co. Four (8.0%) patients were at the age up to 40 years old; 13 (26.0%) patients aged from 41 to 50 years; 14 (28.0%) were at the age from 51 to 60; 19 (38.0 %) patients were above 60. The average age was 55.6±10.6 years. As for the sizes of the hernias the patients were presented as follows: small-sized hernias in 7 (14%) patients, medium-sized: 20 (40%) patients, big hernias: 18 (36%) patients, giant hernias: 5 (10%) patients.

The material for histological examination was taken perioperatively, after the discharge of hypodermic tissue from aponeurosis, while using either ultrasonic harmonic scalpel or high-frequency coagulator. Parts of tissues of subcutaneous fat and aponeurosis after dissection were examined. The material was fixed in 10% solution of neutral formalin, dehydrated, dipped in intervening intermediate medium with subsequent embedding into paraffin. Sections of 5-6 *mcm* width were derived from paraffin blocks and stained with hematoxylin and eosin.

The morphometric examination of histological sections of tissue samples was done using the automated system, including the light optical microscope "Micros", digital video camera "Picxera PVC 600" and applied morphometric program Scion Image, version Beta 4.02 (Scion Corporation, U.S.A.). The measurements of the width of the necrosis zone were done in 10 randomly selected visually seen fields with microscope expansion (x 200).

Cytological examination of exudation discharging from the wound of the patient was done on the 2nd, 4th, 6th and subsequent days, as well as on the day of drainage tubes removal. After drying the glass slides, they were stained by the method of Romanovski-Gimsa.

Upon examination of impression smears 1000 cells were counted and the following cell types were defined: neutrophilic leukocytes, phagocytes and degenerating neutrophilic leukocytes, eosinophilic leukocytes, lymphocytes, histiocytes, macrophages, fibrocytes and fibroblasts. The cell structure was shown in percentage to the total number of cells.

The results of the examinations were processed using the package of applied programs Statistica. Version 6.0 (StatSoft, USA), with the use of parametric and non-parametric statistics data.

RESULTS AND DISCUSSION

Morphological peculiarities of the structure of surrounding tissues in case of different types of tissue dissection

The histological examination of subcutaneous fat adjacent to aponeurosis showed that the removed tissues were presented by the parts of connective and adipose tissue, as well as separate cross-stripped muscular fibers. The adipose tissue was presented by round cells, the core of which was situated along periphery. The groups of lipocytes were divided by interlayers of connective tissues, containing small blood vessels. The connective tissue component was presented by collagen, slightly curled fibers with low quantity of fibrocytes. In all cases, regardless of the dissection type, inflammatory infiltration of different levels was defined. The inflammatory infiltration was mainly presented by lymphocytes with some admixture of neutrophilic leukocytes (Figure 1).

Single transversal striated muscle fibers are presented by muscular tubule with the nuclei on the periphery and well seen cross striation.

When the high frequency coagulator was used, the morphological structure of the dissection edges had significant pathological changes. The connective tissue component underwent coagulative necrosis on the significant depth. Collagen fibers of separate areas were presented by the conglomerate of tightly pressed tissues as a homogeneous pink-red mass (Figure 2).

Blood vessels were defined in the way of round or oblong bands. In single preserved vessels the desquamation of endothelium from basic membrane, as well as dystrophic changes of myocyte and adventitial cells could be seen. The majority of blood vessels was in a "collaptoid" condition with evident manifestation of fibrinoid necrosis. The width of the necrosis zone was 456.5±24.6 *mcm*. In more deep layers the pathological changes in the capillaries, arterioles and venules in the way of fibrinoyd necrosis of vessel walls were defined. The small vessels were presented by homogeneous tubules of pink color. The nuclei of smooth-muscular, endotheliocyte and adventitional cells were not visualized, the collagen fibers were in the state of breakdown and were presented by granular mass of pink-red color (Figure 3).

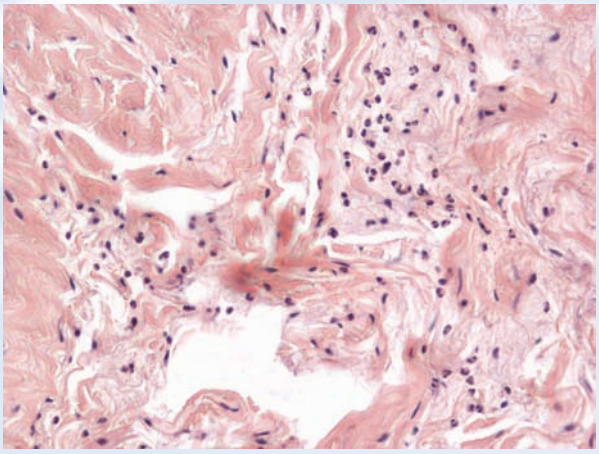

Figure 1. *The part of connective tissue adjacent to the part of dissection. Among collagen fibers inflammatory infiltration is presented by leukocytes and neutrophilic leukocytes. Staining: hematoxylin and eosin. ×200.*

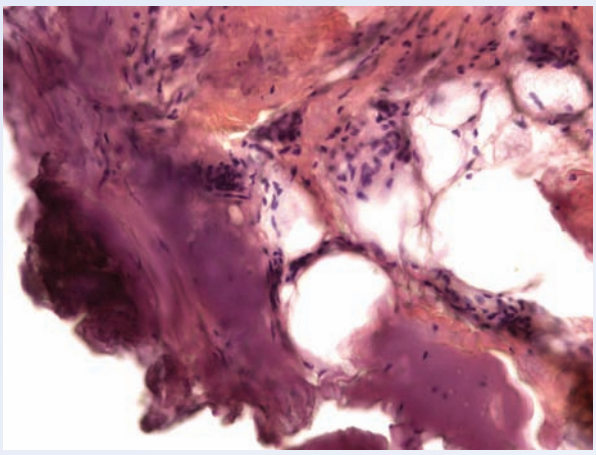

Figure 2. *Part of the soft tissue from the part of dissection using high frequency coagulator. Coagulation necrosis of the connective tissue component in the form of a pink-red conglomerate. Staining: hematoxylin and eosin. ×200.*

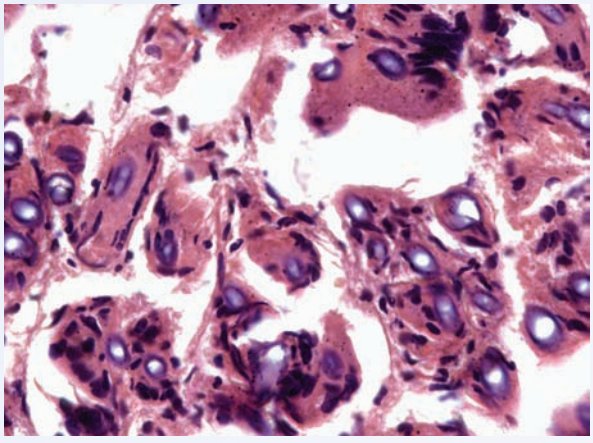

Figure 3. *The part of soft tissue adjacent to the place of dissection when the high-speed coagulator was used. The fibrinoid necrosis of small blood vessels. The lysis of collagen fibers in the way of homogeneous granular mass of pink-red color. Staining: hematoxylin and eosin. ×200.*

In the subcutaneous fat dilated vessels could be seen; in certain vessels the endothelium exfoliated from the basic membrane and was freely situated in their lumina, few vessels had morphological features of hyperemia.

When the ultrasonic scalpel was used, the changes in the soft tissues on the edges of dissection had less evident character. The connective tissue component had the signs of necrotic changes; however, the fascicular structure of fibers could be seen though weak. The zone of necrosis was 268.3±18.5 *mcm*, which was statistically much less than when high-speed coagulator was used ($p<0.001$). In tissues underlying deeper in the necrosis zone, the collagen fibers preserved their organotypic structure. Between the clusters of collagen fibers, the fybrocytes were very well visualized; among the mentioned cells only single ones had dystrophic changes (Figure 4). Small blood vessels had the features of hyperemia and only in single cases the process of endotheliocytes desquamation was noticed.

The subcutaneous fat also preserved organotypic structure, consisted of the lipocytes group, surrounded by the layers of connective tissue, blood vessels had some features of hyperemia, single foci of fibrinoid necrosis could be seen (Figure 5).

Thus, high frequency coagulator and ultrasonic scalpel usage for dissection brings forth a complex of pathological changes in tissues adjacent to the place of exposure, which is visualized by the set of necrotic, dystrophic and exudative reactions. Statistically significant reduction of necrosis zone while exposed to ultrasound scalpel, as compared to high-speed coagulator ($p<0.001$), preservation of small blood vessels adjacent to the zone of the tissue necrosis create condition for faster regeneration of the tissue when the ultrasound scalpel is used.

Cytological characteristics of exudate in case of different types of tissue dissection

In a post-surgery period the cytological examination of the exudate from the drainage tubes was done on the 2nd, 4th and 6th day. The following types of the cells were defined: neutrophilic leukocytes, phagocytes and degenerating neutrophils, lymphocytes, macrophages, fibrocytes and fibroblasts (Table).

The analysis of neutrophilic leukocytes content in the dynamics of observation detected that in the basic group a dynamic decrease of the mentioned cells could be seen on the 4th and 6th days after the surgery ($p=0.012$ and $p=0.005$, correspondingly). In the control group the tendency towards reduction of content of neutrophilic leukocytes could be also seen, although the difference as compared to the previous terms of observation was statistically not significant, which was connected to the big dispersion of the analyzed indices. The comparable analysis between main and control groups showed that

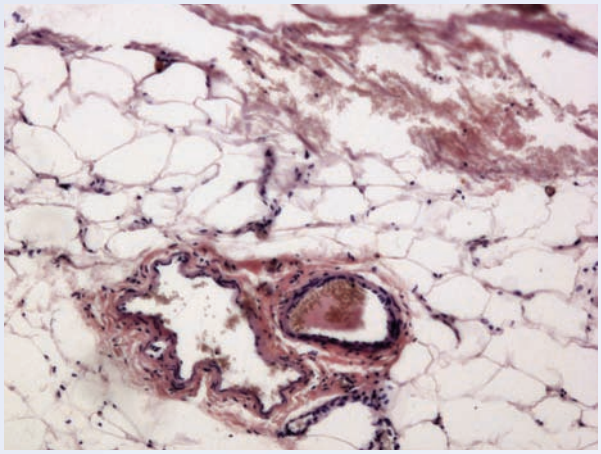

Figure 4. *The part of soft tissue adjacent to the place of dissection when the ultrasonic scalpel was used. Few parts of the zone in place of exposure to ultrasonic device. In deeply situated tissues the clusters of collagen fibers preserve organotypic structure, hyperemia of blood vessels. Staining: hematoxylin and eosin. ×200.*

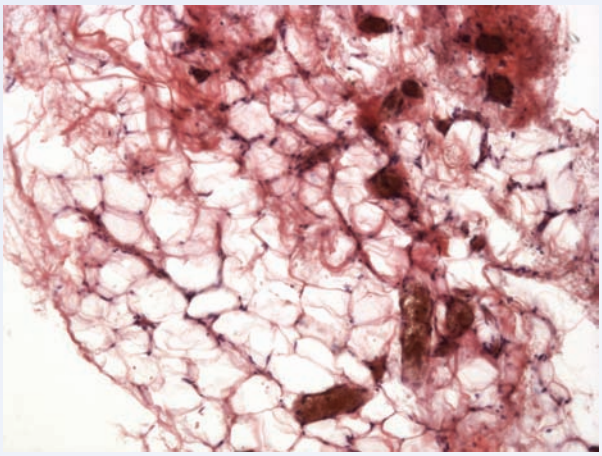

Figure 5. *The part of the soft tissue adjacent to the place of dissection while ultrasonic scalpel was used. The organotypic structure of the adipose tissue, hyperemia of small blood vessels. Small focus of necrosis in the upper-right corner. Staining: hematoxylin and eosin. ×200*

Table.

Indices of cellular composition of wound exudate: patients within basic and control groups

Index	Group	Term of study (days)		
		2	4	6
Neutrophilic leukocytes	Basic	28. 38±2.24 *p*=0.018*	16.33±2.40 *p*=0.012**	5.43±0,.78 *p*=0.046*; *p*=0.005**
	Control	18.3±2.72	15.73±4.71	10.28±2.4
Phagocytes and neutrophilic leukocytes	Basic	2.78±0.21 *p*=0.001*	2.38±0.27 *p*=0.03*	0.44± 0.09 p=0.011**
	Control	0.8±0.11	1.8±0.7	1.06±0.63
Degenerating neutrophilic leukocytes	Basic	3.64±0.69 *p*=0.041*	2.37±0.31 *p*=0.039*	0.67± 0.02 *p*=0.01*; *p*=0.005**
	Control	8.63±0.56	6.12±1.22	4.19±0.83
Lymphocytes	Basic	1.02±0.51	1.32±0.13	0..45±0.04 *p*=0.028*
	Control	0.88±0.55	1.22±0.54	1.06±0.39
Macrophages	Basic	0.71±0.26	0.31±0.01	0.25±0,11
	Control	0.55±0.15	0.26±0.09	0.48±0.06
Fibroblasts and fibrocytes	Basic	1.23±0.37	2.01±0.19	3.46±1.0
	Control	1.00±0.42	2.11±0.52	2.01±0.09

Notes: * - *the difference is statistically significant when compared between basic and control groups;*
** - *the difference is statistically significant when compared with the previous terms of observation within the group.*

the number of cells containing neutrophilic leukocytes from the wound on the 2nd day of observation was significantly higher as compared with the control group (p=0.018). On the 6th day of observation opposite tendency could be seen: the content of neutrophils in the main group was essentially less than in the control one (p=0.046).

The content of phagocyte neutrophilic leukocytes in patients of the main group did not change significantly on the 2nd and 4th day and was reduced on the 6th day (p=0.011). At the same time, the analogical tendency was not seen, while analyzing the phagocyte neutrophilic leukocytes of the control group patients. The comparable analysis of mentioned cells revealed an essential increase of the content on the 2nd and 4th days of observation (p=0.001 and p=0.03, correspondingly). While observing the content of degenerating neutrophilic leukocytes in patients of the main group, a tendency was noted towards the reduction of their contents on the 6th day of observation, and in this case the difference on the 4th and 6th day was statistically significant (p=0.005). The analogical tendency was seen in patients of the control group. In this case the content of degenerating cells of the main group patients was significantly low on the 2nd, 4th and 6th days of observation (p=0.041, p=0.039 and p=0.001, correspondingly).

In the observation dynamics, the content of lymphocytes in patients of the main and control groups did not change significantly. The comparable analysis of their contents showed that on the 6th day of observation, the content of lymphocytes in patients of the main group was significantly lower as compared to the control one (p=0.028).

The content of the macrophages, fibroblasts,

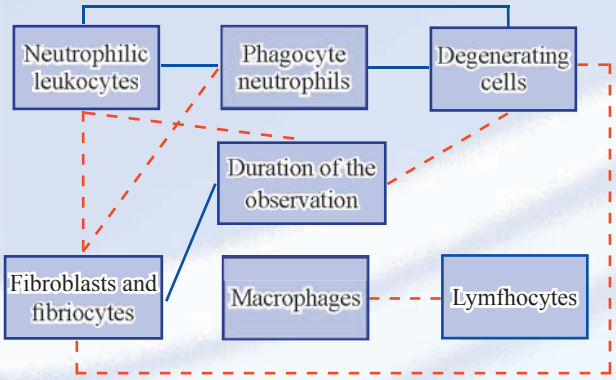

Figure 6. *The character of correlation between the cytological indices of the exudate in patients of the main group patients*

positive correlation

negative correlation

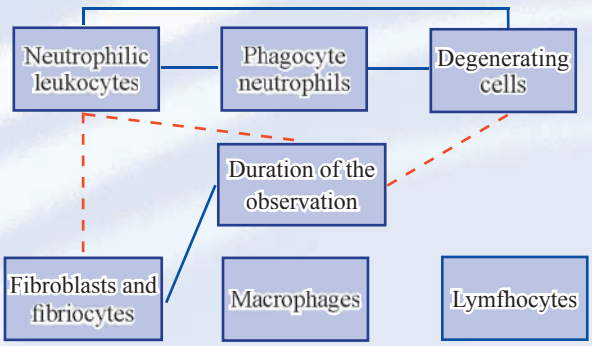

Figure 7. *The character of correlation connection between the cytological indices of the exudate in patients of the control group.*

positive links/correlation

negative links/correlation

and fibrocytes showed the tendency towards growth in the observation dynamics with the patients of both main and control groups. The comparable analysis among the groups and within the observation dynamics did not reveal any statistical significance. However, in the main group the content of fibroblasts and fibrocytes on the 6th day was significantly higher than in the control one.

Correlation analysis done by Spearman's statistics showed that in patients of the main group 4 correlations of positive character and 7 associations of the negative character could be seen. The character of the correlations between cytological indices of the exudate in patients of the main group is shown as Figure 6.

The relations between cytological indices in the observation dynamics reflected traditional character in the period of regeneration processes in the post surgery wound. This tendency was confirmed by the availability of neutrophils and degenerating cells (r=3.89; p=0.001), neutrophilic leukocytes and their phagocyting forms (r=2.43; p=0.023) and negative connections between neutrophilic leukocytes and content of fibroblasts (r=-9.467; p=0.001). The mentioned character of interrelations demonstrated that in the process of post surgery wound healing the increase of neutrophilic leukocytes content is directly coupled with the growth of the number of phagocytes and degenerating forms of neutrophils. In this case, the decrease of the content of neutrophilic leukocytes in the observation dynamics is the manifestation of the inflammatory reaction decay and is followed by stimulation of regeneration processes in the way of fibroblasts content increase.

In the process of postoperative wound healing, statistically significant decline in number of phagocyte neutrophils (r=-2.876; p=0.008) and degenerating cells (r=-5.149; p=0.001) could be seen. The tendency towards an increase in quantity of fibroblasts in the observation dynamics should be noted (r=15.848; p=0.001), which was followed by the decrease in quantity of degenerating cells (r= -4.543; p=0.001). Given changes reflect general biological patterns, when after the cleaning of the wound, plastic processes are stimulated as early fibroblastic reactions.

In control group, the character of interrelations between cytological indices in the observation dynamics had close tendencies as compared to the main group. Although, the quantity and the character of the correlation was somewhat changed. Only 2 connections of positive character and 3 of negative origin were noted. The character of the correlations between cytological indices of the exudate in patients of the main group is shown as Figure 7.

When compared with the main group, the positive interrelations between neutrophilic leukocytes and degenerating cells were preserved (r=4.975; p=0.001), between the duration of observation and content of the fibroblasts (r=5.91; p=0.001). At the same time, the positive correla-

tions between the content of the neutrophils, their phagocyting and degenerating forms were absent. While analyzing the content of immunocompetent cells (macrophages and lymphocytes) the absence of correlation between them in the dynamics of the observation was noted. The decrease of the quantity of connections between the immunocompetent cells in the control group of patients, for whom the high-speed coagulator was chosen as the means of dissection, apparently reflects less level of intensity of immune reactions.

The use of ultrasonic scalpel in the surgical treatment of patients with post-surgery ventral hernias results in growth of neutrophilic leukocytes and their phagocyting forms on the 2nd day of the observation (p=0.18 and p=0.001, correspondingly) and reduction of the neutrophils content on the 6th day of observation (p=0.05), as compared with the high-frequency coagulator, which reflects more expressed intensity of the immune reaction in the early post-surgery period. The content of degenerating forms of neutrophilic leukocytes in case of ultrasonic scalpel application is significantly lower on the 2nd, 4th and 6th day of observation (p=0.001, p=0.03 and p=0.005, correspondingly), which proves less damaging effect of the ultrasonic scalpel on the surrounding tissues and immunocompetent cells.

CONCLUSIONS

Thus, the usage of ultrasonic scalpel caused less evident pathological changes in the adjacent tissues, manifested by less evident necrotic changes in the connective tissue component, as well as by the preservation of the organotypic structure of the small blood vessels. The correlative connections between cellular elements of the exudate from the wound and duration of treatment were characterized by the lower degree of manifestation of inflammatory reactions in patients of the main group. The presence of direct relations between the content of neutrophilic leukocytes, their phagocyting and degenerating forms (p=0.001 and p=0.023, correspondingly), in the aggregate with enhancing of wound cleaning process and increase of fibrocytes content, characterized the higher degree of manifestation of the regeneration processes.

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