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Original Research

Clinical Case of Peripheral Artery Disease with Post-Thrombotic Disease in a Patient with Type 2 Diabetes Mellitus

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Abstract

Introduction: Mesenchymal stem cells have the capacity of multidirectional differentiation and reduced immunogenicity; these cells are widely used in the field of regenerative medicine research. Our aim was to study the effect of stem cell transplantation on the course of the wound process in conditions of combined lesions of the arteries and veins of the lower extremities in a patient with type 2 diabetes mellitus. **Material and Methods:** The study presents Patient S., burned in 1952, with a 20-year history of type 2 diabetes. In our protocol, we used autologous mesenchymal stem cells isolated from the patient›s peripheral blood by magnetic separation using an AutoMACS automatic system (USA). **Results:** The clinical case shows that in patients with combined lesions of the arteries and deep veins and large trophic ulcers, it is advisable to use a step-by-step treatment: first step - the restoration of the arterial blood flow in the extremity; second step - sclerooblitteration of the incompetent perforator veins and the third step - transplantation of mesenchymal stem cells into muscles along the perimeter of trophic ulcer. **Conclusions:** This tactic contributes to the long-lasting and sustained positive clinical effect and rapid healing of trophic ulcers in patients with type 2 diabetes.

Keywords: Autologous mesenchymal stem cells, large trophic ulcers, patient with type 2 diabetes.

Introduction

According to various authors, obliterating atherosclerosis of the lower extremities' arteries is clinically manifested in 2–3% of the total population, and about 15–20% of the elderly (average age is 68 ± 5.5 years). In fact, the number of such patients is 3–4 times greater due to subclinical forms [1, 2, 10–13]. In 60–80% of patients with multiple and distal forms of pathology, severe ischemia develops, leading to limb amputations in 10–20% cases. In the first three years after high amputations, mortality rates increase annually to 69.5% in patients with chronic critical ischemia of the lower extremities [3, 4–6, 14, 18, 22].

Due to increased number of patients with atherosclerotic lesions of several arterial beds in which arterial reconstructive surgery of the lower extremities is impossible or at high risk, as well as a large percentage of patients

with multilevel or distal lesions of the arteries of the lower extremities, the revascularization of lower extremities is not possible in all cases. The absence of an adequate microcirculatory bed is one of the causes of early postoperative thrombosis of bypass shunts [1, 7–9, 11, 23–25].

Therefore, the problem of treating patients with distal lesions of arteries of the lower extremity is one of the most challenging in vascular surgery. Its solution will not only save the limb and improve the quality of life for inoperable patients but also significantly extend their life span [10, 15–17, 20, 26].

Extremely unfavorable are the situations of combined lesions of the limb arteries with chronic venous insufficiency. Venous hypertension is one of the factors in the development of trophic disorders and initiates a whole cascade of pathological responses at subcellular, cellular, and tissue levels. According to the literature, trophic venous ulcers account for more than 70% of all lower extremity ulcers and are found in every fifth patient with chronic venous insufficiency. Using standard conservative therapy that lasts up to 4 months, healing of venous trophic ulcers occurs only in 50% of cases, 20% does not heal after two years of treatment, and after five years in 8% of patient's ulcerative defect remains open [5, 7, 15, 19, 27–29]. The combination of chronic venous insufficiency with chronic arterial disease significantly reduces the effectiveness of traditional treatment of venous trophic ulcers.

The search for new methods of complex treatment of trophic ulcers of the lower extremities is a topical issue of modern medicine, because despite the constant work of morphologists, surgeons and pharmacists to predict the development of the wound process, its healing remains almost an unattainable goal [8, 9, 17, 30].

Nowadays, the methods of stem cell transplantation of various origins in limb ischemia, pancreatic necrosis, diabetes mellitus, tumor hematology, and other diseases are being used in medicine more frequently [7–9, 21, 22]. A certain similarity was observed in the sequence of expression of marker genes and proteins after studying the directed differentiation of stem cells in vitro and in vivo. These cells have the ability to differentiate into different cell types and repair tissues of damaged organs: liver, heart, skin, blood vessels, bones, and cl. [8, 11, 19]. Recent studies have shown that mesenchymal stem cells have the capacity for multidirectional differentiation and poor immunogenicity; these cells are widely used in regenerative medicine research.

However, there is little data in the literature regarding the scientific search for the possibility of using cellular technologies to treat patients with trophic ulcers.

The purpose of the study is to investigate the effect of stem cell transplantation on the course of the wound process in conditions of combined lesions of the arteries and veins of the lower extremities in a patient with diabetes.

In our protocol, we used autologous mesenchymal stem cells (MSC) isolated from the patient's peripheral blood by magnetic separation using an AutoMACS automatic system (USA). MSCs met the following criteria for expression of cell surface markers: ≥95% of the population should express CD105, CD73, and CD90, and ≤2% did not express CD45, CD34, CD14 or CD11b, CD79a or CD19, and MSC class II HLA were able to differentiate into osteoblasts, adipocytes, and chondroblasts in vitro [23, 24].

Case Presentation

Patient S. was admitted to the Department of acute vascular diseases of State Institution "V. T. Zaitsev Institute of General and Urgent Surgery of National Academy of Medical Sciences of Ukraine", complaining of a trophic ulcer on the left ankle (Figure 1), pain in both lower extremities (more exacerbated in the right foot and lower leg), which increase significantly during exercise, and feeling of cold in the fingers. Patient height and bodyweight were 162 cm and 71 kg (body mass index = 26.3), respectively. He was burned in 1952 and had a 20-year history of type 2 diabetes. The patient noted a worsened sleep at night, which was associated with unpleasant sensations and pain in both lower extremities. The length of painless walking distance was about 35–50 m, after which severe pain appeared in the calf muscles of both lower extremities (greater to the right).

Figure 1: Patient S. Trophic ulcer of the right calf.

History of the disease

The patient first felt the pain in the lower extremities during exercise three years ago. She was treated conservatively at a regional hospital. Two years ago, she had acute deep vein thrombosis of the lower extremities. The trophic ulcer appeared nine months prior to being admitted to the hospital. Traditional conservative and topical treatment was unsuccessful as the wound area increased.

During the physical examination, it was noted that the skin was pale and cold on both the lower legs and feet with moderate swelling of the shin. Active and passive movements of the right and left joint extremities were preserved. While the femoral pulse was preserved in the right leg, pulsation of the popliteal, posterior tibial and anterior tibial arteries was absent. Transcutaneous oxygen pressure (TcpO2) of the foot was 9.4 mmHg. Angiography and revascularization of the right lower extremity was performed on 05/10/2019 (Figure 2).

After revascularization, TcpO2 became 39.6 mmHg. Scleroobliteration of incompetent perforator veins under ultrasound control was performed on 10/08/2019 (Figure 3). Aethoxysklerol 0.1–3% was used as a sclerotherapy agent and the sclerosing foam was obtained by the Tessari method (air- sclerosant ratio of 4:1).

Considering the size of the trophic ulcer, it was decided to include in the treatment regimen autologous mesenchymal stem cells which were isolated from the patient's peripheral blood using a magnetic separation method and cultured according to the standard protocol using a CO2 incubator of 5% atmosphere with a 37°C temperature, with further trypsinization and technologic subculturing of cells cultures. The isolation, cultivation, and preservation of mesenchymal stem cells was performed on the basis of the Institute of Cellular Biorehabilitation, licensed by the Ministry of Health of Ukraine for the Bank of Cord Blood, Cells and Tissues (Order of the Ministry

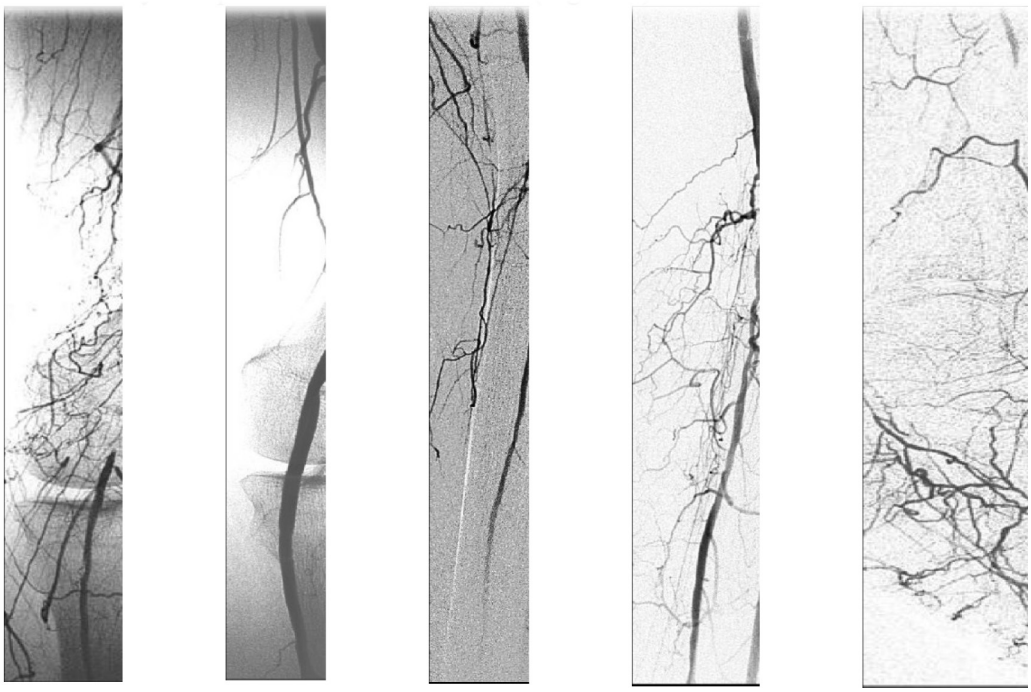

Figure 2: Stenting of the superficial femoral and popliteal artery, balloon angioplasty of the anterior tibial artery.

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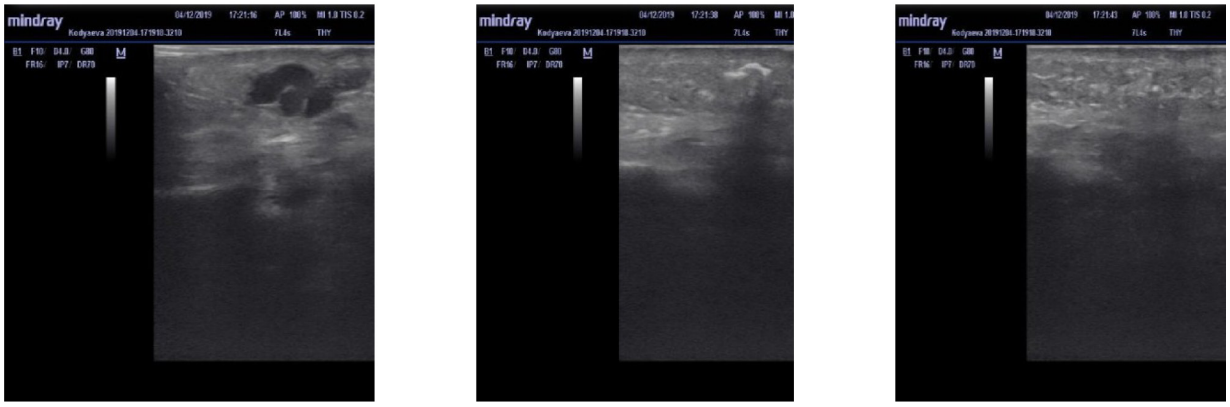

Figure 3: Scleroobliteration of incompetent perforator veins (ultrasound).

of Health of Ukraine No. 1813 dated 04/10/2018 and order of the Ministry of Health of Ukraine on 18.04.2019 No. 877) and is accredited according to the international standard ISO 20387: 2018 - biobank and biotechnology.

During standard therapy (surgical debridement: end result of non keratotic wound edges with a well-vascularized tissue bed; after the debridement was successful, local antimicrobial therapy was sufficient to eliminate bacteria in the ulcer), post-usual therapy local injections of autologous mesenchymal stem cells were used for four days.

On 10/10/2019, transplantation of stem cells into the leg's muscle tissue along the border of the trophic ulcer was performed (Figure 4). The dose was 5 ml of suspension containing $5.0x10^5$ cells per 1 cm^2 of wound area. The pre-cell suspension was tested for the absence of pathogens. Negative results of serological and viral tests - HIV-1 and 2 (HIV Ag/Ab); Hepatitis B Virus Infection, Anti-hepatitis B core antigen (Anti-HBc); Hepatitis C Virus Infection, Anti-HCV; Syphilis specific tests were obtained.

Cell therapy was performed according to the following protocol: after division, the characteristics and subculturing of the stem cells were introduced four times into the ulcer zone in eight points.

The patient was discharged from the hospital with recommendations for local treatment of the wound with ointments based on fat (methyl-uracil 10% ointment), the use of dual disaggregate therapy, blood pressure and glycemic control. Patient monitoring was performed every two weeks for up to 12 weeks of outpatient follow-up.

One month after the cell transplantation, the epithelization of approximately one-third of the wound area was noted (Figure 5).

Two months post cell transplantation, the patient's local status and general condition were improved, had increased ability for working and general activities (Figure 6). Also, TcpO2 was 42.3 mmHg.

Figure 4: Stem cell transplantation in the muscle tissue of the calf.

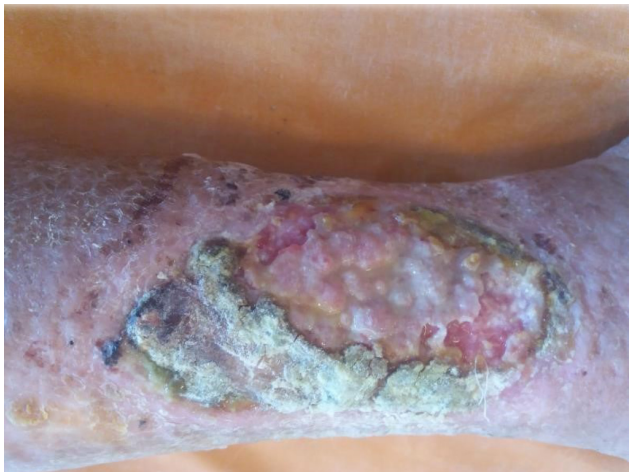

Figure 5: One month after stem cell transplantation.

Figure 6: Two months after stem cell transplantation.

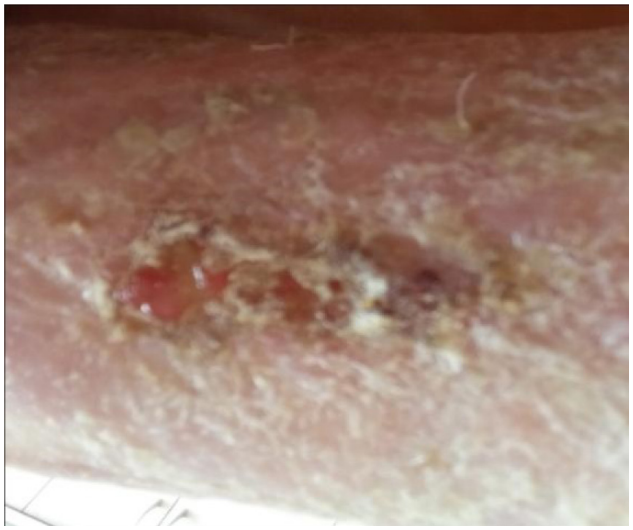

Figure 7: Four months after stem cell transplantation.

The first signs of wound healing were observed four months after the start of therapy (Figure 7). After cell transplantation, the condition of the patient was good, and working ability was preserved, allowing an active life. There was no limb edema, and the wound healed completely.

Discussion

Thus, the above clinical case shows that in patients with combined lesions of the arteries, deep veins and the presence of large trophic ulcers, it is advisable to use a step-by-step treatment: first step - restoration of the arterial blood flow in the affected extremity, second step - sclerooblitеration of the incompetent perforator veins (Figure 3) and the third step - transplantation of mesenchymal stem cells into the muscles along the perimeter of the trophic ulcer (Figure 4). This tactic contributes to the long-lasting and sustained positive clinical effect and rapid healing of trophic ulcers in patients with diabetes type 2.

Conclusion

In the initial stages of diagnosis, the multidisciplinary team should make treatment recommendations based on the severity of ischemia and the presence of infectious lesions. Multidisciplinary collaboration and optimization of treatment regimens are necessary if there is a long-term possibility of preserving any part of the foot or leg. It is clinically proven that the use of mesenchymal stem cell transplantation for patients with combined lesions of the lower extremity arteries and veins significantly increases the chances of successful treatment of trophic ulcers and chronic wounds. It is promising to study the effectiveness of the proposed method on a larger contingent of patients.

Conflict of Interest

The authors declare no conflict of interest.

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