Retrospective analysis of hand and foot function after application of cellular technologies in patients with post–traumatic ischemic and denervation–reinervation injuries of limb muscles

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Abstract

Objective. To determine the effect of cell therapy on the function of the hand and foot in patients with ischemic and denervation–reinervation injuries of the limb muscles.

Materials and methods. Over 9 years (2014 – early 2023), 47 patients with posttraumatic ischaemia and/or peripheral nerve damage were treated at the Institute of Traumatology and Orthopedics. Cell therapy, namely the injection of bone marrow aspirate and adipose tissue suspension (cell substrates) into the target muscles, was used in 32 (68.1%) patients (main group), while 15 (31.9%) patients did not undergo cell therapy (control group). In 16 (50%) patients, cell therapy was applied to the upper limb, and in 16 (50%) – to the lower limb. Patients in the control and intervention groups were divided into three subgroups: with ischemic contracture, peripheral nerve damage, and a combination of these pathologies.

Results. The treatment with the use of cellular technology in patients with ischemic and denervation–reinervation injuries of the muscles of the hand and foot had a statistically significant positive effect. In patients with ischemic contracture of the hand 12 months after the introduction of cell substrates, the loss of its function decreased to less than 30%.

Conclusions. Cell therapy helps to restore hand and foot function in patients with ischemic and/or denervation–reinervation injury of the limb muscles.

Keywords: cellular technology; ischemic injury; denervation–reinervation injury; hand function; foot function.

Over the 9 years of military operations in Ukraine, the number of patients with limb injuries has increased, leading to an increase in the frequency of ischemic and/or denervation–reinervation muscle damage. Every fifth patient treated at our medical facility, or more than 70%, has hand or foot dysfunction due to compartment syndrome and peripheral nerve damage. While the provision of primary care at the first stages of evacuation of the wounded is well established (stabilisation of bone fractures, fasciotomy, vacuum therapy), further treatment of ischemic contracture and/or peripheral nerve damage is not without gaps. The issue of treating patients in the reactive–restorative period of ischemic contracture, i.e. during the formation of muscle necrosis, is particularly acute. According to our research and the scientific literature, it is impossible to perform any reconstructive and restorative interventions during this period, except for osteosynthesis and nerve surgery, so the use of cellular technology is very important in terms of creating conditions to reduce the prevalence of necrosis in the muscles. The same causes a delay in the recovery of limb nerves, which not only negatively affects denervation and re–innervation processes in the muscles, but also leads to direct fibrosis of the muscle fibre.

The aim of the study was to determine the effect of cell therapy on the function of the hand and foot in patients with ischemic and denervation–reinervation injuries of the limb muscles [1–20].

Materials and methods of the study

A study was conducted that corresponded to a case–control study in design and the results of which allowed us to determine the effect of the use of cellular technology on the restoration of hand and/or foot function in patients with ischemic contracture, peripheral nerve damage and a combination of these pathologies.

Patient inclusion criteria: the presence of an injury with ischemic contracture of the hand or foot during the reactive recovery period with or without anatomical damage to the peripheral nerves.

Exclusion criteria: ischemic lesions that had undergone previous surgical treatment in another medical facility; severe somatic pathology, such as diabetes mellitus; children and age over 75 years.

Over the course of 9 years (2014 – early 2023), 47 patients with post–traumatic ischaemia and/or peripheral nerve damage were treated at the Institute of Traumatology and Orthopedics of the National Academy of Medical Sciences of Ukraine. Cell therapy, namely the injection of bone marrow aspirate and adipose tissue suspension (cell substrates) into the target muscles, was used in 32 (68.1%) patients (main group), and 15 (31.9%) patients did not undergo cell therapy (control group). Cell therapy was used in 16 (50%) patients on the upper and 16 (50%) on the lower limb. Patients of both study groups were divided into three clinical subgroups: Group 1...
(patients with ischemic contracture), Group 2 (patients with peripheral nerve damage) and Group 3 (patients with a combination of these pathologies).

In the main group (Table 1), 12 (37.5%) patients with ischemic contracture in the reactive-recovery period (subgroup 1) were treated within 3 to 4 months after compartment syndrome; 16 (50%) patients with peripheral nerve damage (2nd subgroup) – within 4 to 6 months after the injury; 4 (12.5%) patients with ischemic contracture in the reactive recovery period and peripheral nerve damage (3rd subgroup) – within 6 months after the injury. The peripheral nerve damage in all patients was complete (axonotmesis) and required restoration of their integrity by performing autoneuroplasty or suturing.

The location of the lesion was determined in each clinical subgroup. Most often, ischemic contracture developed in patients with compartment syndrome as a result of gunshot wounds. Among the 16 patients in the main group with peripheral nerve damage, 13 (81.3%) had gunshot wounds and 2 (12.5%) had cut wounds.

The location of the lesion was determined in each clinical subgroup. Most often, ischemia was detected in the affected volar bone and fascial case of the forearm (25%) and the deep posterior case of the lower leg (25%). In the main group, the most commonly diagnosed lesions were the median nerve in the upper extremity – 5 (31.3%) observations and the tibial nerve in the lower extremity – 3 (18.8%) observations.

In 1 patient of the main group with combined ischemic and denervation damage, radial nerve damage at the level of the shoulder and ischemic damage to the dorsal and palmar bone and fascial cases of the forearm were diagnosed on the upper limb, and combined damage to the peroneal nerve and lateral and posterior deep cases on the lower limb was diagnosed on the lower limb. In 1 patient with sciatic nerve injury, all the cases of the lower leg were affected by ischemia.

Thus, determination of the topography of ischemic lesions and/or peripheral nerve damage allowed us to identify target muscles for injection of cell substrates in patients of the main group. The cell substrates were injected into the tenor muscles, ulnar flexor carpi ulnaris and ulnar extensor carpi ulnaris muscles on the upper limb and into the tibialis anterior, gastrocnemius, longus fibularis and posterior tibialis muscles on the lower limb. The most frequently targeted muscles in the 2nd subgroup were the tenar muscles – 5 (15.6%) observations, in the 1st subgroup – the ulnar extensor of the wrist – 3 (9.4%) observations, in the 3rd subgroup – the anterior tibialis muscle – 2 (6.3%) observations.

In addition, in the 2nd and 3rd subgroups, patients underwent peripheral nerve repair by neurolysis, suturing or plastic surgery. Of the 20 patients in the 2nd and 3rd subgroups of the main group, 5 (25%) did not require restoration of peripheral nerve integrity, 8 (40%) underwent suturing, and 7 (35%) underwent autoneuroplasty.

In patients undergoing nerve repair, the relevant areas were covered with a mixture of adipose tissue suspension and bone marrow aspirate.

We would like to draw attention to the fact that the assessment of hand function in the main group was difficult, especially in patients of the 1st and 3rd clinical subgroups. The reason was that according to our and literature data, it is impossible to determine the severity of ischemic contracture in the reactive recovery period, and the final severity of ischemic damage can be established only in the residual period. Therefore, we conducted a retrospective comparative analysis of the hand function of 5 (15.6%) patients of the 1st subgroup at 12 months (i.e., when they were in the residual period) and 5 identical patients with ischemic hand contracture who had previously been treated without the use of cellular technology.

All time intervals were counted from the day of introduction of cell substrates (term 0).

In 10 (31.3%) patients of the 2nd clinical subgroup, hand function was assessed at 3, 6 and 12 months after surgery, during which cell substrates were injected into the target muscles.

In Subgroup 3, 1 patient (3.1%) with radial nerve damage in the middle third of the shoulder and ischemic contracture underwent a hand function assessment similar to that in Clinical Subgroup 1 one year after surgery.

Hand function was assessed by the AOOS upper extremity function assessment system in the modification of I. M. Kurinny (1996) [21].

The system for assessing the function of the upper limb included the study of active and passive movements in its joints, the Moberg test, determination of sensitivity in autonomous areas of inertia of the peripheral nerves of the hand and dynamometry of power grips, as well as manual muscle testing.

It was taken into account that other muscles of the forearm in combination with the ulnar flexor carpi ulnaris are

| Table 1. Distribution of patients in the main group (n=32) into subgroups |
|---------------------------|--------------------------|--------------------------|--------------------------|
|                     | **Clinical subgroups** |                     |                     |                     |
|                     | **1st** | **2nd** | **3rd** |                     |                     |                     |
|                     | abs. | %* | abs. | %* | abs. | %* |                     |
| Upper                | 5   | 15,6 | 10   | 31,3 | 1   | 3,1 |                     |
| Lower                | 7   | 21,9 | 6    | 18,8 | 3   | 9,4 |                     |
| Note.               | * – percentage calculated from the total number of patients in the main group. |
involved in both adduction and flexion of the hand, which increased the results of manual assessment of the functioning of these muscles on the scale M0–M5, but the change in the results in patients of the main group allowed to assess the improvement of hand function in patients who received cell substrates in the ulnar flexor carpi ulnaris.

On the lower limb, the function of the tibialis anterior muscle was assessed manually in patients with peroneal and/or sciatic nerve damage. For the manual test, we chose the posterior flexion of the foot at the calcaneonavicular joint and its supination.

The obtained data were entered into the intraclinical database created in Microsoft Access. After entering all the indicators, the index of loss of function of the studied upper limb was automatically obtained in relation to the index of function of the healthy upper limb.

Even greater difficulties arose in determining the function of the foot in each clinical subgroup. Patients in the 1st clinical subgroup were in the reactive–restorative period of ischaemic contracture, so it was impossible to determine the functional loss of the lower limb by analogy with the determination of functional loss of the upper limb. We conducted a retrospective analysis of data from 7 (21.9%) patients with ischaemic contracture of the foot who received cellular substrates and data from 7 patients who had previously been treated without the use of cellular technology. To conduct a comparative analysis of the clinical material of patients with ischaemic foot contracture, we studied the loss of foot function according to the American Orthopaedic Foot & Ankle Society (AOFAS) scoring scale, according to which, with increasing severity of foot injury, both the scores of its individual parts and the total functional score decrease. The patient's condition was assessed depending on the degree of pain and the degree of preservation of the function of the ankle joint, midfoot joints, metatarsophalangeal and interphalangeal joints. For the assessment, total scores were used – the sum of the number of points that were used to evaluate all the joints in each patient. The results of the study were entered into a specially developed database in Microsoft Access.

Considering that the AOFAS foot function assessment scale is not used in patients with lower extremity peripheral nerve damage, the assessment scale by S. S. Strafun and V. V. Gayovich was used in patients of the 2nd and 3rd clinical subgroups [22].

Methods for obtaining a suspension of bone marrow aspirate cells. Using a bone trocar and a 20 ml syringe, 20 ml of bone marrow was aspirated from the pelvic wing, to which the anticoagulant dextrose citrate ADC–A (S. A. Baxter, USA/Belgium) was added. The resulting aspirate was centrifuged for 8 min in a 760 G centrifuge. 1 ml of the upper layer of plasma and leukocyte layer was aspirated from the tube. The resulting aspirate was injected into the target muscle group under sonographic control using a Simens phased array transducer with a frequency of 7.5 MHz (Fig. 1).

The stromal–vascular fraction was obtained before surgery using the following method. The anterior abdominal wall was chosen as a donor site. In the operating room, the sampling site was infiltrated with 100 ml of 0.9% sodium chloride solution, 2 ml of 10% lidocaine solution with the addition of 1 ml of 1.82 mg of epinephrine hydrochloride. Adipose tissue was obtained by microliposuction. The Microlyzer SVF Kit (T–Biotec Technology Ltd) was used. Using a syringe and a long liposuction needle, adipose tissue was harvested; then the material was placed in tubes and centrifuged at 3000 rpm for 10 minutes. The middle fraction was taken into a syringe and the material was crushed by repeatedly passing it through a 100 μm filter, and this substrate was centrifuged again. The lowest fraction, the stromal–vascular fraction, was taken using a 5.0 ml syringe. The approximate time of preparation coincided with the start of the surgical intervention to minimise the period from preparation to administration – about 20–30 minutes.

The resulting bone marrow aspirate cell suspension and adipose tissue were mixed in a ratio of 1 ml of adipose tissue
and 0.5 ml of bone marrow aspirate cell suspension, and the resulting mixture was used to cover the nerve grafting site with 0.1 ml of thrombin (Fig. 2).

**Results**

The total index of hand function assessment in patients with ischemic contracture (1st subgroup of the main and 1st subgroup of the control groups) was calculated as an integral index of discrete assessment indicators and expressed as a percentage of the index of healthy hand function assessment.

According to Table 2, the 1st subgroup of the main and 1st subgroup of the control groups had the highest number of patients – 3 (37.5%) and 2 (25%), respectively, who had a significant loss of hand function – more than 66.1%, on average (78.2 ± 12.6)% of the healthy hand function assessment. After 12 months, 4 (50%) patients of the 1st subgroup of the main group had a deficit of function of the ischaemic limb in the range of 33.1 – 66%, on average (38.4 ± 6.9)%, which in quantitative terms meant an improvement of 1.5 times more than in the control group. It was also positive that the hand function was assessed 12 months after the introduction of cell substrates, i.e. when all patients were in residual ischemic contracture and it was possible to assess the severity of ischemic damage. It turned out that all patients of the 1st subgroup of the main group in the residual period had a mild or moderate degree of ischemic contracture according to our classification.

**Assessment of foot function in patients with ischemic contracture (1st subgroup of the main group and 1st subgroup of the control group).** It is worth recalling that patients in these subgroups did not have damage to the peripheral nerves of the lower extremities. They had purely ischemic damage to the lower leg muscles, which allowed us to immediately assess the loss of foot function using the AOFAS scale.

As can be seen from Table 3, in the 1st subgroup of the main and 1st subgroup of the control groups, 6 (66.7%) patients had a loss of foot function assessed in the range of 31–60 AOFAS points, an average of (48.2 ± 11.7)% of the healthy limb function score. It is possible to state the fact of improvement of foot function in patients with ischemic contracture 12 months after the introduction of cell substrates. There were no patients with a loss of foot function assessed by the sum of points up to 30, i.e. with severe ischemic contracture of the foot, and the number of patients whose foot function was assessed at more than 61 points increased to 3 (33.3%), i.e. they had a mild degree of severity of ischemic contracture.

The total index of hand function assessment in patients with peripheral nerve damage of the upper extremity (2nd subgroup of the main and 2nd subgroup of the control groups), as in ischemic hand contracture, was calculated as an integral index of discrete assessment indicators and expressed as a percentage of the index of healthy hand function assessment.

**Fig. 3** demonstrates that all patients of the 2nd clinical subgroup of the main group improved the total index of hand function assessment. In 5 (35.7%) patients, the deficit of the function of the ischemic limb was in the range of 33.1 – 66%, on average (39.5 ± 7)%, which in quantitative terms meant an improvement of 5 times more than in the control group. The dynamics of restoration of hand function during 0–6 months remained at the same level and at 12 months in 6 (42.8%) patients remained in this range. However, in 4 (28.5%) patients, the loss of hand function decreased to less than 30%, which certainly indicates a positive effect of cell therapy on the restoration of the function of the denervated limb.

**Evaluation of foot function in patients with peripheral nerve damage of the lower extremity (2nd subgroup of the main and 2nd subgroup of the control groups).**

According to **Fig. 4**, 35.8% of patients in the 2nd subgroup of the main group showed a good result of treatment 6 months
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after the administration of cellular preparations. In 1 patient, an excellent result was obtained, i.e., the absence of trophic disorders on the foot, complete restoration of its bearing capacity. All movements in the hip and knee joints were restored, the dorsiflexion of the foot was restored to 15° and, just as importantly, active extension of the toes was restored. There were no excellent results in the control group.

Evaluation of hand function in patients with ischaemic contracture and peripheral nerve damage of the upper extremity (3rd subgroup of the main and 3rd subgroup of the control groups). Restoration of hand function was observed in a patient with a "small" ischaemic contracture and anatomical damage to the median nerve in the lower third of the forearm. Initial indicators of hand function (term 0): cylindrical hand grip of 1.4 kg; Moberg test: 5 subjects were collected with open eyes for 58 s, with closed eyes – 2 subjects for 198 s; during manual testing of the tenar muscles, their strength characteristics were equal to M0; the total index of loss of function was 84%.

After the autoplasty of the median nerve, during which the suture sites were covered with cellular preparations and injected into the tenar muscles under sonographic control, as

<table>
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<th>Clinical subgroup</th>
<th>Loss of foot function according to AOFAS (points)</th>
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<tr>
<td></td>
<td>0 term</td>
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<td></td>
<td>up to 30</td>
<td>31–60</td>
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<td>abs.</td>
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<tr>
<td>1st main group (n=7)</td>
<td>1</td>
<td>11,1</td>
</tr>
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<td>1st control group (n=2)</td>
<td>1</td>
<td>11,1</td>
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Note. * – percentage calculated from the total number of patients with ischaemic foot contracture.

![Fig. 3. Total hand function score in patients with peripheral nerve damage of the upper extremity.](image)

![Fig. 4. Total score of foot function in patients with peripheral nerve damage of the lower extremity.](image)
in the previous groups, intermediate studies of hand function were performed at 3, 6 and 9 months, which showed its gradual recovery. Already after 6 months, an improvement of these indicators was observed by 40%, and after 12 months, the total loss of function was 28%, i.e. the function of the severely injured hand improved by 56%. At the same time, in a similar patient of the 3rd subgroup of the control group, the function of the hand improved by only 32%.

Evaluation of foot function in patients with ischaemic contracture and peripheral nerve damage of the lower extremity (3rd subgroup of the main and 3rd subgroup of the control groups). In all 3 patients of the 3rd subgroup of the main group, foot function was assessed based on the general principles of functional expediency. Since active ankle joint movements were key to the restoration of limb function, most attention was paid to their restoration. Normally, passive movements in the ankle joint within 20–30° plantar flexion and 40–50° plantar flexion provide free gait without limping.

Thus, all patients in the 3rd subgroup of the main group at 0 years had no active extension of the foot in the ankle joint, the foot was in an equinus position of more than 15°. Valgus deviation of the hindfoot with the presence of trophic disorders on the loading surfaces was observed in 2 patients.

In 6 months after the restoration of the lower limb nerves and the introduction of cellular preparations, all 3 patients were diagnosed with a good result: restoration of ankle movements to a neutral position and 5° of dorsal flexion.

**Discussion**

Impaired hand and foot function in ischaemic and denervation–reinnervation injuries of the limb muscles is one of the most common pathologies in wounded military personnel and civilians in our country. The results confirm the effectiveness of cellular technology in patients with ischaemic muscle contracture and indicate the feasibility of introducing cell substrates into such muscles. Therefore, we set out to share our findings to improve the treatment tactics and quality of life of such patients.

**Conclusions**

1. In patients with ischemic hand contracture, 12 months after the introduction of cell substrates, the loss of hand function was less than 30%, which certainly confirms the positive effect of cellular technology on the restoration of the function of the ischemic hand.

2. In 35.8% of patients with peripheral nerve damage of the lower limb, a good result was obtained 6 months after the administration of cellular preparations.

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