

Soft tissues regional blood flow and microvasculature upon different perioperative treatment strategies following indirect revascularization in patients with critical lower limb ischemia, caused by occlusion of distal arteries

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This work was aimed at studying the effect of different perioperative treatment strategies following indirect revascularization on regional blood flow and microcirculatory network of soft tissues in patients with critical lower limb ischemia. The study was conducted in 210 patients (154 men, 56 women) with critical lower limb ischemia aged from 28 to 74 years, who were receiving hospital treatment during 2001-2019, with the ischemia duration of two-months-up-to-four-years. The cause of critical ischemia was the occlusion of the arteries of the femoral-popliteal-tibia and tibia-foot segments of the atherosclerotic and thromboangiogenic genesis. The regional blood flow was examined through non-invasive instrumental techniques (the determination of oxygen saturation of skin, rheovasography, Doppler ultrasonography, ultrasound duplex scanning). The state of the microvasculature and neoangiogenesis was evaluated by complex morphological and immunohistochemical studies of tru-cut biopsy specimens of ischemic limb soft tissues in 80 patients (55 men, 25 women). Non-parametric tests were used to analyze digital data. It was found that in contrast to controls, the combined administration of intravenous laser blood irradiation and cytokine therapy with 'Roncoleukin' in the perioperative period of revascularizing osteotriphalangectomy with intraosseous laser irradiation accelerates neoangiogenesis and increases the number of functionally active microvessels with open lumen in the soft tissues of ischemic limb. The statistically significant improvement in regional blood flow and skin oxygen saturation was observed after indirect revascularization as well. The combination of intravenous laser blood irradiation and cytokine therapy in the perioperative period of indirect revascularization by osteotriphalangectomy with bone marrow laser irradiation creates favorable conditions for increasing regional blood flow and promoting neoangiogenesis to improve microvasculature in critically ischemic lower limb.

Keywords: Occlusion of arteries, critical ischemia, indirect revascularization, laser irradiation, cytokine therapy, regional hemodynamics, microvasculature

INTRODUCTION

The treatment and prognosis of the critical lower limb ischemia (CLLI), caused by the occlusion of their distal arteries have not lost their relevance. Reconstructive surgery of the arteries of lower extremities in CLLI is the main treatment strategy for this severe category of patients; however, in 5.8-16.5% of cases there are adverse outcomes, and in 8.8-20.4% – major amputations

(Бокерия и соавт., 2011; Буров и соавт., 2019; Затевахин и соавт., 2011; Казаков и Жук, 2019; Покровский и соавт., 2018; Нац. рекомендации, 2019; Biancari et al., 2007). X-ray endovascular surgery does not always provide a complete revascularization of the limb (Затевахин и соавт., 2011; Казаков и Жук, 2019; Ховалкин и соавт., 2019; Bosh et al., 2007; Clair et al., 2005). Conservative therapy as an alternative to disabling surgery (disabling sur-

gical intervention) is also ineffective (Нац. Рекомендации, 2019). In the surgical treatment of patients with CLLI, a separate group is made up of indirect methods of revascularization which includes revascularizing osteotriphalangectomy (ROT), lumbar sympathectomy (LSC) in combination with other surgical interventions and various modifications of perioperative complex therapy (Гасанов и Косаев, 2019; Зусманович, 1999; Косаев, 2011; 2012а; 2012б; Кротовский и Зудин, 2005; Суховатых и Орлова, 2013; Aslyayev et al., 2016). The state of the microvasculature of the affected soft tissues of the lower extremities of patients with CLLI under different schemes of perioperative treatment with indirect revascularization remains poorly understood, especially from the standpoint of modern morphology and non-invasive instrumental diagnostics (Nowak-Sliwinska et al., 2018). Meanwhile, an objective assessment of the morphological and functional state of micro-vasculature would not only optimize perioperative therapy in indirect revascularization, but also improve the prognosis in patients with CLLI.

OBJECTIVES

The purpose of this research is a comprehensive non-invasive-instrumental and morphological study of regional blood flow and microvasculature of soft tissues under various perioperative treatment regimens following indirect revascularization in patients with critical lower limb ischemia (CLLI) caused by the occlusion of their distal arteries.

MATERIALS AND METHODS

A permission to conduct the study was obtained from the Ethics Committee of the Scientific Center of Surgery named after Acad. M.A. Topchubashev. The study contingent was composed of 80 patients who were familiarized with all aspects of the upcoming treatment before it began and gave written consent to carry it out.

Twenty-eight-to-seventy-four years old patients of both sexes (55 men and 25 women) suffering from critical ischemia from 2 months up to 4 years were hospitalized in the Department of Vas-

cular Surgery of the Scientific Center of Surgery named after Acad. M.A. Topchubashev in the period from 2001 to 2019. The reason for the development of critical ischemia was the unreconstructable occlusion of femoral-popliteal-tibial and tibial-foot segments of arteries, which was of atherosclerotic and thromboangiolytic origins. As concomitant diseases, there were arterial hypertension, coronary heart disease, chronic cerebrovascular disease, chronic obstructive bronchopulmonary diseases, as well as pyloroduodenal erosive lesion and renal failure.

In order to establish a diagnosis of critical lower limb ischemia and evaluate the effectiveness of the proposed method, clinical and instrumental studies were carried out: the determination of oxygen saturation of skin, rheovasography, Doppler ultrasonography, ultrasound duplex scanning, and multi-spiral computed tomography angiography. Using instrumental methods of research, we determined such indicators of arterial and venous blood flow as the rheographic index (RI), linear velocity of blood (LVC) in the popliteal artery, gradient of regional systolic pressure (GRSP), gradient of post-occlusive venous pressure (GPOVP), venous arterial index (VAI), which was measured in the standing and supine positions. Oxygen saturation of skin in the distal part of the foot was also studied.

In the perioperative period, 34 patients received standard treatment, 32 patients - standard treatment plus intravenous laser blood irradiation, 32 patients - standard treatment plus cytokine therapy with 'Roncoleukin', 33 patients - standard treatment plus intravenous laser irradiation of blood along with the cytokine therapy with the 'Roncoleukin', 31 patients - in the ROT with intraosseous laser irradiation - standard treatment plus intravenous laser blood irradiation along with the cytokine therapy with «Roncoleukin».

The state of regional arterial and venous blood flow in patients was studied upon their admission to the clinic and upon the completion of treatment. Some of the parameters of regional arterial and venous blood circulation were compared to the identical indicators of 48 healthy individuals (the "reference group").

The microvasculature of ischemic soft tissues of the lower extremities was studied in a total of 80 patients (55 men, 25 women). It was performed by non-traumatic tru-cut biopsy of soft tissue

es of the foot (skin, subcutaneous tissue, fascio-aponeural layer and muscles), lower leg and thigh, along with general histological (hematoxylin-eosin, picrofuxin) and immunohistochemical analyzes (VEGF, CD31, CD34, collagen of IV type and Ki67; “Roche Diagnostics”) according to the standard procedure (Dey, 2018).

To determine the possible correlations of the studied parameters with various schemes of perioperative treatment, the obtained clinical and instrumental data was processed by using non-parametric tests with the calculation of the Pearson's chi-squared test (χ^2) and correlation coefficient (r) at a confidence level of $P = 0.95$ (Юнкеров В.И. и соавт., 2011).

RESULTS AND DISCUSSION

Regional arterial and venous blood flow was characterized by controversial changes. Upon admission to the clinic, patients had severe blood flow disturbances, which were also accompanied by a significant suppression of the level oxygen saturation of skin. Revascularizing osteotrepation, in general, had a positive effect on the studied parameters of regional blood flow, which was confirmed by an increase in the number of patients with positive clinical dynamics. Moreover, the best results were observed for operations of revascularizing osteotrepation with simultaneous laser irradiation of bone marrow in the postoperative period during 7-8 days. It is pertinent to mention that upon completion of treatment, 25-27 out of 31 patients showed a significant improvement in almost all the considered indicators of regional blood flow in the critically ischemic limb. Of particular note are the rheographic index and the gradient of regional systolic pressure, the improvement of which was recorded in 87.1% of patients (Table 1).

In parallel with instrumental non-invasive examination of regional blood circulation, neoangiogenesis and the density of microvasculature in the small soft tissue biopsy specimens of critically ischemic limb were also comprehensively studied based on a combination of a number of morpholo-

gical parameters. In particular, neoangiogenesis analysis revealed that after the completion of complex treatment, 40 patients out of the examined 80 (50.0%) showed an acceleration of the formation of new microvessels in previously necrotic tissues, especially in the endomysium of muscles and fascial-adipose tissue. The maximum stimulation of this process was inherent to the subgroup of patients who received intraosseous and intravenous laser irradiation in combination with cytokine therapy in the perioperative period (14 patients with an increase of neoangiogenesis; 77.8%; Table 2).

The above-mentioned data allowed us to suggest that laser stimulation of the bone marrow and venous lining in combination with immunomodulatory therapy in the perioperative period can promote the formation of microvessels, which is consonant with modern understanding of angiogenesis (Nowak-Sliwinska et al., 2018).

An analysis of the microvasculature density indices upon completion of treatment of patients revealed a similar pattern. Thus, the total density of various microvessels (arterioles, precapillaries, hemocapillaries and postcapillary venules) tended to increase in 34 patients out of 80 examined (42.5%). As in the case of neoangiogenesis, the maximum increase in the average number of microvessels was detected in patients with intraosseous and intravenous laser irradiation in combination with cytokine therapy in the perioperative period (11 patients; 61.1%; Table 3).

It should be noted that the morphologically verified microvessels of a specific necrobiotic-altered (but not necrotic) tissue microzone were roughly divided into two types: functional (with preserved structure and lumen) and non-functional (without a clearly structured wall and lumen less than $4.1 \mu\text{m}$). Based on the results, the proportion of functional microvessels during perioperative therapy accompanying the revascularizing osteotrepation with bone marrow laser irradiation was higher in those patients who underwent complex perioperative treatment with intravenous laser irradiation in combination with cytokine therapy.

Table 1. The indicators of regional blood flow and oxygen saturation of skin under the usage of various perioperative therapy strategies for indirect revascularization in patients with critical ischemia of the lower extremities of distal arterial occlusive origin (number of patients, χ^2 ; p; r *)

| Indicators | Study groups | Cont. group n=48 | ROT operation n=42 | | LST operation n=51 | | ROT operation + LST n=38 | | ROT operation with BMLI n=31 | |
|--------------|--------------|------------------|--------------------|-----------------------------------|--------------------|------------------------------------|--------------------------|-------------------------------------|------------------------------|-------------------------------------|
| | | | | | | | | | | |
| VAI lying | decreased | 18 | 25 | $\chi^2=4,355$ p<0,05 r=0,3 | 32 | $\chi^2=6,304$ p<0,05 r=0,4 | 28 | $\chi^2=11,162$ p<0,001 r=0,5 | 26 | $\chi^2=16,414$ p<0,001 r=0,6 |
| | not changed | 30 | 17 | | 19 | | 10 | | 5 | |
| VAI standing | decreased | 17 | 25 | $\chi^2=5,230$ p<0,05 r=0,3 | 34 | $\chi^2=9,668$ p<0,01 r=0,4 | 28 | $\chi^2=12,451$ p<0,001 r=0,5 | 25 | $\chi^2=15,474$ p<0,001 r=0,6 |
| | not changed | 31 | 17 | | 17 | | 10 | | 6 | |
| GRSP | decreased | 21 | 28 | $\chi^2=4,473$ p<0,05 r=0,3 | 33 | $\chi^2=4,380$ p<0,05 r=0,3 | 27 | $\chi^2=6,411$ p<0,05 r=0,4 | 27 | $\chi^2=14,844$ p<0,001 r=0,6 |
| | not changed | 27 | 14 | | 18 | | 11 | | 4 | |
| GPOVP | decreased | 19 | 26 | $\chi^2=4,464$ p<0,05 r=0,3 | 31 | $\chi^2=4,446$ p<0,05 r=0,3 | 29 | $\chi^2=11,604$ p<0,001 r=0,5 | 26 | $\chi^2=15,070$ p<0,001 r=0,6 |
| | not changed | 29 | 16 | | 20 | | 9 | | 5 | |
| RI | increased | 20 | 27 | $\chi^2=4,593$ p<0,05 r=0,3 | 36 | $\chi^2=8,418$ p<0,05 r=0,4 | 28 | $\chi^2=8,816$ p<0,01 r=0,4 | 27 | $\chi^2=16,131$ p<0,001 r=0,6 |
| | not changed | 28 | 15 | | 15 | | 10 | | 4 | |
| LVC | increased | 18 | 26 | $\chi^2=5,339$ p<0,05 r=0,3 | 32 | $\chi^2=6,304$ p<0,001 r=0,4 | 28 | $\chi^2=11,162$ p<0,001 r=0,5 | 26 | $\chi^2=16,414$ p<0,001 r=0,6 |
| | not changed | 30 | 16 | | 19 | | 10 | | 5 | |
| OSS | increased | 18 | 25 | $\chi^2=4,355$ p<0,05 r=0,3 | 33 | $\chi^2=7,328$ p<0,01 r=0,4 | 29 | $\chi^2=12,894$ p<0,001 r=0,5 | 26 | $\chi^2=16,414$ p<0,001 r=0,6 |
| | not changed | 30 | 17 | | 18 | | 9 | | 5 | |

Note: ROT - revascularizing osteotrepation; LST - lumbar sympathectomy; ROT + LST - revascularizing osteotrepation + lumbar sympathectomy; ROT with BMLI - revascularizing osteotrepation with bone marrow laser irradiation; VAI - venous arterial index; GRSP - gradient of regional systolic pressure, GPOVP - gradient of postocclusal venous pressure; RI - reographic index; LVC - linear blood velocity; OSS - oxygen saturation of skin;

*- χ^2 , p, r were calculated between the corresponding indicators of the control and individual surgical groups of patients

Table 2. The intensity of neoangiogenesis under the conditions of applying various perioperative therapy regimens for revascularizing osteotrepation in patients with critical lower limb ischemia of distal arterial occlusive origin (at the end of treatment; number of patients, χ^2 ; p; r*)

| Group of Patients | Indicator | The intensity of neoangiogenesis | χ^2 p r |
|---|---------------|----------------------------------|-----------------------------------|
| Comparison (control; n=15) | increased (↑) | 5 | |
| | not changed | 10 | |
| Intravenous laser irradiation (n=15) | increased (↑) | 9 | $\chi^2=2,143$ p>0,05 r=0,4 |
| | not changed | 6 | |
| Cytokine therapy with 'Roncoleukin' (n=15) | increased (↑) | 8 | $\chi^2=1,222$ p>0,05 r=0,3 |
| | not changed | 7 | |
| Intravenous laser irradiation + cytokine therapy with 'Roncoleukin' (n=17) | increased (↑) | 11 | $\chi^2=3,137$ p>0,05 r=0,4 |
| | not changed | 6 | |
| Bone marrow laser irradiation + intravenous laser irradiation + cytokine therapy with 'Roncoleukin' n=18) | increased (↑) | 14 | $\chi^2=6,617$ p<0,05 r=0,6 |
| | not changed | 4 | |

Note: * - χ^2 , p, r - were calculated between the corresponding indicators of the examined groups of patients

Table 3. The density of microvessels under the conditions of applying various schemes of perioperative therapy for indirect surgical revascularization in patients with critical ischemia of the lower extremities of distal arterial occlusive origin (at the end of treatment; number of patients, χ^2 ; p; r *)

| Group of patients | Indicator | Density of microvessels | χ^2 p r |
|--|---------------|-------------------------|-----------------------------------|
| Comparison (control; n=15) | increased (↑) | 4 | |
| | not changed | 11 | |
| Intravenous Laser Irradiation (n=15) | increased (↑) | 8 | $\chi^2=2,222$ p>0,05 r=0,4 |
| | not changed | 7 | |
| Cytokine Therapy with 'Roncoleukinum' (n=15) | increased (↑) | 7 | $\chi^2=1,292$ p>0,05 r=0,3 |
| | not changed | 8 | |
| Intravenous laser irradiation + cytokine therapy with 'Roncoleukinum' (n=17) | increased (↑) | 10 | $\chi^2=3,348$ p>0,05 r=0,4 |
| | not changed | 7 | |
| Bone marrow laser irradiation + intravenous laser irradiation + cytokine therapy with 'Roncoleukinum' (n=18) | increased (↑) | 11 | $\chi^2=3,915$ p<0,05 r=0,5 |
| | not changed | 7 | |

Note: * - χ^2 , p, r - were calculated between the corresponding indicators of the examined groups of patients

CONCLUSIONS

1. The combination of generally accepted conservative treatment regimens (protocols) with bone marrow and intravenous laser irradiation in the perioperative period during revascularizing osteotriphication operations contributes to the partial repair of the microvasculature, as evidenced by the results of the instrumental and morphological examinations of the regional blood circulation and microcirculation in patients with critical low limb ischemia caused by distal arterial occlusion.
2. Intravenous laser irradiation in combination with cytokine therapy in the perioperative period of revascularizing osteotriphication with bone marrow laser irradiation produced comparatively better long-term outcomes in correction of the regional blood flow and neoangiogenesis in patients with critical lower limb ischemia caused by distal arterial occlusion.

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Aşağı ətrafların arteriyalarının distal okklüziyaları mənşəli kritik işemiyası olan xəstələrdə dolayı revaskulyarizasiya zamanı perioperasion terapiyanın müxtəlif sxemlərində regionar qan axınının və yumşaq toxumaların mikrosirkulyasiya şəbəkəsinin vəziyyəti

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Tədqiqatın məqsədi arteriyaların distal steno-okklüziyası fonunda aşağı ətrafların kritik işemiyası olan xəstələrdə regionar qan axınının və yumşaq toxumaların mikrosirkulyasiya şəbəkəsinin vəziyyətini dolayı revaskulyarizasiya əməliyyatları zamanı perioperasion dövrdəki müxtəlif müalicə sxemləri şəraitində öyrənməkdən ibarət olmuşdur. Tədqiqat kontingenti 2002-2019-cu illərdə müalicə almış 2 ay – 4 il müddətində kritik işemiyalı 28-74 yaşlı 210 xəstədən ibarətdir (154 kişi və 56 qadın). Kritik işemiyanın bilavasitə səbəbi - bud-dizaltı-baldır və baldır-ayaq pəncəsi seqmentləri arteriyalarının aterosklerozla və trombangitlə şərtlənmiş okklüziyasıdır. Regionar arterial və venoz qan axını göstəriciləri, dərinin oksigenlə saturasiyası qeyri-invaziv instrumental ((reovazoqrafiya, ultrasəs dopplerografiya, ultrasəs duplex sonografiya və s.), 80 xəstədə (55 kişi, 25 qadın) neoangiogenezi və mikrosirkulyasiya şəbəkəsinin vəziyyəti isə - kritik işemiyalı aşağı ətrafların yumşaq toxumaları tru-cut biopsiyalarının kompleks morfoloji-immunohistokimyəvi analizləri ilə öyrənilmişdir. Kəmiyyət göstəriciləri qeyri-parametrik statistika üsulları ilə təhlil edilmişdir. Müəyyən edilmişdir ki, sümükiliyinin lazer şüalanması ilə birlikdə aparılan revaskulyarizasiyaedici osteotropanasiya əməliyyatı zamanı perioperasion dövrdə lazerlə venadaxili şüalandırma və sitokinoterapiya aşağı ətrafların kritik işemiyaya məruz qalmış yumşaq toxumalarında neoangiogenezi sürətləndirir, mənfəzləri sərbəst (funksional-fəal) mikrodamarların sıxlığını artırır, arterial və venoz qan axınlarının əsas göstəricilərini, həmçinin dərinin oksigenlə saturasiyası səviyyəsini, müqayisə qrupu ilə nisbətə, əhəmiyyətli dərəcədə - bəzən isə statistik-etibarlı xarakterdə - yaxşılaşdırır. Qeyd edilən prose-

durların birlikdə aparılması əməliyyatdan və müalicədən sonrakı bir necə illik dövrdə də xəstələrin klinik vəziyyətinin və öyrənilmiş göstəricilərin nisbi-qənaətbəxşliyinə şərait yaradır.

Açar sözlər: *Arteriyaların okklüziyası, kritik işemiya, dolayı revaskulyarizasiya, lazer şüalanması, sitokinoterapiya, regionar hemodinamika, mikrosirkulyasiya*

Состояние регионарного кровотока и микроциркуляторного русла мягких тканей при различных схемах периоперационной терапии при непрямой реваскуляризации у больных с критической ишемией, вызванной окклюзией дистальных артерий нижних конечностей

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Целью настоящего исследования являлось изучение состояния регионарного кровотока и микроциркуляторной сети мягких тканей у больных критической ишемией на фоне дистальной окклюзии артерий нижних конечностей в зависимости от лечебной тактики в периоперационном периоде при непрямой реваскуляризации. Исследование проводилось у 210 больных (154 мужчин, 56 женщин) с критической ишемией нижних конечностей в возрасте от 28 до 74 лет, находящихся в стационарном лечении в 2001-2019 гг., продолжительностью ишемии в течении от 2-х мес. до 4-х лет. Причиной критической ишемии явилась окклюзия артерий бедренно-подколенно-берцового и берцово-стопного сегментов атеросклеротического и тромбангитического генеза. Неинвазивными инструментальными исследованиями (реовазография, ультразвуковая доплерография, ультразвуковое дуплексное сканирование и др.) были изучены показатели регионарного кровотока. В tru-cut биоптатах мягких тканей ишемизированной конечности у 80 больных (55 мужчин, 25 женщин) комплексными морфологическими и иммуногистохимическими исследованиями же было изучено состояние микроциркуляторного русла и неоангиогенеза. Цифровые показатели были обработаны статистическими методами для непараметрических критериев. Выявлено, что применение внутривенного лазерного облучения крови и цитокинотерапии ронколейкином в периоперационном периоде при реваскуляризирующей остеотрепанации с внутрикостномозговым лазерным облучением, по сравнению с контрольной группой, ускоряет неоангиогенез, увеличивает число функционально-активных микрососудов со свободным просветом в мягких тканях ишемизированной конечности. В сравнении с контрольной группой, непрямые методы реваскуляризации статистически достоверно улучшают показатели регионарного кровотока и сатурацию кожи кислородом. Сочетанное применение внутривенного лазерного облучения крови и цитокинотерапии в периоперационном периоде при непрямой реваскуляризации остеотрепанацией с костномозговым лазерным облучением создает благоприятные условия для стимуляции регионарного кровотока и активации неоангиогенеза с улучшением микроциркуляторного русла в критически-ишемизированных нижних конечностях.

Ключевые слова: *Окклюзия артерий, критическая ишемия, непрямая реваскуляризация, лазерное облучение, цитокинотерапия, регионарная гемодинамика, микроциркуляторное русло*