DOI: 10.17816/PTORS5325-36

# CLINICAL AND MORPHOLOGICAL CHARACTERISTICS OF THE VASCULAR BED OF HYPERTROPHIC SCAR TISSUE IN DIFFERENT PERIODS OF ITS FORMATION

© ***O.V. Filippova[1], K.A. Afonichev[1], I.N. Krasnogorsky[1], R.V. Vashetko[2]***

[1] The Turner Scientific Research Institute for Children's Orthopedics, Saint Petersburg, Russia;
[2] Saint Petersburg I.I. Dzhanelidze Research Institute of Emergency Medicine, Saint Petersburg, Russia

Received: 03.05.2017 Accepted: 10.08.2017

**Background.** The state of the microcirculatory bed in the scar tissue is extremely important for determining the most appropriate methods of conservative and surgical treatments. Only few studies have assessed the vascular features of scar tissue.
The objective was to study and analyze the morphological features of the vascular bed of scar tissue and their clinical implications.
**Materials and methods.** Fifty-four patients with hypertrophic post-burn scars were examined. The study used a clinical method and performed histological analysis of the scars biopsy specimens, including a survey light microscopy, a morphometric assessment of the vascular bed as well as the verification of the collagen fibers of scar tissue with an immunohistochemical (IHC) analysis with specific monoclonal antibodies (AT) (Novocastra, Bond) to Type I and III collagens.
**Results.** A significant increase in the total area of the vessels of the rumen in the first 6 months of its formation was observed in comparison with intact skin and later maturation of the scar tissue (in % in 1 $mm^2$ of intact skin – 8.50, in the rumen in terms of up to 6 months – 13.10). The average number of vessels in the scar tissue and the total area of their lumen in the maturing rumen from 2 to 5 years decreased in comparison with that in the intact skin. The nodes were detected in the scars by an early appearance of the clinical signs of vascular disorders including blisters and erosions on thickened and hyperemic scar tissues.
**Discussion.** In the developing hypertrophic rumen, the circulatory conditions gradually deteriorated due to the compression and obliteration of the vessels of the skin with collagen. The enhancement in perfusion recorded using laser Doppler fluorometry may be associated with a significant dilatation of the rumen vessels, rather than because of actual enhanced perfusion.
**Conclusions.** 1. The increase in the vascular cross sectional area in the early stages of scar formation is attributable to the expansion of their lumen vessels. In the ripened rumen, the number of vessels is 3 times less than that in intact skin. 2. Hyperemia of the hypertrophic scar is caused by a substantial widening of the vessels of the scar tissue, and not by an increase in their number. 3. The use of a hypertrophic scar for the creation of rotational and other flaps is associated with a high risk of trophic complications.

**Keywords:** rumen vessels, blood circulation in the scars, trophic scar tissue, morphological examination of the scars.

# КЛИНИКО-МОРФОЛОГИЧЕСКИЕ ОСОБЕННОСТИ СОСУДИСТОГО РУСЛА ГИПЕРТРОФИЧЕСКОЙ РУБЦОВОЙ ТКАНИ В РАЗНЫЕ СРОКИ ЕЕ ФОРМИРОВАНИЯ

© ***О.В. Филиппова[1], К.А. Афоничев[1], И.Н. Красногорский[1], Р.В. Вашетко[2]***

[1] ФГБУ «НИДОИ им. Г.И. Турнера» Минздрава России, Санкт-Петербург;
[2] ГБУ «Санкт-Петербургский научно-исследовательский институт им. И.И. Джанелидзе», Санкт-Петербург

Статья поступила в редакцию: 03.05.2017 Статья принята к печати: 10.08.2017

**Актуальность.** Состояние микроциркуляторного русла в рубцовой ткани имеет большое значение для выбора тактики консервативного и хирургического лечения. Литературные данные, посвященные изучению сосудистых особенностей рубцовой ткани, немногочисленны.
**Цель исследования:** изучить и проанализировать некоторые морфологические особенности сосудистого русла рубцовой ткани, их влияние на клиническую картину.

■ For citation: Filippova OV, Afonichev KA, Krasnogorsky IN, Vashetko RV. Clinical and morphological characteristics of the vascular bed of hypertrophic scar tissue in different periods of its formation. *Pediatric Traumatology, Orthopaedics and Reconstructive Surgery*. 2017;5(3):25-35. doi: 10.17816/PTORS525-36

**Материал и методы.** Были обследованы 54 ребенка с гипертрофическими послеожоговыми рубцами. В исследовании использовались клинический метод и гистологическое изучение биоптатов рубцов, включающее обзорную световую микроскопию, морфометрическую оценку сосудистого русла, а также определение некоторых типов коллагеновых волокон рубцовой ткани при помощи иммуногистохимического (ИГХ) исследования (с использованием специфических моноклональных антител (АТ) к коллагену I и III типов [Novocastra, Bond]).
**Результаты исследования.** Выявлено достоверное увеличение суммарной площади поперечного сечения сосудов рубца на единицу площади (1 мм$^2$) в первые 6 месяцев его формирования по сравнению с интактной кожей и на более поздних сроках созревания рубцовой ткани (в % в 1 мм$^2$ интактной кожи — 8,50, в рубце в сроки до 6 месяцев — 13,10). Отмечалось уменьшение средних значений количества сосудов в рубцовой ткани и суммарной площади их просвета в сроки созревания рубца от 2 до 5 лет по сравнению с интактной кожей. Узлы обнаруживались в рубцах с ранним появлением клинических признаков сосудистых расстройств в виде пузырей и эрозий на утолщенной и гиперемированной рубцовой ткани.
**Обсуждение.** В формирующихся гипертрофических рубцах кожи условия кровообращения постепенно ухудшаются за счет сдавления и облитерации сосудов кожи коллагеном. Усиление перфузии, регистрируемое с помощью лазерной доплеровской флуометрии, может быть связано со значительным расширением сосудов рубца, а не с усилением перфузии.
**Выводы.** 1. Увеличение площади сосудистого сечения в ранние сроки формирования рубца происходит за счет расширения просвета сосудов. В созревшем рубце количество сосудов уменьшено в 3 раза по сравнению с интактной кожей. 2. Гиперемия гипертрофического рубца обусловлена резким расширением сосудов рубцовой ткани, а не увеличением их количества. 3. Использование гипертрофического рубца в составе ротационных и других лоскутов связано с высоким риском развития трофических осложнений.

**Ключевые слова:** кровеносные сосуды рубца, кровообращение в рубцах, трофика рубцовой ткани, морфологическое исследование рубцов.

## Background

The condition of the microvasculature in scar tissues is crucial for making a decision regarding the choice between conservative and surgical treatment. Vascular changes are most clearly reflected in the clinical picture. However, literature shows that few studies have explored the vascular features of scar tissues.

Page et al. (1983) studied microcirculation in post-burn hypertrophic scars and skin autografts at various stages of maturity using capillary microscopy. They found that the sympathetic innervation of large vessels under and inside the scar tissues was present exclusively in fully mature scars, indicating an association between the processes of reinnervation and maturation of the scars [1].

In 2010, using contact optical probe videocapillaroscopy, a group of scientists conducted *in vivo* studies to assess the differences between the microcirculatory characteristics of post-burn scar and the healthy skin of upper limbs. This study included 15 scar zones and 12 healthy skin areas in 12 patients. The density, length, and diameter of the capillaries were evaluated; further, the pattern of capillary distribution (point, reticular, and directional) and the presence of microhemorrhages and neoangiogenesis were assessed. We found that the diameter and length of the capillary loop, the density of the capillary distribution, and the level of neoangiogenesis were significantly higher in the post-burn scars than in the controls. Further, the study revealed significant differences between the diameter of the capillaries and the level of neoangiogenesis in actively growing hypertrophic scars and mature scar tissues, indicating that the evaluation of the microvasculature in the scars could be crucial for evaluating and predicting outcomes of burn injuries [2].

Several researchers have used laser Doppler flowmetry to study circulation in scar tissues.

In 1986, Hosoda et al. suggested that in patients with hypertrophic scars, there is increased microcirculatory perfusion in the scar area. Using laser Doppler flowmetry, the authors studied the microcirculatory blood flow in patients with hypertrophic and normotrophic post-burn scars. The study was performed at three-week intervals and revealed higher values of cutaneous blood flow in patients with hypertrophic scars, indicating a connection between increased microcirculatory blood flow and hypertrophic scar formation. The researchers also suggested that an early increase in microcirculatory blood flow is a potential early indicator of the appearance of hypertrophic scars [3].

In 1989, a group of Chinese researchers evaluated the microcirculation in post-burn hypertrophic scars in 50 patients using laser Doppler flowmetry. The average follow-up period in this study was

20 months. The microcirculatory response in the scars to a vasodilatation (thermal) stimulus was investigated, and significant differences ($p < 0.0001$) were found between hypertrophic scars of different clinical maturity and healthy skin. Thus, laser Doppler flowmetry may be considered useful for the clinical evaluation of scar maturity [4].

According to Ehrlich et al. (1992), the intense hyperemia in scar tissues in the early stages of their formation indicates a relationship between scar maturation and the vascular changes, suggesting a local increase in the microcirculation. To measure the changes in the blood flow in the scars, the scientists also used a laser Doppler blood flow monitor. The assessment of the scars in patients with recently healed wound surfaces revealed an average blood flow level of 365 ± 325 mV ($n = 131$). The values ranged from 98 to 1450 mV, 18 times higher than the average value in intact skin with average values of 43 ± 13 mV ($n = 212$). Further evaluation of the blood flow after 16-18 weeks revealed a fall of up to 32 ± 21 mV ($n = 7$) in the values at the site of the healed wound where a normotrophic scar was developing. Moreover, the values were in the range of 148 ± 78 mV ($n = 59$) at sites that showed clinical signs of scar tissue hypertrophy. This value was three times greater than that in the intact skin and four times greater than that in the normotrophic scar. The level of blood flow in the hypertrophic scars remained elevated (102 ± 34 mV [$n = 10$]) after 38-50 weeks [5].

In 2003, a team of researchers used the laser Doppler perfusion imaging (LDI) method to measure the perfusion in normal and post-burn scar tissue. Single-wavelength (635 nm), stepped LDI scanning as well as double wavelength (633 and 780 nm), continuous LDI scanning were used. Measurements were performed in 20 patients who had hypertrophic post-burn scars (1 month to 8 years after the trauma); the scars were clinically evaluated using the modified Vancouver scale. Perfusion of each scar zone and the intact skin of the symmetrical contralateral area was measured. In all the comparisons, a significant positive correlation was observed between the perfusion indices and the severity of the clinical picture [6].

Considering the importance of diagnosing the state of microcirculation in the scars for the prediction of prognosis, Fourman et al. (2015) tested the capability of LDI and angiography using indocyanine green dye (ICG) in predicting the development of burn scars 28 days following the injury, using the previously tested model of inflicted burn injury in pigs wherein a vertical scar was predicted. After the burn injuries were inflicted on the animals, the researchers performed linear regression analyses to compare the results of perfusion with wound reduction on the 28$^{th}$ day after the injury. ICG angiography showed a peak linear correlation [$r$ (2) 0.63 (95% CI 34-92)] 48 hours after the burn, which was significantly different from the linear regression of LDI ($p < 0.05$), which was measured at $r$ (2) 0.20 (95% CI 0.02-0.39). The linear regression of angiography with ICG exceeded LDI at all time points. The authors concluded that ICG angiography may have significant potential for predicting the long-term prognoses of burn injuries [7].

To assess the perfusion in the cover tissues, a laser speckle perfusion imaging system (LSPI) was used. The LSPI is a new, non-invasive method that enables rapid and reproducible measurement of tissue perfusion. The high resolution and frame rate of LSPI make it more informative than the conventional method of LDI and enable the detection of the temporary changes in blood flow during the healing of skin wounds. Stewart et al. (2006) conducted an experimental study wherein skin perfusion was measured after inflicting burn injury in the pigs. After the injury, the wounds were measured and photographed once every week, and normalized blood flow values were determined using the LSPI system. The tissue perfusion values became available for measurement after the complete epithelialization and removal of the eschar on day 21. At this time, the blood flow in the wound had significantly increased compared with that in the surrounding intact skin. Later on, the blood flow consistently reduced and approached normal values on the 35$^{th}$ day following the injury [8].

In 2016, Liu et al. used the laser point contrast imaging method to study blood flow in keloids and the adjacent skin. A total of 61 keloid scars in 21 patients were studied. Using this method, significantly higher perfusion was determined in the tissues of the keloids and the adjacent skin than in the peripheral areas ($p < 0.05$). The mean and range values (CI 95%) were as follows: $K/N = 2.41$ (2.28-2.54) and $A/N = 1.33$ (1.28-1.37). A heterogeneous manifold of perfusion was commonly observed. The average perfusion in the keloids and the adjacent skin in the chest region

was n observed significantly higher than that on the back ($p < 0.05$). However, there was no statistically significant difference in the *K/N* at different sites ($p > 0.05$) [9].

Biochemical and immunohistochemical methods were also used to determine the trophic status of the scar tissue.

Ueda et al. (2004) studied the biopsy samples of scar tissue using morphometry and biochemical test results. Vessels were counted in the keloids, hypertrophic and atrophic scars in a certain area, and the cross-sectional area of their internal lumens, and the concentration of lactate in the tissue were measured. The average number of vessels and the average area of the vascular cross-section were the smallest in the keloids. The lactate content was 39.4 (13.5) mmol/g protein in the keloids; 23.8 (7.5) in the hypertrophic red scars, 23.8 (7.6) in the pink scars, and 13.3 (7.3) in the white scars. In addition, the content of adenosine triphosphate (ATP) in the keloids remained high for a long time. The results reveal that against a background of reduced perfusion and pronounced hypoxia, ATP can be formed in keloids by anaerobic glycolysis [10].

It has been established that angiogenesis plays a significant role in tissue regeneration. Kumar et al. (2009) attempted to study the angiogenesis in scars after surgical wounds using immunohistochemical techniques. They determined vascular endothelial growth factor (VEGF) that stimulates angiogenesis through the receptor kinases VEGF-R1 and VEGF-R2 and the co-receptors neuropilin Np1 and Np2 in the biopsy specimens of the scar tissue. Quantitative determination of the microvessel density (MVD) was also performed using the Chalkley network; the expressions of VEGF, VEGF-R1, VEGF-R2, Np1, and Np2, were found to be correlated with MVD endothelial growth factors and scar age. The biopsy specimens of the scars were collected from the patients in the period from 3 days to 2 years after the surgery. Further, during the surgery, biopsy samples of normal skin were also collected. Data analyses showed that the total MVD was significantly higher in the scars than in the controls ($p = 0.011$) and was associated with scar age ($p = 0.007$). The expressions of VEGF, VEGF-R2, Np1, and Np2 significantly increased in all scars and correlated with MVD. In contrast, VEGF-R1 expression of the scar was reduced and correlated with an increase in the VEGF and VEGF-R2 expressions. The study results indicate that VEGF-receptor complexes play a significant role in early wound healing. The increased expression of VEGF and an increase in the microvessel density are prolonged in the scar tissue and indicate that structural remodeling continues for at least two years after surgery [11].

Previous studies indicate a higher information value of invasive research methods in comparison with that of LDI techniques. Despite the increased perfusion in keloid and hypertrophic scars detected using laser Doppler fluorometry, the examination of the biopsy specimens of scar tissue indicate pronounced hypoxia. Thus, further study of the microvascular features of hypertrophic scars remain a topical issue that will complement and contribute to the integration of the available scientific data.

**Aim.** In the present study, we aimed to investigate and analyze the morphological features of the vascular bed of scar tissue and their effect on the clinical picture.

## Materials and methods

We examined 54 patients with multiple post-burn scar deformities that necessitated multistage surgical treatment. All patients and their representatives provided their consent for study participation and the use of personal data.

Clinical and histological methods were used in this study. The clinical methods included collecting information pertaining to patient complaints and anamnestic data as well as an objective clinical examination.

The following parameters were evaluated:

1) Subjective feelings of the patient (sensitivity, pain, and/or itching)

2) Objective scar characteristics, including color, thickness and size of the scar, and the presence or absence of trophic changes (peeling, vesicles, erosion, and/or microtrauma).

The scar tissue excised during the surgery was histologically examined. The histological method included an evaluation of the morphometric parameters of the vascular bed. The number of biopsy specimens obtained from a single patient at the different stages of scar formation ranged from 2 to 4.

The general characteristics of the biopsy materials are presented in Table 1.

A total of 42 biopsy specimens of intact skin were studied using morphological methods.

The biopsy specimens of intact skin were represented by insignificant surpluses of the full-layer skin autografts used for reconstructive surgery.

The surgical material, represented by fragments of scar and intact skin, was immediately placed in a 10% neutral formalin solution after excision for primary fixation. The duration of the primary fixation averaged 1.5-2 days. Thereafter, 1.5 × 0.4 cm blocks were excised from the most altered areas. The biopsy samples were subjected to histologic evaluation in isopropanol for diagnosis, using a carrousel-type tissue processor Microm STP 120 (Carl Zeiss, Thermo Scientific, Germany). Subsequently, the tissue fragments were embedded in paraffin using a station for paraffin embedding of blocks Microm EC350 (Carl Zeiss, Thermo Scientific, Germany). Paraffin sections with a thickness of 3.5-4.0 μm were prepared using paraffin blocks on the sliding microtome Microm HM 430 (Carl Zeiss, Thermo Scientific, Germany). Thereafter, the histological sections were de-embedded in xylene and stained with hematoxylin and eosin (HE) and picro-Mallory trichrome.

Then, microscopic examination of histological preparations and their photographing was performed using the light microscope Axio Scope A1 (Carl Zeiss, Germany). Morphometric measurements in the studied tissues were performed using a light microscope Leitz (Wetzlar, Germany) with an object-micrometer of transmitted light OMP (LOMO, Leningrad, value of one scale division is 0.01 mm), ocular morphometric grids with dots, and a grid divided by 256 small squares.

The morphometric examination provided information regarding the changes in the vascular cross section and in the number of vessels and number of cells.

*Table 1*

General characteristics of the biopsy material

| Terms after epithelialization | Number of biopsy specimens of the scar tissue (morphological examination) |
|---|---|
| up to 6 months | 39 |
| up to 2 years | 69 |
| 2–5 years | 48 |
| Total | 156 |

For verification in the tissues of the collagen fibers, an immunohistochemical study was performed using specific monoclonal antibodies (AB) (Novocastra, Bond) to collagen I and III types.

The collected data were processed with Excel, SPSS 17.0, and Statistica for Windows 6.0 applications. The normality of the data was evaluated using the Shapiro–Wilk test. All data are presented in terms of median values with 25% and 75% quartiles. As per the standard followed in medical research, a $p$ value < 0.05 was considered statistically significant.

## Results of the study

The histological examination of scar tissue revealed significant differences in the state of the dermal vascular network of the scars at the different stages of development and in comparison to that in the intact skin.

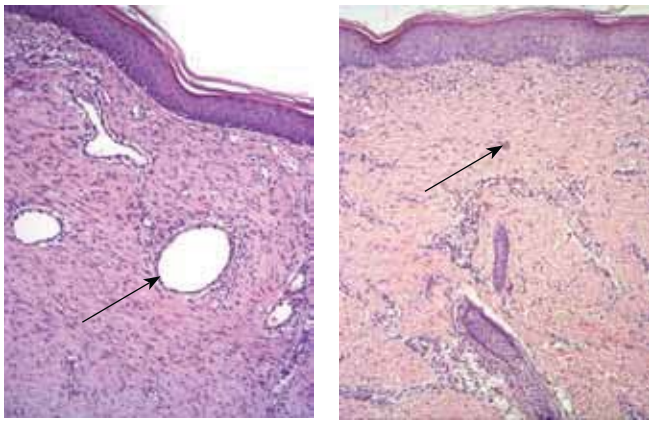
In the first months following the injury, when collagen synthesis in the reticular layer of the scar occurs optimally, the total cross-sectional area of the vessels of the papillary layer of the scar was significantly higher than the values characteristic for intact skin; however, there was a decrease in the total number of vessels in the scar tissue (Table 2).

*Table 2*

Dynamics of the morphometric parameters of the vascular bed of the scar at different stages of development

| Morphometric parameters (in 1 mm$^2$) | Intact skin | Scar | | |
|---|---|---|---|---|
| | | up to 6 months | up to 2 years | 2–5 years |
| Number of the vessels in the papillary layer | 120 (70; 140) $n$ = 38 | 95 (60; 125) $n$ = 28 | 90 (50; 130) $n$ = 54 | 44 (30; 75) $n$ = 34 |
| Number of the vessels in the reticular layer | 235 (120; 320) $n$ = 26 | 185 (110; 285) $n$ = 30 | 155 (90; 230) $n$ = 58 | 60 (40; 130) $n$ = 38 |
| Total area of the vessels in the papillary layer, % | 8.50 (4.5; 11.0) $n$ = 38 | 13.10*, 1, 2 (11.5; 16.5) $n$ = 28 | 6.20 (3.5; 9.5) $n$ = 54 | 2.96 (2.3; 7.5) $n$ = 34 |
| Total area of the vessels in the reticular layer, % | 14.10 (8.5; 19.5) $n$ = 26 | 11.40 (8.5; 15.8) $n$ = 30 | 6.90 (5.5; 11.5) $n$ = 58 | 5.36 (4.8; 8.9) $n$ = 38 |

* $p < 0.05$ unlike the norm; [1] $p < 0.05$ from 6 months to 2 years; [2] $p < 0.05$ 2–5 years.



*a* *b*

**Fig. 1.** Scar: Enlarged vessels with varying diameters in the connective tissue layer, 4 months after epithelialization (*a*). Intact skin. Vessels with normal diameter in the connective tissue layer (*b*). Staining with hematoxylin and eosin, ×200

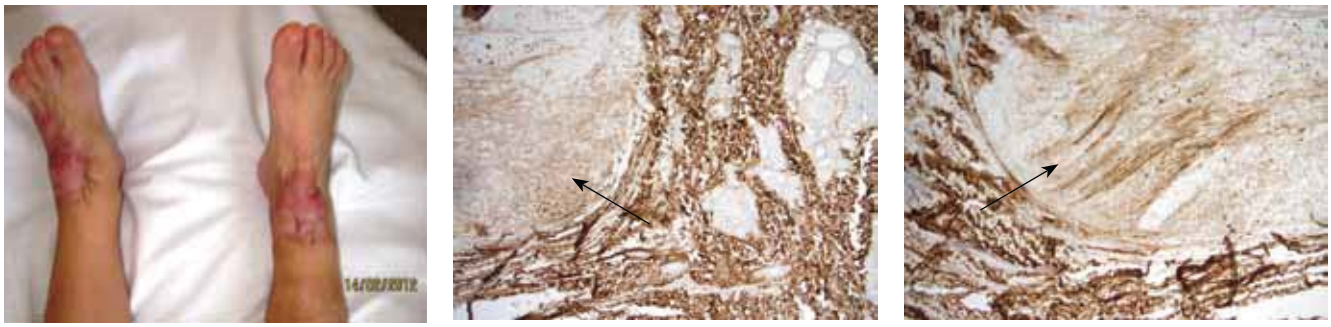
Histological data also revealed that the increase in the area of the vascular cross-section observed during the early stages of scar formation is not attributed to an increase in the number of vessels but to the expansion of their lumen (Fig. 1).

Significant increase in the vascular lumen is accompanied by an increase in the vascular permeability, tissue impregnation with plasma proteins, and adsorption by their unchanged fibrous structures along with subsequent precipitation. This mechanism triggers the formation of nodes in the fibrous connective tissue: the bundles of the collagen fibers lose fibrillation and merge into a homogeneous dense cartilage-like mass, the cellular elements are compressed and undergo atrophy (Fig. 2*a-d*).

In several cases, the signs of vascular disorders are revealed at 4-5 weeks after epithelialization, indicating intensive stimulation of fibroblasts and active collagen synthesis (Fig. 3).

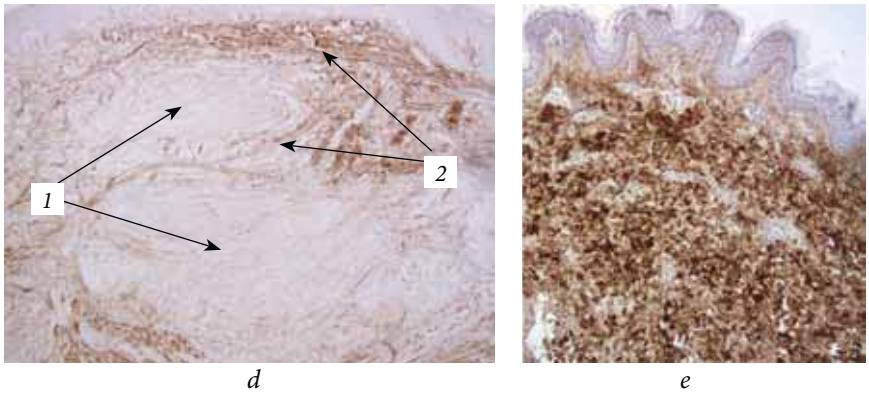
The early appearance of vascular disorders in the scar tissue is an unfavorable prognostic sign because it triggers the formation of nodal structures and increases the resistance of a scar to collagenase therapy as the nodes do not contain collagen structures.

The clinical picture in the first 3-6 months after the epithelialization indicates a disorder in the



*a* *b* *c*

**Fig. 2*a*.** Hypertrophic scars in the ankle joint 1 year after wound epithelialization.
**Fig. 2*b*, *c*.** Node in the scar tissue (1 year after wound epithelialization): hyalinized avascular zone surrounded by bundles of collagen fibers. Diffuse brown staining of the collagen fibers around the node (*b*). IHC with antibodies to collagen type III, ×200 (*c*). IHC with antibodies to collagen type I, ×200




*d* *e*

**Fig. 2*d*, *e*.** Nodes in the connective tissue layer of the scar (2 years after wound epithelialization): no brown staining (*1*). Around the nodes of the interlayer of collagen fibers: uneven light brown staining (*2*). IHC with antibodies to collagen type III, ×200 (*d*). Intact skin: uniform brown staining of the collagen fibers. IHC with antibodies to type I collagen, ×200 (*e*)

**Fig. 3.** Patient A., 9 years old (case history No. 12/1243). Donor site after cutting a thick split autograft. Four weeks after the epithelialization: induration and thickening of the scar tissue, formation of epidermal vesicles and erosion

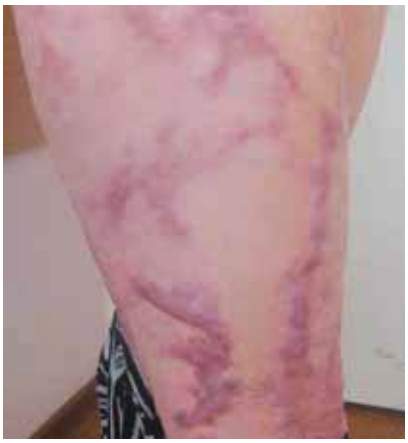
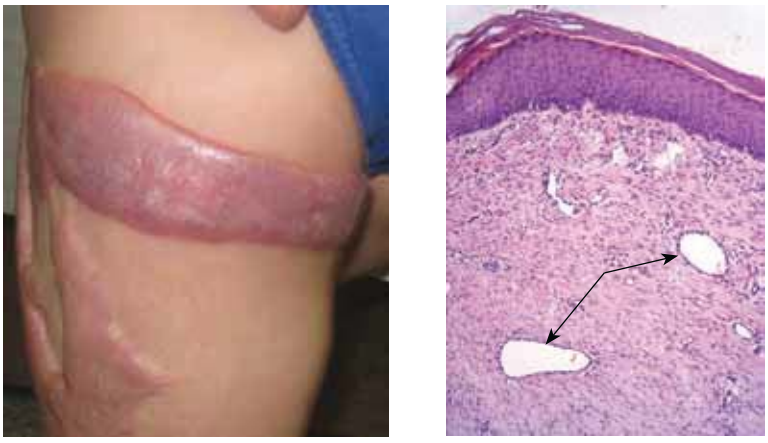


**Fig. 4.** Patient K., 11 years old (case history No. 14/1012). Post-burn scars of the thigh, 4 months after the epithelialization. Pronounced cyanosis



*a* *b*

**Fig. 5.** Patient B., 3 years old (case history No. 11/2045). Scarring 5 months after the epithelialization: edema and cyanosis (*a*). Pronounced enlargement of the vessels of the connective tissue layer of the scar. Staining with hematoxylin and eosin, ×200 (*b*)

venous outflow in the developing hypertrophic scar, manifested as cyanosis and edema (Fig. 4, 5).

Thereafter, starting from 5-6 months after the epithelialization, the mosaic sections of the enlarged vessels and the vessels compressed with bundles of collagen fibers are commonly noted (Fig. 6).

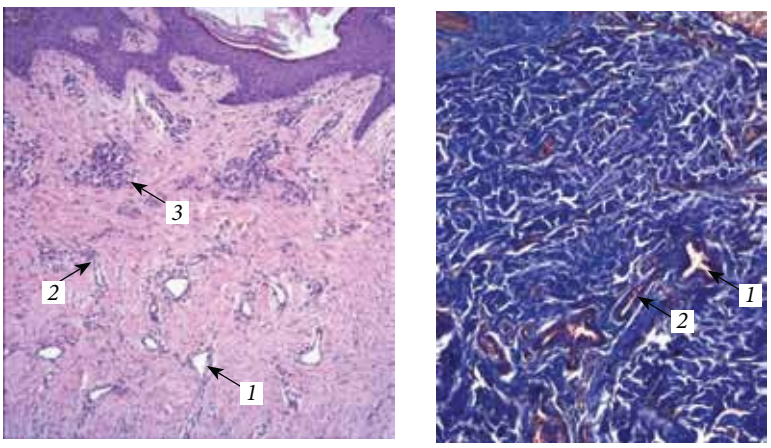
The clinical picture at this stage is represented by decreased edema, increased roughness of the relief of the scar surface, and variegated color wherein the pale areas alternate with bright pink areas. The growing volume of the collagen fibers is believed to compress the arterioles, resulting in the signs of trophic disorders in the form of hyperkeratosis and skin cracks (Fig. 7, 8).

The clinical manifestation of reduction in the arterial blood circulation is most commonly the initial observation, starting at 7-8 months after the epithelialization as mosaic coloration of the scar with alternating pale and bright areas, uneven relief, scar peeling, and appearance of recurrent erosion in the functionally active zones (Fig. 8).

In the period from 12 months to 2-3 years after the injury, there was an increase in the number of blood vessels compressed with scar tissue. In addition, vessels that did not contain the formed elements were commonly observed (Fig. 9).

Clinically, such a scar color approaches to intact skin; however, its surface remains uneven with increased density (Fig. 10). Symptoms of itching and burning are alleviated.

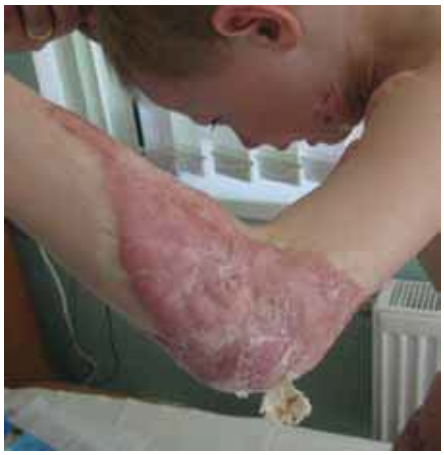
However, blood circulation in the scar is irreversibly reduced because of obliteration of



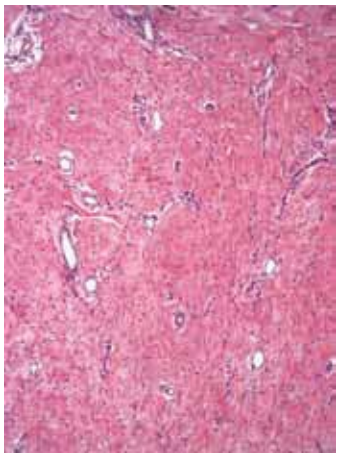

*a* *b*

**Fig. 6.** Scar: 5 months after wound epithelialization. Staining with hematoxylin and eosin, ×300 (*a*). Picro-Mallory trichrome, ×300 (*b*). Scar tissue with areas of the enlarged vessels (*1*) and slit-like vessels compressed with bundles of collagen fibers (*2*), lymphocytic infiltration in the upper layers of the dermis (*3*)

**Fig. 7.** Patient K., 9 years old (case history No. 09/1104). Scar of the knee joint region, 8 months after wound epithelialization



*a*



*b*

**Fig. 8.** Patient B., 3 years old (case history No. 11/2045). Scar at the stage of vascular restructuring (phase of arterial disorders): peeling, tuberous relief (*a*). Reduction of the lumen of the dermal vessels due to compression by the bundles of collagen fibers. Staining with hematoxylin and eosin, ×200 (*b*)

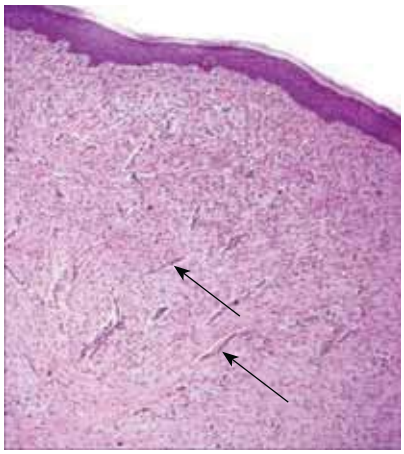


**Fig. 9.** Scar: Slit-shaped vessels of the dermis compressed by compactly located connective tissue fibers. Twelve months after wound epithelialization. Staining with hematoxylin and eosin, ×200

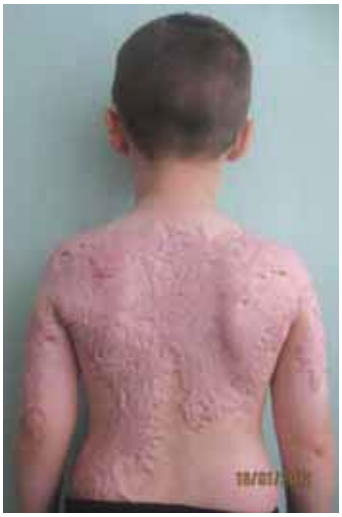
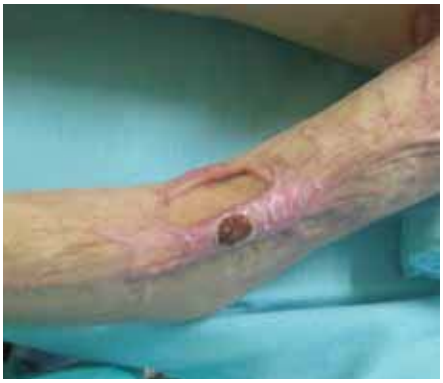
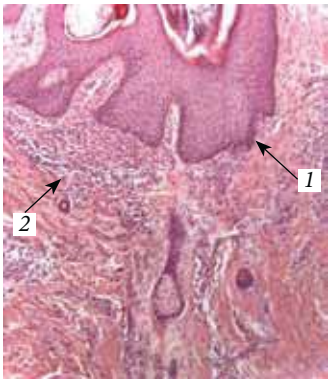


**Fig. 10.** Patient G., 7 years old (case history No. 12/0134). Mature hypertrophic scars on the back and the shoulders. Three years after wound epithelialization



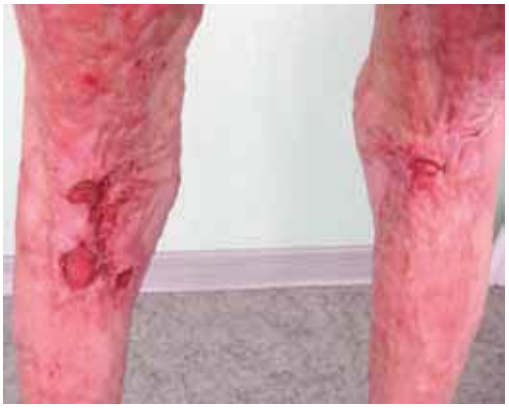
*a*



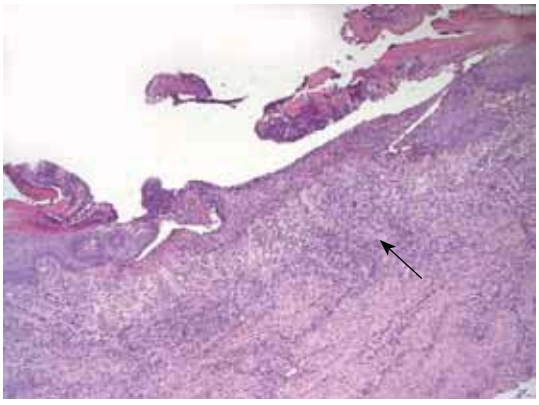

*b*

**Fig. 11*a*.** Patient S., 8 years old (case history No. 11/3899). Post-burn scars 11 months after skin restoration – erosion of scar tissue in the area of the elbow joint with marginal epithelialization

**Fig. 11*b*.** Histologic specimen: Epithelialized epidermal erosion of the hypertrophic scar: inflammatory growth of the epithelium (*1*), expressed diffuse lymphocytic infiltration of the papillary layer (*2*). Staining with hematoxylin and eosin, ×200



*a*



*b*

**Fig. 12*a*.** Patient G. (case history No. 09/4532). Chronic recurrent ulcers of the scar tissue in the popliteal region. Two years after skin restoration

**Fig. 12*b*.** Histologic specimen: Violation of the epidermis integrity and the papillary layer of the scar. Expressed diffuse lymphocytic infiltration. Staining with hematoxylin and eosin, ×200

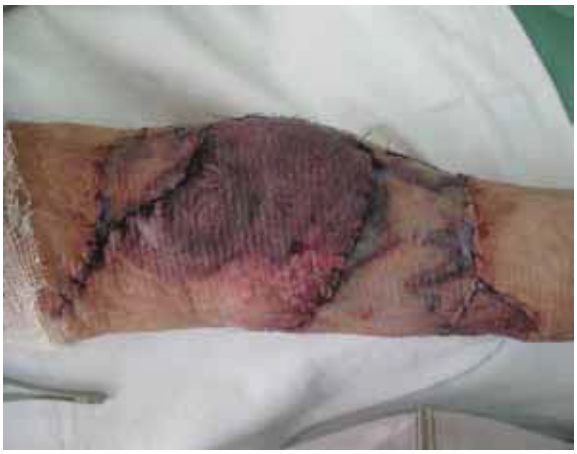
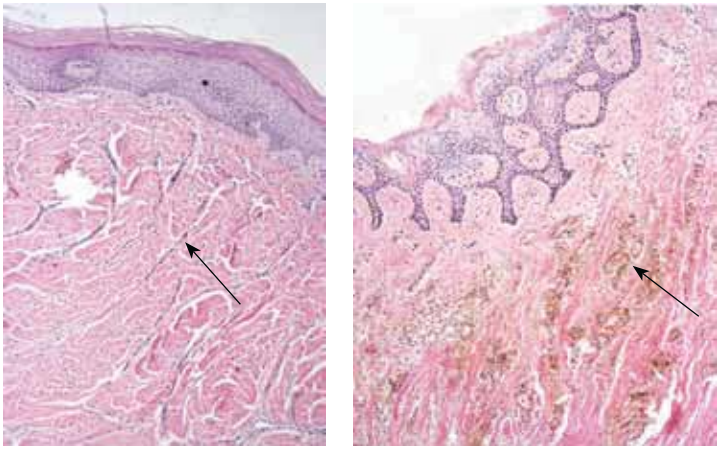


**Fig. 13.** Patient G. (case history No. 09/4532). A macroscopic picture of venous outflow disorder in a rotary fasciocutaneous flap, including the scar skin of the popliteal region. Day 4 after the surgery: edema, cyanosis



*a* *b*

**Fig. 14.** Slit-like vessels in the connective tissue layer of the scar before surgery (*a*). Abrupt enlargement of the vessels of the connective tissue layer of the scar with microbleeding on the 4th day after the surgery (*b*). Staining with hematoxylin and eosin, ×200

the vessels compressed with collagen. When the scar is localized in the functionally active zone, the increased need for oxygen and hypoxia often result in the emergence of signs indicative of local decompensation of the circulation. The functional load activates the arterial influx, including that to the cover tissues, causing venule overflow and provoking violation of the venous outflow. Vein overflow and increased venous permeability lead to the formation of vesicles and erosions, with characteristic features of sluggish epithelialization and frequent recurrence. In the future, erosion may transform into a recurrent trophic ulcer (Fig. 11).

Violation of the epidermis integrity causes additional activation of the inflammatory cells that are known to exert a stimulating effect on the fibroblasts through fibrogenic cytokines, resulting in exacerbated fibrotic changes (Fig. 12).

This self-sustaining process can cause prolonged delay (for years) of the transition of the scar to the next stage of scar formation, leading to the rapid development of severe contractures.

Analyses of the clinical and histological pattern indicate a significant deterioration of the circulatory conditions in the scar tissue because of the compression of the dermal vascular network. This phenomenon should be considered when performing reconstructive surgeries using scar tissues. As clinical evidence shows, intraoperative disruption of the links between the dermal vascular plexus and the deep vessels often leads to the death of a $^1/_2$ flap or the vertices of the displaced flaps, even when the intact fascia is included in the scar flap (Fig. 13).

Figure 14 shows the morphological changes before and after the surgical intervention. Prior to the surgery, histological sections revealed dermal vessels compressed by the connective tissue fibers, coming from the depth to the surface of the skin, represented on the cut with long strands of endothelial lining, stained with blue (Fig. 14, *a*). On the 4th day after the surgery, while studying the microscopic picture, an abundant network of enlarged, full blood vessels and microbleeding (Fig. 14, *b*) were detected.

Later, necrosis of the flap developed (Fig. 15).

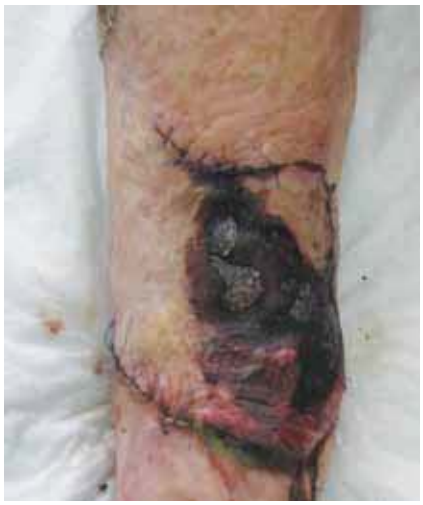


**Fig. 15.** Necrosis of the displaced adipo-dermal flap in the popliteal region: the formation of a dry crust. Day 6 after the surgery

The inconsistency of the vasculature of the scar dermis, believed to be caused by the partial destruction of the network of the intradermal vascular anastomoses with excess collagen, increases the significance of the subcutaneous vessels for the maintenance of the viability of the scar. The obtained clinical and morphological picture indicates irreversible change in the vascular architectonics in the scar tissue and indicates the risk of using it for grafting purposes.

## Discussion

The morphological analyses performed in the dynamics indicates that during the formation of the hypertrophic scar, the circulatory conditions gradually deteriorate; these changes include excessive and intensive collagen synthesis leading to an irreversible change in the vascular architectonics and a reduction in the number of vessels as well as the area of the total vascular lumen owing to their gradual compression and obliteration. The increased perfusion recorded using laser Doppler fluorometry may be associated to a significant enlargement of the scar vessels with deteriorated trophicity [1, 2, 4, 5, 7, 8]. Deterioration of the trophicity in the scar tissue is indicated not only by a significant increase in the lactate content, but also by postoperative complications [11].

## Conclusions

1. Increase in the vascular cross sectional area during the early stages of scar formation is not attributable to increased number of vessels, but to the expansion of the vessel lumen. With scar maturation, the number of vessels in the scar tissue and the total area of their lumen are reduced. In mature scars, the number of vessels is reduced by more than 3 times than that in the intact skin.

2. Hyperemia of the hypertrophic scar is caused by the abrupt enlargement of the vessels of the scar tissue, not by a rise in the number of vessels or by the intensification of perfusion.

3. It is advisable to avoid the use of hypertrophic scar tissue for grafting in the form of mobilized cutaneous, adipo-dermal, and fasciocutaneous flaps because of the high risk of trophic complications caused by a reduced trophic status of the scar tissues.

## Information on funding and conflict of interest

This work was performed on the basis and with support of the Turner Scientific and Research Institute for Children's Orthopedics under the Ministry of Health of Russia.

The authors declare no obvious and potential conflicts of interest related to the publication of this article.

## References

1. Page RE, Robertson GA, Pettigrew NM. Microcirculation in hypertrophic burn scars. *Burns Incl Therm Inj*. 1983;Sep;10(1):64-70. doi: 10.1016/0305-4179(83)90130-4.
2. Gangemi EN, Carnino R, Stella M. Videocapillaroscopy in postburn scars: *in vivo* analysis of the microcirculation. *Burns*. 2010Sep;36(6):799-805. doi: 10.1016/j.burns.2010.02.002.
3. Hosoda G, Holloway GA, Heimbach DM. Laser Doppler flowmetry for the early detection of hypertrophic burn scars. *J Burn Care Rehabil*. 1986Nov-Dec;7(6):496-7. doi: 10.1097/00004630-198611000-00010.
4. Leung KS, Sher A, Clark JA, et al. Microcirculation in hypertrophic scars after burn injury. *J Burn Care Rehabil*. 1989Sep-Oct;10(5):436-44. doi: 10.1097/00004630-198909000-00013.
5. Ehrlich HP, Kelley SF. Hypertrophic scar: an interruption in the remodeling of repair a laser Doppler blood flow study. *Plast Reconstr Surg*. 1992Dec;90(6):993-8. doi: 10.1097/00006534-199212000-00009.
6. Bray R, Forrester K, Leonard C, et al. Laser Doppler imaging of burn scars: a comparison of wavelength and scanning methods. *Burns*. 2003May;29(3):199-206. doi: 10.1016/s0305-4179(02)00307-8.
7. Fourman MS, McKenna P, Phillips BT, et al. ICG angiography predicts burn scarring within 48 h of injury in a porcine vertical progression burn model. *Burns*. 2015Aug;41(5):1043-8. doi: 10.1016/j.burns.2014.11.001.
8. Stewart CJ, Gallant-Behm CL, Forrester K, et al. Kinetics of blood flow during healing of excisional full-thickness skin wounds in pigs as monitored by laser speckle perfusion imaging. *Skin Res Technol*. 2006Nov;12(4):247-53. doi: 10.1111/j.0909-752x.2006.00157.x.
9. Liu Q, Wang X, Jia Y, et al. Increased blood flow in keloids and adjacent skin revealed by laser speckle contrast imaging. *Lasers Surg Med*. 2016Apr;48(4):360-4. doi: 10.1002/lsm.22470.
10. Ueda K, Yasuda Y, Furuya E, Oba S. Inadequate blood supply persists in keloids. *Scand J Plast Reconstr Surg Hand Surg*. 2004;38(5):267-71. doi: 10.1080/02844310410029552.
11. Kumar I, Staton CA, Cross SS, et al. Angiogenesis, vascular endothelial growth factor and its receptors in human surgical wounds. *Br J Surg*. 2009Dec;96(12):1484-91. doi: 10.1002/bjs.6778.

*Information about the authors*

**Olga V. Filippova** — MD, PhD, professor, leading researcher of the department of trauma effects and rheumatoid arthritis. The Turner Scientific Research Institute for Children's Orthopedics. E-mail: OlgaFil-@mail.ru.

**Ivan N. Krasnogorskiy** — MD, PhD, senior research associate histologist of the scientific and morphological laboratory. The Turner Scientific Research Institute for Children's Orthopedics. E-mail: krasnogorsky@yandex.ru.

**Konstantin A. Afonichev** — MD, PhD, professor, head of the department of trauma effects and rheumatoid arthritis. The Turner Scientific Research Institute for Children's Orthopedics. E-mail: afonichev@list.ru.

**Rostislav V. Vashetko** — MD, PhD, professor, head of Department of Pathological Anatomy. Saint Petersburg I.I. Dzhanelidze Research Institute of Emergency Medicine.

**Ольга Васильевна Филиппова** — д-р мед. наук, ведущий научный сотрудник. ФГБУ «НИДОИ им. Г.И. Турнера» Минздрава России. E-mail: OlgaFil-@mail.ru.

**Иван Николаевич Красногорский** — канд. мед. наук, старший научный сотрудник-гистолог научно-морфологической лаборатории ФГБУ «НИДОИ им. Г.И. Турнера» Минздрава России. E-mail: krasnogorsky@yandex.ru.

**Константин Александрович Афоничев** — д-р мед. наук, руководитель отделения последствий травмы и ревматоидного артрита. ФГБУ «НИДОИ им. Г.И. Турнера» Минздрава России. E-mail: afonichev@list.ru.

**Ростислав Вадимович Вашетко** — д-р мед. наук, руководитель отделения патологической анатомии, ГБУ СПб НИИ СП им. И.И. Джанелидзе.