Amador et al, J Regen Med 2020,9:3
DOI: 10.37532/jrgm.2020.9(3).166

Journal of Regenerative Medicine

Research Article a SciTechnol journal

Microvascular Effect After the Application of Cell Therapy with A Concentrate of Hematopoietic Stem Cells in Patients with Peripheral Arterial Disease with Non-Critical Limb Ischemia and Diabetes

Lauro Fabián Amador Medina[1]*, Blanca Olivia Murillo Ortíz[1], Luis Carlos Padierna García[2], Carlos Villaseñor Mora[2], Enrique Castro Camus[3], Goretti Hernández Cardoso[3], Juan Manuel Malacara Hernindez[4] and M ar a del Rosario Sinchez Navrro[5]

Abstract

Background: Bone marrow derived cell therapy is an experimental treatment for critical limb ischemia. Little is known about the microvascular changes in hematopoietic stem cell (HSC) therapy obtained from the bone marrow in peripheral arterial disease (PAD) with non-critical limb ischemia and type 2 diabetes mellitus.

Objective: To evaluate the microvascular effect after the application of cell therapy with a concentrate of hematopoietic stem cells in patients with PAD with non-critical limb ischemia and diabetes.

Methods: Patients were randomly to receive HSC therapy, in addition to standard care, or standard care alone. HSC therapy consisted of intramuscular application to the lower extremities of a concentrated obtained from bone marrow stimulated with granulocyte colony stimulating factor. Microvascular evaluations were performed after 6 to 8 weeks by means of infrared imaging, Terahertz imaging and Doppler ultrasound of interdigital arteries.

Results: A total of 24 patients who met the inclusion criteria were randomized to the study groups. The microvascular effect evaluated by Doppler ultrasound of the interdigital arteries showed a decrease in the resistance index of the right foot (0.80 vs 0.71; p = 0.02), of the left foot (0.83 vs 0.75; p = 0.004), with a beneficial effect on the therapy group. No beneficial microvascular changes evaluated with the other methods were demonstrated.

Conclusions: The application of cell therapy with hematopoietic stem cells in patients with Peripheral Arterial Disease with non-critical limb ischemia and Diabetes shows beneficial microvascular changes, under demonstrable evaluation via Doppler ultrasound

Keywords

Hematopoietic stem cell, Peripheral arterial disease, Diabetes

***Corresponding author:** Lauro Fabián Amador Medina, Instituto Mexicano del Seguro Social, High Specialty Medical Unit, No 1 del Bajío, Clinical Epidemiology Research Unit, Blvd. López Mateos Esq Insurgentes S / N, Colonia Los Paraísos, CP 37320, León, Guanajuato, Mexico, Tel: +52 477 718 3039; E-mail: lafab2013@gmail.com

Received: June 05, 2020 **Accepted:** August 22, 2020 **Published:** August 29, 2020

Introduction

Peripheral Arterial Disease (PAD) is defined as the complete or partial obstruction of one or more peripheral arteries. PAD is also referred to as an atherosclerotic occlusive disease of the lower extremities [1]. The prevalence of PAD in patients older than 60 years is 20%, and this prevalence increases with age [2]. Several epidemiological studies base the diagnosis of PAD on Ankle Brachial Index (ABI), which is defined as an ABI <0.9 [3]. PAD is a common cardiovascular complication in patients with type 2 diabetes mellitus. The risk of developing PAD is much higher in patients with diabetes, and the disease is more severe and progresses faster than in non-diabetic individuals. Moreover, the presence of PAD is a potent marker of increased cardiovascular risk. If PAD is identified on the basis of an ABI of <0.90, its prevalence in patients with diabetes may be as high as 29%[4].

The Therapeutic Angiogenesis using Cell Transplantation (TACT) trial was the first clinical trial to evaluate the use of unselected, autologous bone marrow mononuclear cells (BM- MNCs) in the treatment of PAD. The trial found that there was a statistically significant improvement in ABI, transcutaneous oxygen pressure (TcO_2), rest pain, and pain free walking time associated with BM-MNC treatment. This landmark trial was the first to demonstrate improvement in clinical markers of PAD severity after the administration BM-MNCs [5]. In recent clinical trials, patients had critical limb ischemia (CLI), that is the most severe form of atherosclerotic PAD [6,7].

It is not clear whether administration of stem cells at an earlier stage of the disease could be more beneficial, and which subset BM-MNCs is optimal for treatment, (endothelial progenitor cells, mesenchymal stem cells or hematopoietic stem cell) that can stimulate angiogenesis.

The physiologic endpoint such as ABI and TcO2 are commonly used surrogates for microvascular disease in clinical trials, but there is no gold standard for microcirculatory assessment with which to compare these methods.

There are many methods used in the assessment of microvascular disease of lower extremities [8]. Novel technologies such as infrared imaging, Terahertz imaging, and Doppler ultrasound of interdigital arteries can evaluate the microvascular effect of stem cell therapy [9-11].

These three novel technologies were employed to accomplish the objective of the present clinical trial, which was to evaluate the microvascular effect of applying cell therapy with a concentrate of hematopoietic progenitor cells in patients with PAD with non-critical limb ischemia and type 2 diabetes.

Materials and Methods

Study Design: Randomized controlled trial with blinding (for the observer evaluating treatment goals). The protocol was approved by The Guanajuato University Ethics Committee (CIBIUG-P14-2018) and the National Commission for Scientific Research of the Mexican Institute of Social Security (R-2018-785-031) and registered on

clinicaltrials.gov (Identifier: NCT03635970). The study was conducted in accordance with the Declaration of Helsinki. Written informed consent for participation was obtained from all the patients.

Inclusion criteria: Eligible patients were men and women, ranging from 50 to 80 years old, with peripheral arterial disease, with non-critical limb ischemia, and, with more than 10 years of diagnosed diabetes mellitus.

Exclusion Criteria: Main exclusion criteria were severe sepsis process, restrictions with the use of filgrastim, and loss of patients during follow-up.

Procedure for obtaining cell therapy with HSC concentrate: The treatment procedure involved aspiration and processing of bone marrow stimulated with granulocyte colony stimulating factor (G-CSF), and intramuscular injections of autologous bone marrow CD34+ cell concentrate into the gastrocnemii muscles. The usual G-CSF dose was 10 µg/kg/ day divided into two subcutaneous injections for 5 days. On the fifth day of filgrastim administration the procedure was carried out under conscious sedation and aseptic conditions. A total volume of 100mL of uncoagulated bone marrow was aspirated from multiple sites of the posterior superior iliac crest in heparinized syringes under local anesthesia using an 11-gauge Jamshidi needle. The product of the bone marrow aspirate is poured into 4 sterile 50 mL falcon tubes inside a laminar flow hood. To the tubes, 15 mL of 6% HES (pentalstarch 6 g/100 mL) was added and gently homogenized. Falcon tubes with the bone marrow aspirate were placed in a refrigerated centrifuge at 3,500 rpm for 15 minutes at 8°C. They were removed from the centrifuge and the supernatant was aspirated into the laminar flow hood with sterile transfer pipettes. With an automatic pipette of up to 5 mL and a sterile blue tip, the buffy coat (from mononuclear cells) was acquired, trying to contain as few red blood cells as possible, and it was placed in a sterile falcon tube and re-suspension with plasma of the supernatant was performed. up to a total volume of 45mL. A small aliquot of 1 mL was collected from the aspirated bone marrow and sample was analysed for cell counts, sterility, and viability. The administration of the CD34+ cell concentrate was performed by intramuscular application in the gastrocnemii muscles, with a 25-gauge needle and administering 0.5 to 1 mL at each puncture site. This procedure was carried out under standard asepsis and antisepsis techniques.

Infrared thermographic imaging: The patients stayed for 15 minutes to acclimatize to the room conditions (18°C to 22°C and humidity of 65%). When the patients arrived at the laboratory, they removed their shoes, and sat in a normal position waiting for the acquisition of the infrared images, achieving the time, a stage where the foot was placed respectively to take infrared images per foot. These images were acquired with an infrared camera Xenics Gobi 640 with 50 mm lens, the camera was always kept at a distance 53 cm. The infrared thermographic data were analysed using the MATLAB software.

Terahertz imaging: A platform was built to emit Terahertz radiation. Before each measurement, the platform was cleaned with isopropyl alcohol. The patient was asked to remove their shoes before climbing onto the platform. Then, the patient was sat in a chair located on the platform, and placed his bare feet on the platform. Afterwards, the technician taking the sample accommodated the patient's feet within the measurement window. The patient was asked to maintain contact with the window and avoid foot movement. Finally, the picture was taken. The scan lasted approximately 10 minutes.

Ultrasound microvascular evaluation of interdigital arteries: A linear transducer with a variable ultrasound frequency of 9-15 MHz was used. The transducer was placed over an artery for cross-sectional analysis and is then rotated 90° for longitudinal analysis. The operator had to rotate or move the transducer gently to maintain visualization of the artery. The evaluation was usually carried out with the patient placed in a supine position. The measurement of the 5 interdigital arteries of the right and left foot was performed, with which the following values were obtained: peak systolic velocity (PSV), velocity at the end of diastole (EDV), and resistance index (IR). No previous studies, were identified that, specify the normal limits of these variables, in the territory of interdigital arteries; however, it is stated in analogy to other arterial territories that greater IR, leads to a greater degree of arterial obstruction.

Endpoints: The primary endpoint of this clinical trial was to assess the microvascular effect after the application of cell therapy with a concentrate of hematopoietic progenitor cells in patients with PAD with non-critical limb ischemia and type 2 diabetes mellitus. This microvascular evaluation was carried out by 3 novel non-invasive imaging methods infrared imaging (energy emissivity in Joules), Terahertz imaging (percentage of hydration) and Doppler ultrasound of interdigital arteries (resistance index).

Statistical Analysis: Continuous variables were described as medians and interquartile ranges and categorical variables were summarized as proportions. Medians were compared using the Mann Whitney U test, and percentages using the χ^2 test and Fisher´s exact test. All analyses were performed using the SPSS software (version 22).

Results

From August 2018 to November 2019, 90 patients with type 2 diabetes mellitus over 10 years from diagnosis were evaluated, in which the determination of the ABI was performed and 24 patients (27%) with altered ABI (0.99- 0.6) were identified and considered to establish the diagnosis of PAD with non-critical limb ischemia.

Patients who met the inclusion criteria were randomized in a 1: 1 ratio in the study groups (control group and cell therapy group), and their data remained blinded to the observer who evaluated the treatment goals. In each study group, an initial evaluation of the microvascular characteristics was carried out by each of the 3 evaluation methods (ultrasound of the interdigital arteries, Terahertz image and infrared image), and they were evaluated over a period of 6 to 8 weeks with the same imaging methods. There were no losses to follow up (Figure 1) shows the distribution of patients in each of the study groups.

Baseline clinical characteristics of the enrolled patients are summarized in (Table 1). the baseline biochemical characteristics such as HbA1c, lipid profile and blood chemistry were similar in both groups.

Components of cell therapy administered in the experimental group: The HSC concentrate administered to the cell therapy group resulted in a median mononuclear cell doses of 297 x10^6 (interquartile range,188 to 500); median CD34+ cell doses of 1.84 x 10^6 (interquartile range, 0.79-2.84), and median cell viability of 59.5% (interquartile range, 39.4-76.2).

Adverse Events: There were no adverse effects in the cell therapy treatment group, with the exception of mild lower limb edema that resolved within a few days after HCT administration, 2 patients in the

Table 1: Patient demographic and clinical characteristics.

Characteristic	Control Group (n=12)	Cell therapy group (n=12)	P-value
Age, median years (IQR)	69 (53-74)	64 (53-69)	0.68*
Female sex, n (%)	9 (75)	7 (58.3)	0.38**
BMI, (kg/m^2), median (IQR)	28.3 (24.9-28.8)	28.0 (26.2-30)	1*
Hypertension, n (%)	4 (33.3)	4 (33.3)	1**
Smoking, n (%)	2 (17)	6 (50)	0.08**
Dyslipidemia, n (%)	2 (17)	3 (25)	0.61**
Comorbidities, n (%)			
Ischemic heart disease	0 (0)	0 (0)	0.98**
Stroke	0 (0)	0 (0)	
Chronic renal insufficiency	2 (17)	1 (8)	
Amputation	1 (8)	1 (8)	
TASC II recommendations, n (%)			
Lipid lowering drugs	3 (25)	2 (17)	
Antihypertensive drugs	5 (42)	5 (42)	
Antithrombotics	5 (42)	4 (33.3)	0.98***
Dietary recommendations	7 (58.3)	7 (58.3)	
Exercise therapy	6 (50)	7 (58.3)	
Fontaine classification, n (%)			
I/II	12 (100)	12 (100)	1**
III/IV	0 (0)	0 (0)	
Rutherford classification, n (%)			
0/1	12 (100)	10 (83.3)	
2/3	0 (0)	2 (17)	0.33**
4/5	0 (0)	0 (0)	
Abnormal ABI (<1), n (%)			
Right ABI involvement	5 (42)	3 (25)	
Left ABI involvement	2 (16)	2 (17)	0.66**
Bilateral ABI involvement	5 (42)	7 (58)	

IQR, interquartile range. BMI, body mass index.

*Mann-Whitney U test, **Fisher exact test***, chi^2 test.

TASC: Trans-Atlantic Inter-Society Consensus Document on Management of Peripheral Arterial Disease

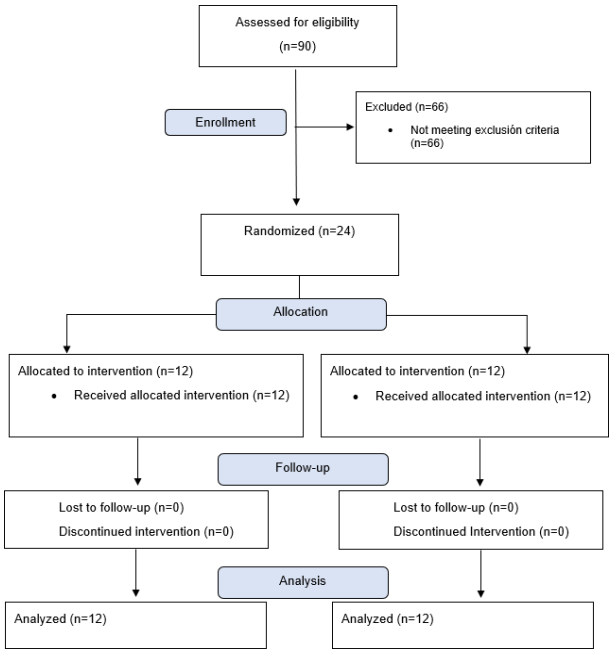

Figure 1: Consort flow chart showing the distribution of patients.

cell therapy group presented some areas of ecchymosis at declining sites of the foot that also resolved in a few days. One patient in the cell therapy group had a toe infection that required amputation as a therapeutic measure. There were no significant differences in the rate of adverse events between cell therapy group and control group.

Efficacy endpoints: Infrared imaging (energy emissivity in Joules), Terahertz imaging (percentage of hydration) and Doppler ultrasound of interdigital arteries (resistance index) were used as non-invasive techniques for assessing microvascular blood flow and tissue oxygen tension after autologous bone marrow concentrated intramuscular treatment. The (Tables 2-4) show the changes in energy emissivity in Joules, percentage of hydration and resistance index from baseline to 6-8 weeks after the administration of cell therapy, respectively

Discussion

The main results found in this work were that the group of patients with diabetes and PAD with non-critical ischemia undergoing cell therapy with a concentrate of HSC showed beneficial microvascular changes by reducing the resistance index (IR) of their interdigital arteries and in comparison, with the control group via Doppler ultrasound. However, we did not find beneficial microvascular changes in cell therapy when the evaluation was performed either using infrared imaging or Terahertz.

An analysis of previous clinical trials also establishes a beneficial effect of stem cell therapy with autologous implantation, in patients with PAD with critical ischemia of the lower extremities, showing improvement of ITB, of TcPO2, decrease in the amputation rate, and increase in ulcer healing rate, the latter two especially in the subgroup of patients with type 2 diabetes. [7,12]

This work was carried out with the intention of studying the possible microvascular effect of cell therapy with a concentrate of CPH and at an earlier stage of PAD, by excluding patients with critical limb ischemia. This contrasts with previous studies where they evaluate patients with more severe PAD, especially without treatment option other than limb amputation [13-19]. It is noteworthy that cell therapy shows beneficial microvascular effects from earlier stages in patients with PAD and type 2 diabetes.

Table 2: Microvascular Evaluation Using Infrared Imaging.

Component analyzed*	Control group (n=12)			Cell therapy group (n=12)			***intergroup p-value
	initial	final	**p-value	Initial	final	**p-value	
Right toes	1,30,330	1,40,407	0.71	1,58,259	1,53,968	0.51	0.57
Left toes	1,38,012	1,58,526	0.58	1,67,787	1,35,476	0.23	0.38
Most affected right toe	1,06,795	1,29,047	0.38	1,30,848	1,31,669	0.65	0.9
Most affected left toe	1,17,857	1,44,157	0.3	1,45,721	1,19,030	0.29	0.32
Right instep	3,02,049	2,58,258	0.05	3,25,668	2,62,106	0.007	0.86
Left instep	3,27,338	2,73,354	0.05	3,46,352	2,67,274	0.01	0.8
Right calf	3,44,113	2,89,403	0.03	3,51,104	2,66,764	0.009	0.44
Left calf	3,46,534	3,01,981	0.06	3,54,023	2,71,441	0.02	0.28

*Average energy measured in Joules (J) of the analyzed component; ** Student's t-test for related samples; *** Student's t-test for independent samples with respect to final evaluations of the control group and the cell therapy group

Table 3: Microvascular evaluation using Terahertz image.

Component analyzed*	Control group (n=12)			Cell therapy group (n=12)			***intergroup p-value
	initial	final	**p-value	Initial	final	**p-value	
Right absolute average	47.92	48.25	0.67	49.9	49.5	0.68	0.39
Left absolute average	47.5	47.1	0.65	49.3	49.5	0.87	0.12
Absolute right toe	57.42	56.75	0.68	44.42	47.25	0.74	0.16
Absolute left toe	51	51.75	0.67	49.9	54.58	0.43	0.59
Absolute right heel	44.17	48.75	0.33	44.33	44.25	0.99	0.48
Absolute left heel	43.42	48.83	0.2	30.5	50.17	0.87	0.52
Red left	40	39.17	0.77	31.25	32.33	0.75	0.21
Red right	37.5	36.8	0.75	29.08	29.08	1	0.21
Green left	24.58	22.5	0.59	28.58	27.5	0.88	0.54
Green right	27.42	24.75	0.62	29.58	28	0.83	0.68

* (%) hydration of the analyzed component; ** Student's t-test for related samples; *** Student's t-test for independent samples with respect to final evaluations of the control group and the cell therapy group

Table 4: Microvascular evaluation by measurement of interdigital arteries by Doppler ultrasound.

Component analyzed*	Control group (n=12)			Cell therapy group (n=12)			***intergroup p-value
	initial	final	**p-value	Initial	final	**p-value	
Average right foot resistance index	0.77	0.8	0.05	0.75	0.71	0.23	0.02
Average left foot resistance index	0.76	0.83	0.0005	0.73	0.75	0.62	0.004

Average of the resistance indices of the interdigital arteries ** Student's t-test for related samples; *** Student's t-test for independent samples, with respect to final evaluations of the control group and the cell therapy group

Until now, the measurement of TcPO2 is the most frequently used method to measure tissue oxygenation in patients with PAD; however, it is not entirely available in hospital centers to establish itself as a unique method of evaluating microvascular effects [20]. Due to the above, we implemented three imaging methods to evaluate microvascular changes with the use of cell therapy with HSC.

From the parameters analyzed by Doppler ultrasound, we conclude that the resistance index (RI) of the interdigital arteries is the most representative measure of the microvascular state since, up to now, the normal parameters of these measurements in the vascular territory of arteries are not precisely known.

The components analyzed by Doppler ultrasound of the interdigital arteries of both feet were: Resistance Index (IR), Peak Systolic Speed (PSV) and Speed at the end of Diastole (EDV); of these, we take IR as the most representative measure of the microvascular state since, up to now, the normal parameters of these measurements are not precisely found in the vascular territory of interdigital arteries [21,22]; We established before and after comparisons in the cell therapy group and in the control group, a situation that allowed us to detect changes and evaluate the microvascular effects of the treatment groups.

Zhang et al, [23] have reported higher RI levels of DMT2 patients compared to the control group ($p < 0.001$), even the analysis of subgroups by duration of diabetes greater than 5 years shows higher levels compared to duration of DMT2 younger than 5 years ($p < 0.01$).

In our study, the RI (right and left foot) in the control group showed a significant increase, which translates into a higher degree of microvascular obstruction; On the other hand, the IR (of the right and left foot) of the cell therapy group did not show a significant increase, which is interpreted as a beneficial change due to the cell therapy.

When making the final comparisons between the control group and the cell therapy group, we can see that the cell therapy group shows lower IR; which indicates improvement in the microvascular component. The reason why no beneficial microvascular changes were found with the other evaluation methods may have several explanations.

In the particular case of microvascular evaluation using infrared imaging of patients with PAD and diabetes, we decided to analyse 4 sites in order to detect microvascular changes: toes, the most affected toe, the instep and the calf of both feet. The analysed sites did not show significant changes and therefore no clinical benefit for patients is concluded, even some components analysed showed areas of energy decrease with statistical significance, a situation that does not demonstrate a benefit, contrary to the results found using Doppler Ultrasound.

Infrared thermographic imaging measures skin temperature and not perfusion directly, changes in tissue perfusion often result in a change in tissue temperature. This is the principle of microvascular imaging thermography, using an infrared thermal imaging camera to determine the temperature of human body tissue. However, the skin temperature can also be modified by other factors in addition to tissue perfusion, these factors are mainly inflammatory or infectious, including factors external to the patient, such as ambient temperature, a situation that was not controlled in the analysis of the patients. Since it measures tissue temperature, it is at best a surrogate measure of change in microvascular perfusion and as such has gained limited acceptance as an imaging method for microvasculature [24].

Regarding the Terahertz image, a novel method for the evaluation of diabetic foot syndrome, which has been proposed as a screening method that could help for the prevention and follow-up of medical treatment of diabetic foot, more specifically evaluates the degree of hydration of the feet in patients with diabetes mellitus, and not directly the microvascular state, of patients with PAD and diabetes [10].

Indeed, dehydration of the skin of the feet of patients with diabetes is a central factor in the evolution towards diabetic foot syndrome, attributed to PAD, but we must recognize that it is not the only factor associated with dehydration of the skin. However, time domain spectroscopy in the Terahertz band is a recent technique with high potential for development and application in different areas, including biology and medicine, and several studies will be required to define its role in the evaluation of diabetic foot syndrome.

A patient in the cell therapy group had an amputation of one of his toes after the application of cell therapy, this amputation was due to an infectious process that could not be resolved with antibiotics, this could indicate on the one hand that cell therapy has a medium-term effect or a lack of response to cell therapy in cases of active infectious processes.

In our study, we did not find that the dose of CD34 + cells influenced the response to cell therapy, although there is data indicating that higher doses of CD34 + cells correlate with a longer amputation-free survival [25]

To our knowledge, this is the first study that attempts to evaluate the microvascular effect of cellular therapy with HSC using three non-invasive imaging methods in patients with PAD with non- critical ischemia and diabetes.

In our work, the evaluation technique in which significant differences were found with HSC therapy is the ultrasound of the interdigital arteries. We believe that it may be a good method to evaluate the microvascular component.

Future research will often further validate the routine use of Doppler ultrasound of the interdigital arteries as a method of evaluating microvascular changes, with the use of novel story therapies such as cell therapy

Conclusions

This study suggests a beneficial effect of cellular therapy with HSC in patients with PAD with noncritical ischemia and diabetes. We propose that this therapy could be used at an earlier stage of PAD to prevent disease progression.

The application of cell therapy with a concentrate of hematopoietic progenitor cells in patients with Peripheral Arterial Disease with non-critical ischemia and Diabetes shows microvascular changes, under evaluation by Doppler ultrasound of interdigital arteries, specifically regarding decreased resistance indices

Acknowledgements

We want to acknowledge to the Guanajuato state public health Laboratory and institutional supporting FIS/IMSS/PROT/MD18/1798. Also, we thank to the Secretaria de Innovación, Cienciay Educacion Superior (SICES) of the Guanajuato state, research project IJ-19-36, for the financial support to implement the infrared thermographic imaging method.

References

1. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA (2007) Inter-

society consensus for the management of peripheral arterial disease (TASC II). J Vasc Surg 45:S5-S67.

2. Selvin E, Erlinger TP (2004) Prevalence of and risk factors for peripheral arterial disease in the United States: results from the National Health and Nutrition Examination Survey, 1999–2000. Circulation 110:738-743.
3. Yang SL, Zhu LY, Han R, Sun LL, Li JX (2017) Pathophysiology of peripheral arterial disease in diabetes mellitus. J Diabetes 9:133-140.
4. Marso SP, Hiatt WR (2006) Peripheral arterial disease in patients with diabetes. J Am Coll Cardiol 47(5):921-929.
5. Tateishi-Yuyama E (2002) Therapeutic Angiogenesis using Cell Transplantation (TACT) Study Investigators Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. Lancet 360:427-435.
6. Rigato M, Monami M, Fadini GP (2017) Autologous cell therapy for peripheral arterial disease: systematic review and meta-analysis of randomized, nonrandomized, and noncontrolled studies. Circ Res 120:1326-1340.
7. Gao W, Chen D, Liu G, Ran X (2019) Autologous stem cell therapy for peripheral arterial disease: a systematic review and meta-analysis of randomized controlled trials. Stem Cell Res Ther 10:140.
8. Chen Q, Rosenson RS (2018) Systematic review of methods used for the microvascular assessment of peripheral arterial disease. Cardiovasc Drug Ther 32:301-310.
9. Saminathan J, Sasikala M, Narayanamurthy V, Rajesh K, Arvind R (2020) Computer aided detection of diabetic foot ulcer using asymmetry analysis of texture and temperature features. Infrared Phys Techn 105:103219.
10. Hernandez-Cardoso GG, Rojas-Landeros SC (2017) Terahertz imaging for early screening of diabetic foot syndrome: A proof of concept. Sci Rep-UK 7:42124.
11. Hwang JY (2017) Doppler ultrasonography of the lower extremity arteries: anatomy and scanning guidelines. Ultrasonography 36:111-119.
12. Procházka V, Gumulec J, Jalůvka F, Šalounová D, Jonszta T (2010) Cell therapy, a new standard in management of chronic critical limb ischemia and foot ulcer. Cell Transplant. 19:1413-1424.
13. Walter DH, Krankenberg H, Balzer JO, Kalka C (2011) Intraarterial administration of bone marrow mononuclear cells in patients with critical limb ischemia: a randomized-start, placebo-controlled pilot trial (PROVASA). Circ-Cardiovasc Inte 4:26-37.
14. Iafrati MD, Hallett JW, Geils G, Pearl G, Lumsden A, et al. (2011) Early results and lessons learned from a multicenter, randomized, double-blind trial of bone marrow aspirate concentrate in critical limb ischemia. J Vasc Surg 54:1650-1658.
15. Idei N, Soga J, Hata T, Fujii Y, Fujimura N (2011) Autologous bone-marrow mononuclear cell implantation reduces long-term major amputation risk in patients with critical limb ischemia: a comparison of atherosclerotic peripheral arterial disease and Buerger disease. Circ-Cardiovasc Inte 4:15-25.
16. Benoit E, O'Donnell TF, Iafrati MD, Asher E, Bandyk DF (2011) The role of amputation as an outcome measure in cellular therapy for critical limb ischemia: implications for clinical trial design. J Transl Med 9:165.
17. Powell RJ, Comerota AJ, Berceli SA, Guzman R, Henry TD (2011) Interim analysis results from the RESTORE-CLI, a randomized, double-blind multicenter phase II trial comparing expanded autologous bone marrow-derived tissue repair cells and placebo in patients with critical limb ischemia. J Vasc Surg 54:1032-1041.
18. Powell RJ, Marston WA, Berceli SA, Guzman R, Henry TD (2012) Cellular therapy with Ixmyelocel-T to treat critical limb ischemia: the randomized, double-blind, placebo-controlled RESTORE-CLI trial. Mol Ther 20:1280-1286.
19. Losordo D, Kibbe M, Mendelsohn F, Marston W, Driver V, et al. (2012) A randomized, controlled pilot study of autologous CD34+ cell therapy for critical limb ischemia. Circ-Cardiovasc Inte 5:821-830.
20. Mennes OA, van Netten JJ, Slart RH, Steenbergen W (2018) Novel optical techniques for imaging microcirculation in the diabetic foot. Curr Pharm design 24:1304-1316.
21. Patel M, Conte M, Cutlip D, Dib N, Geraghty P, et al. (2015) Evaluation and treatment of patients with lower extremity peripheral artery disease: consensus definitions from Peripheral Academic Research Consortium (PARC). J Am Coll Cardiol 65:931-941.
22. Gerhard-Herman M, Gardin JM, Jaff M, Mohler E, Roman M, et al (2006) Guidelines for noninvasive vascular laboratory testing: a report from the American Society of Echocardiography and the Society for Vascular Medicine and Biology.Vasc Med 11:183-200.
23. Zhang T, Xia LH, Bian YY, Feng B, Wang C, et al. (2013) Blood flow of the acral finger arterioles in patients with type 2 diabetes by quality Doppler profiles. Cell Biochem Biophys 67:717-725.
24. Bharara M, Schoess J, Armstrong DG (2012) Coming events cast their shadows before: detecting inflammation in the acute diabetic foot and the foot in remission. Diabetes Metab Res 28:15-20.
25. Madaric J, Klepanec A, Valachovicova M, Mistrik M, Bucova M, et al. (2016) Characteristics of responders to autologous bone marrow cell therapy for no-option critical limb ischemia. Stem Cell Res Ther 7:116.

Author Affiliations

Top

[1]*Clinical Epidemiology Research Unit, Mexican Institute of Social Security, San Francisco del Rincón, Guanajuato, Mexico*

[2]*University of Guanajuato-Campus León. Science and Engineering Division, León Guanajuato, Mexico*

[3]*Centro de Investigaciones en Óptica A.C, Lomas del Campestre, León, Guanajuato, Mexico*

[4]*University of Guanajuato-Campus León, Department of Medical Sciences, León Guanajuato 37320, Mexico*

[5]*Instituto de Salud Pública del Estado de Guanajuato*