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Review Article

Surgical and Cell Therapy in Critical Limb Ischemia: Current Evidence and Rationale for Combined Treatment with Special Focus on Diabetic Patients

Ottorino Del Foco[1,2], Antonio A. Bencomo-Hernandez[1], Yandy M. Castillo-Aleman[1], Pierdanilo Sanna[1,2], Stefano Benedetti[1,2], Enrico Dassen[1,2]

[1]Abu Dhabi Stem Cells Center, [2]Yas Clinic Khalifa City, Abu Dhabi, United Arab Emirates

Abstract

Critical limb ischemia (CLI) is considered the end-stage of peripheral arterial disease, with a prevalence between 2% and 4% in the general population and more than 15% in older adults. One-year major amputation rate can reach 30%, and diabetic patients are five times more likely to develop CLI than nondiabetics. The vascular damage and the complexity in the anatomical extension of the lesions are also worse in people with diabetes with poorer outcomes after vascularization attempts. Following the classifications suggested by international guidelines, we can define the presence of CLI and have a precise evaluation of the amputation risk and the best revascularization procedure for the patient. Nowadays, new endovascular techniques and devices make it possible to treat tibial vessels and even arteries below the ankle with promising initial results. Nevertheless, the re-occlusions rate and the need to re-do treatments at 1 year remain between 30% and 50%. The disease progression and hyperplasia can because it. However, the damage at the microcirculatory level can also lead to a decrease in tissue runoff and an increase in peripheral resistance, which determine the revascularization failure. In the last 20 years, several trials have been designed to avoid amputation in patients with no surgical options. The aim is to find a valid cellular base therapy to create a new vessel web in the ischemic tissue based on the angiogenetic power that stem cells have already demonstrated *in vitro* and animal studies. Different types of cells have been tested with different concentrations and administration routes with promising results. $CD34^+$ Mononuclear cells, Mesenchymal stem cells, growth factors have demonstrated their contribution to the neo-angiogenesis in ischemic areas. At Abu Dhabi Stem Cells Center, we created a cellular cocktail as an adjunct treatment to surgical revascularization. We think that acting at the microcirculatory and immunological level. We may reduce postsurgery hyperplasia and increase tissue perfusion, ultimately prolonging the patency of revascularization procedures.

Keywords: Critical limb ischemia, endovascular, major amputation, mesenchymal stem cells, peripheral arterial disease, surgical revascularization

Introduction

Ischemic rest pain, tissue loss, gangrene, and nonhealing ulcers, are all signs of a clinical syndrome, critical limb ischemia (CLI) that can be considered the terminal stage of peripheral arterial disease (PAD). The risk of amputation and cardiovascular events are highly probable in these patients. Vascular lesions determine a reduction of distal perfusion and an impairment of blood flow and nutrient exchange to the tissues, severely affecting the microcirculation. Some studies revealed a strong association between CLI and 1-year major amputation rate that can reach 30% of all cases with an overall mortality rate of 25%.[1]

The prevalence of PAD in the general population is 2%–4%, but this value reaches 15% in those over 70 years of age.[2] CLI in older adults (60–90 years old) is estimated at 1% (0.5%–1.2%); the sex ratio male to female is 3:1. From the first assessment, one in ten patients with asymptomatic PAD or claudication will progress to CLI within 5 years.

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Address for correspondence: Dr. Ottorino Del Foco, Department of Vascular Surgery, Yas Clinic Khalifa City, Abu Dhabi, United Arab Emirates.
E-mail: ottorino.foco@adscc.ae

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It has been demonstrated in several studies that over 50% of CLI patients do not complain of any PAD symptoms 6 months prior to the onset of CLI. Diabetes is the major risk factor for PAD; however, smoking, hyperlipidemia, and hypertension need to be considered. Diabetic patients are at least five times more likely to develop CLI than nondiabetic patients [Figure 1]. The anatomical pattern of atherosclerotic distribution in diabetic patients is mostly infragenicular with severe tibial and foot vessels [Figure 2].[3] According to the 2021 International Diabetes Federation statistics, the United Arab Emirates (UAE) has one of the world's highest prevalence rates of diabetes at 16.4% (worldwide diabetes prevalence, 9.8%; 2021).[4] In addition, approximately 40.7% of adults (aged 20–79 years) with type 2 diabetes mellitus are unaware that they have the condition.

Classifications

There have been many clinical classifications to define the degree of limb ischemia. Initial classifications were based on an assessment of clinical and functional limitations. The Fontaine classification with four stages evaluated the severity of claudication, rest pain, and the presence of lesions. Rutherford classification with six stages defines more precisely the severity of claudication. CLI should be considered when patients present signs and symptoms of Stage III and IV of Fontaine or 4, 5, and 6 of Rutherford classification [Table 1].

Both classifications do not assess the risk of amputation and the extent and severity of vascular lesions. These limitations prevent the characterization of subgroups of patients who may need more aggressive revascularization attempts and stricter follow-up.

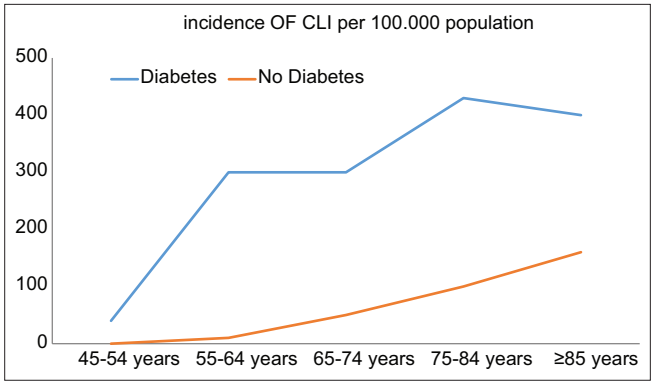

Figure 1: Critical limb ischemia in relation to diabetes (from critical limb ischemia OXVASC 2015)

Table 1: Preferred initial revascularization strategies for infrainguinal disease in average-risk patients with suitable autologous vein conduit available for bypass

Classification	Stage	Clinical description
Fontaine	I	Asymptomatic
	IIa	Mild claudication
	IIb	Moderate-to-severe claudication
	III	Rest pain
	IV	Ulceration or gangrene
Rutherford	0	Asymptomatic
	1	Mild claudication
	2	Moderate claudication
	3	Severe claudication
	4	Rest pain
	5	Minor tissue loss
	6	Severe tissue loss or gangrene

From global (ESVS, SVS, WFVS) vascular guidelines on CLTI management. ESVS: European society for vascular surgery, SVS: Society for vascular surgery, WFVS: World federation of vascular societies, CLTI: Chronic limb-threatening ischemia

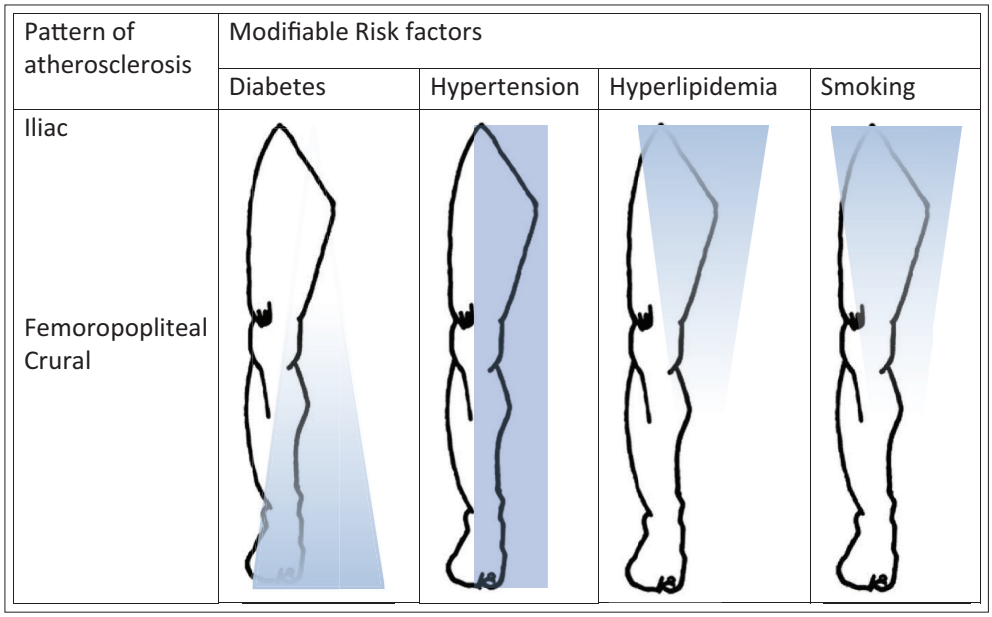

Figure 2: The blue overlay on the anatomic cartoon illustrates the Association of the modifiable risk factor with patterns of atherosclerotic disease[3]

The Global Vascular Guidelines (ESVS, SVS, WFVS) promote clinical staging of threatening ischemia with the WIfi Classification [Figure 3]. This staging considers the presence of wounds, the severity of the ischemia, and the staging of foot infection. The Global Anatomical Staging System defines the complexity and extent of the vascular lesions to define the best surgical or endovascular approach to prevent the amputation, heal the wounds, control the foot infection and restore a pain-free limb [Figure 4].

The decision-making workflow follows the denominate PLAN concept, Patient risk, Limb stage, and ANatomic complexity of disease to tailor the surgical intervention to patient general and local conditions.[5]

Endovascular Treatment

Over the past two decades, we have seen a valuable development of new endovascular tools to solve increasingly extensive and severe stenosis and occlusions. Studies on endovascularly treated patients, their follow-up, and the analysis of the treated arteries' changes have shed light on the mechanisms that lead to restenosis and re-occlusion. Anatomical areas considered unfit for treatment until a few years ago, such as the tibial arteries and the plantar arch, have finally been treated thanks to these advances. These advances made it possible to revascularize extremities with ever greater degrees of ischemia and lesions, showing us the problems that determine the hyperplasia and re-occlusion of the procedures during the follow-up.

Due to intravascular ultrasound, a direct view of the vessel from inside with a 360° live image of all the vascular districts to treat makes it possible to understand the effects of angioplasty on the arterial wall and mechanical lesions in the intima and barotrauma. The nature of the lesions, the presence of thrombosis, fibrosis, calcification, and the actual diameter of the vessels can be evaluated.

New tools such as debulking devices allow us to perform a remote endarterectomy that eliminates obstructions determining an increase of the lumen of the treated artery. The shockwave drastically reduces calcifications, including those limited to the media. Scoring balloons reduce intimal dissections after angioplasty, and balloons and stents coated with drugs such as Paclitaxel and Sirolimus (rapamycin) due to their antimitotic effect reduce intimal hyperplasia, increasing the permeability of the procedures, and reducing the target lesion recurrence. The use of stents is currently considered a bail-out technique, and its use has been drastically reduced. New biodegradable scaffolds will soon hit the market following this trend of reducing the number of implants.

Despite all this, the rate of re-occlusions and the need for re-do treatments at 1 year remains between 30% and 50%. The progression of the disease and hyperplasia can be a cause of it. However, damage at the microcirculatory level can also influence a decrease in tissue runoff and an increase in peripheral resistance that ultimately determines the revascularization failure. The concept of desert foot clearly illustrates a typical hemodynamic situation of diabetic patients. Angiographically, there is a total absence of the microcirculation web at the foot level. In these cases, vascular surgeons have introduced the new techniques of "arterialization" of the distal venous bed by creating arteriovenous fistulas to replace the missing arterial network with the venous one. The results of these techniques have yet to be evaluated. However, the level of ischemia that limits the possibilities of revascularization has passed from the tibial and plantar arteries to the microcirculatory level.

Patients in which, due to the extension of the necrotic lesions or when any revascularization technique is not feasible, are considered a no option (NO). In these cases, according to the PLAN workflow, the only possible treatment is a primary amputation versus palliation treatment and wound care.

Cell Therapy

For more than 25 years, different treatments with stem cells and growth factors (GFs) have been implemented for those cases of ischemia without surgical possibilities. Since the use of vascular endothelial growth factor (VEGF) in 1996 in cases of PAD,[6] several cell lines from different locations have been tested in both animal and human studies, confirming the

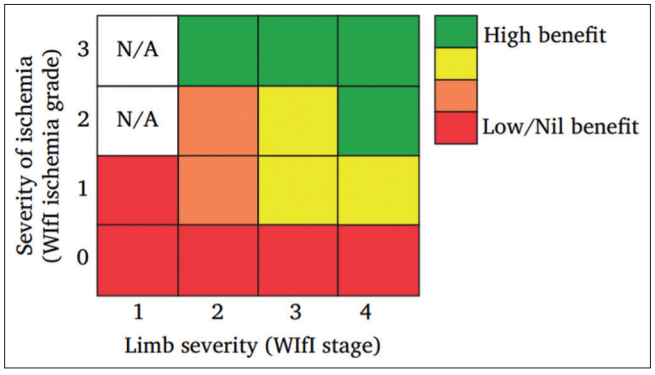

Figure 3: The benefit to performing revascularization increase with the degree of ischemia and with the severity of limb thread (Wound Ischemia and Foot infection WIFi stage). From Global (ESVS, SVS, WFVS) Vascular Guidelines on chronic limb-threatening ischemia Management. Chronic limb-threatening ischemia

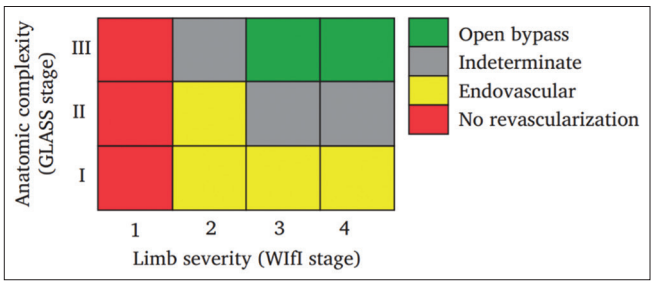

Figure 4: Preferred initial revascularization strategies for infrainguinal disease in average-risk patients with suitable autologous vein conduit available for bypass. From Global (ESVS, SVS, WFVS) vascular guidelines on chronic limb-threatening ischemia management

efficacy of Mononuclear cells (MNCs), endothelial progenitor cells (EPC),[7] and mesenchymal stem cells (MSCs).[8]

The use of GFs with angiogenetic stimulation abilities such as VEGF, hepatocyte growth factor, and fibroblast growth factor-2[6,9,10] have given promising results in stimulating the differentiation towards neo-angiogenesis of somatic stem cells. However, its efficacy is related to the presence of a good "stem cell niche." From what is known so far, in advanced CLI phases, especially in diabetic patients, oxidative stress, glycol-toxicity, and the presence of chronic inflammation act as negative factors for the maintenance of a good "niche." Therefore, the response to GFs is impaired due to the cellular dysfunction that affects both proliferation and mobilization.[11]

MSCs and MNCs have been evaluated in preclinical studies and have shown some angiogenetic activity[12] demonstrated *in vitro* and *in vivo*. This activity appears mediated by the secretion of immunomodulatory solid and pro-angiogenetic power mediated by a paracrine pathway.[13]

The recent interest in MSCs and their potential angiogenetic efficacy was stimulated by the possible allogenic use.[14] The possible use of allogeneic MSCs would make it possible to have "off-the-shelf" cell preparations to avoid the harvesting procedure and reduce the costs related to cell expansion.[15]

Stem cell-based therapeutic angiogenesis, with MNCs transplantation obtained from bone marrow (BM) or peripheral blood (PB), is being used increasingly in clinical trials that attempt to treat NO patients.[16-24] Several phase I/II trials have confirmed the potential therapeutic benefits of MNCs transplantation, its safety, and its feasibility have been confirmed by several phase I/II trials. However, the curative effect has not been confirmed in the different population studies. Several trials demonstrate the positive therapeutic efficacy of MNC or purified $CD34^+$ cell transplantation in treating NO patients to avoid major amputations and increase wound healing.[23,8,25,26] Conversely, other studies have observed an insignificant moderate prognosis following such therapeutic approaches relative to conservative treatments or placebo.[16,20]

The reason for these results lies in two aspects that the same studies have begun to specify. One of them is the importance of $CD34^+$ cells. The low dosage of transplanted $CD34^+$ cells is crucial for ineffective revascularization and restoration of blood supply.[27] Meta-analyses have revealed that patients do not respond favorably to a low dosage of transplanted $CD34^+$ cells.[5,27-29]

Second, limiting these treatments only to NO patients with vast areas of necrosis and extensive damage in the macro and microcirculation make the possible increase in vascularization clinically ineffective.

The Theoretical Argument for Combined Treatment

Considering all studies to date, GFs, MSCs, and $CD34^+$ cells offer immense potential. However, the continuous progress in endovascular revascularization techniques nowadays allows treatment areas considered without surgical option until a few years ago, especially the arteries below the knee and ankle. The latter has drastically reduced the number of NO patients and opened the door to an increasingly comprehensive treatment.

Despite the immediate results of the new endovascular techniques, the number of treated patients who require new revascularization attempts at 6 months or 1 year remains high.

Many reasons are attributed to this; concepts such as neo-intimal hyperplasia due to chronic inflammation, calcification, recoil, runoff, angiosomes, thrombosis, dissections are cited singly or jointly as responsible for the reduction of permeability.

The potential effect of neo-angiogenesis of $CD34^+$ cells, the immunomodulatory power of the MSCs, and the GF effect on stimulation and differentiation and facilitating the homing of the transplanted cells can play a crucial role in increasing microcirculation. The decrease in peripheral resistance can contribute in a tangible way to maintaining the permeability of endovascular procedures.

Cell Therapy Proposals

The Global (ESVS, SVS, WFVS) Vascular guidelines restrict therapeutic angiogenesis to registered clinical trials. Particular attention is drawn to the determination of biomarkers imaging that assists in understanding the mechanism of action and determining if the cell-based therapies can improve clinical outcomes as an adjunct to surgical revascularization [Table 2].

Earlier studies were based on observing the angiogenetic response of cells from the BM without a unique typing. In the latest phases studies, different cells have been selected to evaluate their power in favoring neovascularization. Neo-angiogenesis involves different cells and factors and

Table 2: European society for vascular surgery, Society for vascular surgery, World federation of vascular societies guidelines on chronic limb-threatening ischemia management

8.1. Restrict use of therapeutic angiogenesis to CLTI patients who are enrolled in a registered clinical trial	
Grade	**Level of evidence**
1 (strong)	B (moderate)
Research priorities for biologic and regenerative medicine approach in CLTI	
	Recommendations
8.1	Identify surrogate markers (biomarkers, imaging) that would assist in understanding the possible mechanisms of action of gene-and cell-based therapies in CLTI
8.2	Determine whether gene-or cell-based therapies can serve as an adjunct to revascularization to improve clinical outcomes in subsets of CLTI patients

CLTI: Chronic limb-threatening ischemia

transplanting $CD34^+$ cells into an ischemic area, whether due to intramuscular injections or intra-arterial infusion, offers serious problems of cell homing due to ischemic tissue conditions and chronic inflammation. The immunomodulatory effect of MSCs and the chemotactic and the activating effect of GFs, induce better engrafting and angiogenetic effects.

Work is underway at our institution to create a complete cellular cocktail adjunct to surgical revascularization treatment in controlled trials. We propose that acting at the microcirculatory and immunological level can reduce postsurgical hyperplasia, increase tissue perfusion, and ultimately prolong the patency of revascularization procedures.

Cellular Products

As pointed out above, $CD34^+$ stem cells, MSCs, and the GFs are the main cell therapy $CD34^+$ stem cells are obtained from PB by apheresis harvesting after mobilization with granulocyte colony-stimulating factor (G-CSF).[30] Our protocols describe autologous blood donation after G-CSF mobilization in a closed bag system to collect $CD34^+$ enriched MNCs by density gradients. With this method, 60 ml of plasma rich in MNCs with a percentage of monocytes between 20% and 35%, and a median of 25 $CD34^+$ cells/μL are obtained (unpublished results). The advantage is the collection of a high $CD34^+$ cell count, avoiding adverse reactions from apheresis collection, particularly in patients not eligible for this procedure.[31] On the other hand, a comprehensive characterization of this product is necessary, which includes detecting EPCs with the co-expression of CD133 and CXCR4 in the $CD45^-$cell fraction and the expression of stemness markers in the cellular cocktail.[32,33]

MSCs have immunosuppressive abilities and are weakly immunogenic in humans after allogeneic infusion, and are capable of differentiating into many cell lineages, including bone, cartilage, tendon, muscle, or adipose tissue, produce a significant number of vascular GFs, and have been confirmed *in vitro* to differentiate myocardium and endothelial cells.[34] Also, MSCs are obtained from different tissues such as adipose tissue, umbilical cord matrix (Wharton's gelatin), BM, periosteum, the villous chorion, and dental pulp.[35] Since many cells are needed in clinical protocols (2×10^6 cells/kg of body weight), it is necessary to carry out *ex vivo* expansion of these cells.[36]

Our strategy is to produce MSCs following good manufacturing practices in a bioreactor system that enables a higher harvest rate with increased cell proliferation and recovery rates. An appropriate MSCs characterization is also required to understand better the factors that can contribute to efficacy. MSCs cell surface markers have wide variability related to source and manufacture that can influence results.[37]

The use of GFs as a supplement to improve tissue remodeling has been widely studied in the literature. Platelets are a source of GFs that potentially improve angiogenic function for cell therapy in treating ischemic tissues, especially VEGF.[38]

GFs derived from platelet-rich plasma (PRP) can induce stem cell differentiation, proliferation, and adhesion. Therefore, the combination of both therapies could be an advantage in regenerative medicine treatments.[39] Standardization in obtaining PRP is desirable because platelet count influences GFs concentration that can cause variability in clinical results. We obtain GFs from PRP by ultrasonic waves that is an effective method, and the product can be stored without reducing its biological activity.[40]

Conclusions

Diabetic patients present CLI at younger ages, with a generalized anatomical distribution and more aggressively due to the involvement of the microcirculatory network and the depletion of the pool of somatic stem cells. Surgical techniques, especially endovascular ones, have undergone important advances that allow most patients to be treated. However, the reoperation rate is still high.

Cell therapies experienced for more than 20 years are already a reality with promising results. Thanks to the high typification of the cellular products clinical studies for a combined surgical and cellular treatment of patients with CLI will be possible. Close collaboration between physicians and scientists is critical for comprehensive management of patients with CLI.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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