STEM CELL THERAPY FOR NO-OPTION CRITICAL LIMB ISCHEMIA

A DISSERTATION SUBMITTED TO

THE TAMIL NADU Dr M.G.R MEDICAL UNIVERSITY

In partial fulfilment of the regulations for the award of the

M.S DEGREE EXAMINATION

BRANCH 1 GENERAL SURGERY

DEPARTMENT OF GENERAL SURGERY

STANLEY MEDICAL COLLEGE & HOSPITAL

THE TAMIL NADU Dr M.G.R MEDICAL UNIVERSITY CHENNAI

APRIL 2016

CERTIFICATE

This is to certify that the dissertation titled " STEM CELL THERAPY FOR NO-OPTION CRITICAL LIMB ISCHEMIA" is the bonafide work done by Dr Kitaka Sukhato Wotsa, Post Graduate student (2013 - 2016) in the Department of General Surgery, Government Stanley Medical College & Hospital, Chennai under my direct guidance and supervision, in partial fulfillment of the regulations of The Tamil Nadu Dr M.G.R Medical University, Chennai for the award of M.S Degree (General Surgery) Branch-1, examination to be held in April 2016.

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DECLARATION

I, Dr KITAKA SUKHATO WOTSA, solemnly declare that this dissertation titled "STEM CELL THERAPY FOR NO-OPTION CRITICAL ISCHEMIA" is a bonafide work done by me in the Department of General Surgery, Government Stanley Medical College & Hospital, Chennai, under the guidance and supervision of my unit chief, Prof. Dr C. BALAMURUGAN.

This dissertation is submitted to the Tamil Nadu Dr M.G.R Medical University, Chennai in partial fulfillment of the University regulations for the award of M.S Degree(General Surgery) Branch-1 Examination to be held in April 2016.

Place : Chennai **Dr KITAKA SUKHATO WOTSA**

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INTRODUCTION

Critical limb ischemia(CLI) is defined as persistent, recurring ischemic rest pain requiring opiate analgesia for atleast 2 weeks with resting ankle systolic pressure lower than 50 mm Hg or ulceration/gangrene of the foor with the same resting pressures. CLI represents the end-stage peripheral arterial disease(PAD). The clinical course of Peripheral artery disease patients without CLI is mostly stable, however, once it progresses to CLI, the morbidity increases. Approximately 40% of patients with CLI are not amenable for revascularisaton, either by open surgery or endovascular methods. Such patients are categorized as No-Option CLI and 40 % of patients with No-option CLI end up with major amputation within 6 months[1]. Major amputation is associated with poor prognosis for life and rehabilitation[2].

A SAGE group published article concludes that PAD afflicts over 30 million

in India and almost 3 million suffer from CLI. The background clinical problem is atherosclerosis or thromboangitis obliterans(TAO). Patienst with CLI due to TAO present at much younger age. The amputation rate is 3-10 times higher among smokers and 30 times higher in diabetics. Diabetics represent 50% to 70% of CLI patients. With 40 million diabetics, India has become known as the 'Diabetis Capital', and may soon become " CLI capital of the world ".

Hence, newer treatment strategy need to be developed to handle this increasing number of patients and ameliorate the burden of CLI.

Knowledge about angiogenesis arising out of cancer research has raised interest in using therapeutic angiogenesis as an alternative treatment in patients with No-option CLI. The aim of therapeutic angiogenesis is the induction and augmentation of collateral artery growth which is the most

important physiologic repair mechanism in the peripheral arterial occlusive disease [3].

The landmark studies of Asahara, Isner and colleagues pioneered the use of cell therapy in the treatment of limb ischemia [4]. Several experimental studies have documented the ability of bone marrow-derived mononuclear cells or of purified fractions of such cells, primarily EPCs or mesenchymal stem cells to relieve ischemia-induced perfusion defects and to increase vessel density in ischemic tissues [5-7].

ABBREVIATIONS

1. **CLI – Critical Limb Ischemia**
2. **PAD – Peripheral Arterial Disease**
3. **TAO – Thromboangitis Obliterans**
4. **EPC – Endothelial Progenitor Cells**
5. **VEGF – Vascualar Endothelial Growth Factor**
6. **PDGF – Platelet derived Growth Factor**
7. **BM – Bone marrow**
8. **ABI- Ankle Brachial Index**
9. **TACT- Therapeutic Angiogenesis using Cell Transplantation**
10. **BMAC- Bone marrow aspirate concentrate**

Review of literature

Critical limb ischemia(CLI) is defined as persistent, recurring ischemic rest pain requiring opiate analgesia for atleast 2 weeks with resting ankle systolic pressure lower than 50 mm Hg or ulceration/gangrene of the foor with the same resting pressures. The incidence of CLI is 500-1000 individuals per million persons per year [8-9] .As CLI progresses most cases eventually result in amputation of the affeceted limb. Incidence of amputation is 20-30 times higher in diabetics with CLI [8]. The 1 year mortality associated with CLI is approximately 25% and it increases to 45% after amputation [10]. Patients with CLI have similar quality of life index scores like patients with end-stage cancers [11].

Despite all the recent advances in open and endovascular techniques, approximately 40% of the CLI cases are not candidates for revascularisation procedures and such cases are termed as No Option CLI [12-13]. With no

effective pharmacological therapy available [14], amputation remains the only option available. However even after amputation full mobility is observed only in 25-50%, as the peri-operative mortality is around 5-20% and a subsequent re-amputation at higher level is done in approximately 25-30% of patients [9]. Post amputation rehabilitation related cost is also estimated to be twice that of patients with successful revascularization[15]. Considering the increased mortality and morbidity associated with No Option CLI, it is of outmost importance to explore new treatment strategies for revascularizing the ischemic limbs. Several studies have identified bone marrow(BM) derived stem cells to increase vessel density and improve perfusion in ischemic tissues.

<u>Bone marrow derived stem cells and neovascularization: background</u>

Asaharaa *et al* demonstrate that peripheral blood contains endothelial progenitor cells or mesenchymal stem cells, which can differentiate into functional endothelial cells [16]. Since then several studies have confirmed that

the number of circulating EPC increase in conditions of ischemia [17-18], and these EPCs are specifically seen in more concentrations at sites of ischemic limb and myocardium [18-20]. BM-derived cells also promote neovascularization through paracrine effect on resident EC by secreting cytokines and proangiogenic factors like VEGF, PDGF, etc [21]. In a animal hind limb ischemia model, autologous BM-derived mesenchymal stem cell injected intramuscularly (IM) was shown to improve angiogenesis and collateral vessels increased significantly [22] . In another study involving rat's hind limb ischemia model, both IM and IA delivery of BM-derived mesenchymal stem cells showed similar results [23] . Kalka *et al* observed increased capillary density and blood flow in ischemic hind limb of athymic nude mice when human EPC was injected IM [24] . Human umbilical cord blood derived mesenchymal stem cells was also observed to significantly augment neovascularization in ischemic limbs and myocardial ischemia [25].

Several large, randomized clinical trials studying the benefits of BM derived mesenchymal stem cells or EPC in promoting therapeutic angiogenesis in ischemic limbs have already been conducted and many more ongoing. Results of which are promising.

The pioneering study on efficacy amd safety of BM derived stem cells for therapeutic angiogenesis in CLI was published by the TACT in 2002. Twenty five patients with CLI received IM injections of BM-derived stem cells in leg with CLI and saline as control in the contralateral limb. Improved ankle-brachial index(ABI), transcutaneous oxygen pressure(tcpO2) was noted with relief of rest pain and increased claudication distance at 4 and 24 weeks. In the second part of the trial, twenty two patients with bilateral limb ischemia were enrolled. BM derived stem cells were injected in one limb and peripheral blood mononuclear cells in the other limb. ABI, tcpO2 and pain at rest improved in the BM-derived stem cells injected leg, while the other leg showed

much smaller increases. The study concluded that EPC containimg CD 34 + cells and proangiogenic factors, present in the bone marrow, but significantly less in peripheral blood, was responsible for the efficacy of the treatment.

Higashi *et al* reported that acetylcholine(Ach) could improve the blood flow in limbs indicating normalization of endothelium[26].

Several trials on combined IM and IA injections of autologous BM derived stem cells have also shown significant increase of ABI, tcpO2 and pain free walking distance [27-29].

Thus, progenitor cell-based therapy for CLI is, without doubt, a safe and efficacious treatment modality to promote neovascularization in ischemic tissues resulting in limb salvage, improved quality of life and CLI downstaging.

Clinical application: many questions yet to be answered

Stem cell therapy has shown encouraging results but due to lack of larger randomized, placebo - controlled, blinded trials, many questions still remain unanswered. Questions regarding best route of delivery, the dose, impact of bone marrow cell dys function needs to be answered.

Routes of administration: In both animal and human studies, the three routes of administration- IM, IA and combination of IM and IA, are found to be safe and promising. IM implantation results in local concentration of the stem cells in the target muscles and promotes neovascularization [30]. Some studies, however, on stem cell viability after implantation into ischemic myocardium of animal models showed that only 10% of the cells survive four days after the implantation [31, 32]. No human studies have been done yet. With IA administration, the stem cells reach the borders of the ischemic muscles by blood flow [33-35]. Stem cell survival is optimum due to favourable environment

of adequate nutrients and oxygen available in circulation. But stem cell uptake from circulation may be limited as it involves migration of the stem cells out of the vessel into the surrounding ischemic tissues [36]. No published studies are available at present which compares the efficacy of different routes of stem cells administration in patients wit CLI.

<u>Optimal dose</u>: In studies involving bone marrow stem cell administration, the amount of bone aspirated varied from 70 ml to 1 litre. In TACT trial and another trial by Higashi *et al* [37] , 500 ml of bone marrow was aspirated and around 1.6 x 10^9 Mononuclear cells were obtained. CD34+ cells in the isolated mononuclear cell population ranges from 0.6% to 2.4 % [38,39]. Studies on animal models of myocardial infarction show dose dependent potential of CD34+ cells for neovascularisation[40].

Bone marrow cell dysfunction : It has been documented that persons with risk factors for cardiovascular disease have decreased levels of EPC and certain degrees of EPC dysfunction [41]. In diabetic patients with PAD, the EPCs have reduced adhesiveness and clonogenic capacities [42]. BM-derived stem cells from persons with chronic ischemic heart diseases were also found to have greatly reduced capacity for neovascualrisation [43] and thus raises the question of therapeutic potential.

Several drugs are being studied to improve the EPC function. Statins can improve proliferation, migration and adhesive properties of EPCs [44-47]. Pretreatment of BM-derived stem cells with nitric oxide synthase improves migratory capacity and significantly better neovascularization is seen in ischemic hind limb models [48]. Hypoxic preconditioning is also proven to have similar effects [49]. In vitro studies, following VEGF gene transfer, enhanced EPC proliferation and incorporation into endothelial cell was seen [50].

Dietary supplementation of vitamin E, C and L-arginine along with IV administration of autologous BM cells resulted in significantly better neovascularization in mouse ischemic hind limb models [51].

<u>Clinical evaluation</u> : A major weakness in neovascularization research is related to scientific assessment of vascular growth [52].

According to TASC 2 recommendations, multiple parameters should be assessed to evaluate treatment success, like ankle pressure, toe pressure, tcpO2, laser Doppler fluxometry as well as radiographic imaging [53]. Quality of life questionnaires like EuroQoL 5D-5L and Short Form 36 can provide sunjective information on effects of the therapy. Questionnaires for pain like Visual analog scale can also be used to evaluate effectivenss of therapy. Ulcer healing status can be assessed by grading ulcers before and after therapy using Wagner ulcer grade classification. Rutherford and Fontaine criterias can be used to assess the limb status. High resolution Magnetic resonance

angiography(MRA) with contrast can provide excellent quality of images to assess neovascularization, size of collaterals and changes in local tissue perfusion.

BM derived stem cells for the treatment of No-Option CLI is a potential new therapeutic target and several clinical trials have shown promising results. Hopefully, in the near future, studies will throw more light on the mechanisms underlying the beneficial effects of stem cell therapy, which will help in designing more specific and targeted therapy.

<u>Future perspectives</u>

Stem cell therapy is still being developed and optimized for the treatment of PAD and PAD with No-Option CLI specifically. Combining gene therapy could be a logical option for future research. To potentiate IM or IA administered stem cells, a pre-treatment with gene therapy in the target calf muscles can be tried. Gene therapy of stem cells ex-vivo can enhance

differentiation capacity, migration and incorporation into the endothelial cells. EPC dysfunctions can be managed by pharmacological interventions with drugs like HMG-CoA reductase inhibitors [54] , erythropoietin [55] and AT 2 receptor antagonists [56]. *Ex-vivo* pre-treatment of the stem cells with statins and certain anti-hypertensive drugs is also believed to improve function and neovascularization *in-vivo* [57-63].

Technically, *In vitro* filtering out of unwanted and pro-atherosclerotic cells and administering only the pro-angiogenic cells can improve therapeutiv efficacy. However as of now the exact /or combinations of cells responsible for neovascularization is not known.

The need for newer therapeutic treatments for No-Option CLI is urgent but possibility of adverse effects should not be disregarded. With currently available data the side effects appear to be minimal or none. Patients treated

with stem cell therapy should have continued monitoring and long term follow-up to provide strong evidence to make long-term safety conclusions.

AIM AND OBJECTIVES

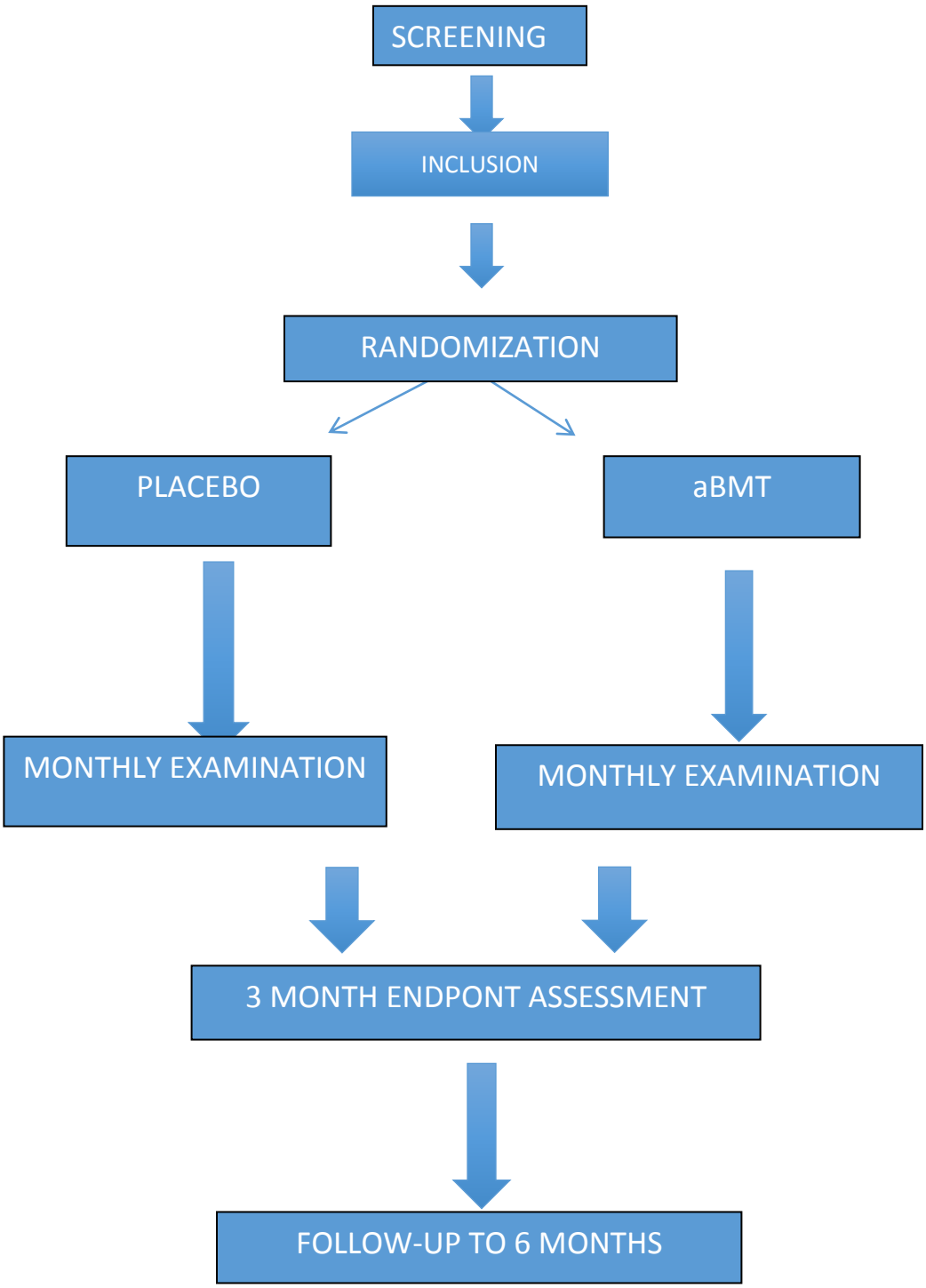
Aim. **The aim of this study is to assess the safety and efficacy of transplantation of autologous bone marrow concentrates in "no-option" patients to restore blood perfusion by collateral flow and limb salvage.**

Objectives. **40 consecutive patients affected by CLI with no option for revascularization will be enrolled. One arm with 20 subjects will be treated with a concentrate of autologous bone marrow cells which will be injected at multiple sites into the ischemic limb. In the other arm, study subjects will be managed conservatively. At 3 months, a combined primary endpoint of major amputation or persisting critical limb ischemia(no clinical or perfusion improvement) will be evaluated. Secondary endpoints are death, wound healing, quality of life, walking distance, ABI, Absolute ankle blood pressure, minor amputations, and cancer incidence. Post study follow up is upto 6 months.**

Time course of study and follow-up activities

	0	1m	3m	6m
Inclusion/ Exclusion criteria	**X**			
Clinical assessment	**X**	**X**	**X**	**X**
Study intervention	**X**			
ABI	**X**	**X**	**X**	**X**
AE assessment		**X**	**X**	**X**
Qol, VAS	**X**	**X**	**X**	**X**
Walking distance	**X**	**X**	**X**	**X**
Endpoint assessment			**X**	**X**
Tumour screening				**X**

Methods and design



Investigator initiated, prospective, randomized, single-blind, single center trial in Chennai,India. Start in November 2014, expected end of recruitment March 2015, final results September 2015.

<u>Study hypothesis</u>: in otherwise untreatable CLI, implantation of bone marrow cells into ischemic limb can:

1) **effectively improve critical ischemia**
2) **reduce the number of major amputations**

<u>Sample size and randomization</u>

40 CLI patients will be included. They will be randomly assigned in a 1:1 manner to aBMT or placebo-procedure. Randomization will be done using block randomization method.

<u>Inclusion criteria</u>:

1) **Ankle-brachial index less than or equal to 0.4**
2) **Absolute ankle blood pressure less than 50 mmHg**
3) **Severe PAD (Rutherford category 4,5 & 6)**
4) **No surgical or interventional option for revascularization as confirmed by a vascular surgeon, or failed revascularization attempt.**
5) **Age 18 -80 years**
6) **Signed informed consent**

Exclusion criteria:

1) **Expected life span less than 6 months**

2) **Known bone marrow diseases which preclude transplantation(eg leukemia,myelodysplastic syndrome, bone marrow metastases,marrow lymphoma, and similar)**

3) **End stage renal failure on regular dialysis treatment**

4) **Current smoker**

5) **Previous(5 years) or current history of neoplasms**

6) **Preganacy**

7) **Acute life threatening complications of limb ischemia with the need for immediate limb amputation to avoid death or clinical deterioration.**

Study procedures

aBMT: Patients randomized to the aBMT procedure will undergo aspiration of 240 ml bone marrow by standard Yamshidi technique from iliac crest under analgosedation with propofol. The bone marrow cell aspirate will be processed 'point of care' bedside with the Harvest Smart Prep centrifuge. The resulting concentrated bone marrow cell suspension with a volume of 40 ml will be injected at multiple sites into the muscles of the ischemic limb, following the anatomic course of the obstructed arteries. Injections will be guided by angiography and administered into areas of lowest perfusion and distally thereof. Injections will start about 2 cm proximal to the arterial

obstruction and will be 1.5-2 cm apart. If wound is present, upto 5 injections will be given around the wound and into the wound bed itself.

Conservative management: Patients randomized to the conservative arm will receive antiplatelet drugs, cilastrazole for duration of the study.

Duration and follow up

Following conclusion of the study procedure, monthly study visits until end of study protocol are performed by an examiner. At 3 months, the patient, in conservative arm, is offered aBMT. Further follow-up examinations are performed at 6 months.

Endpoints

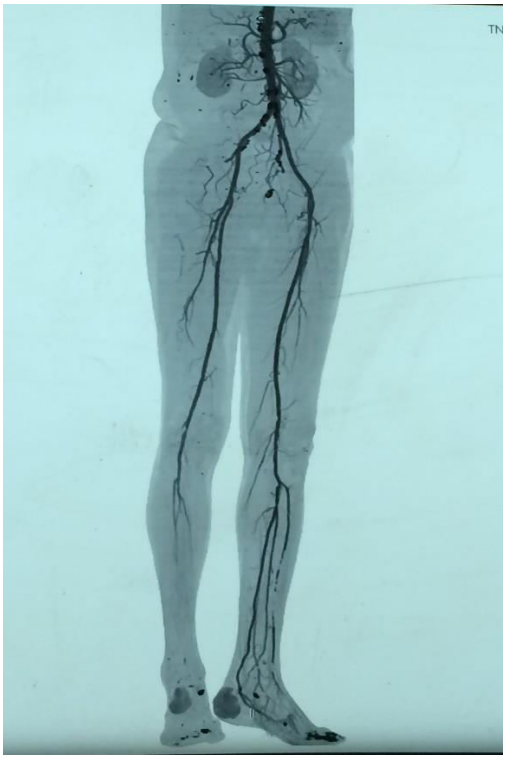
The primary endpoint is the combined incidence of either major amputation(above ankle) of the index limb after 3 months, or persisting,

unchanged critical limb ischemia of the index limb after 3 months. Persisting unchanged CLI is defined as less than 15% change in ABI or absolute ankle pressure.

Secondary endpoints are survival, wound healing(wound size, wound stage according to the Wagner classification9) , analgesics use, Rutherford/fontaine classification, walking distance, quality of life(EQ-5D questionnaire), tcpO2, ABI, absolute ankle perfusion pressure and the rate of minor(below ankle) amputations in the index limb. All these endpoints will be registered in the follow-up period as well

Assesment	1 month	3 months	6 months
Major amputation	X	X	X
Persisting CLI	X	X	X
Wound stage	X	X	X
Analgesics use	X	X	X
Rutherford & fontaine classification	X	X	X
Walking distance	X	X	X
Quality of life(EQ-5D-5L questionnaire)	X	X	X
SF 36V2	X	X	X
ABI	X	X	X
Absolute ankle perfusion pressure	X	X	X
Minor(below ankle) amputations	X	X	X

Statitistical analysis: All statistical analysis will be performed usng Microsoft excel 2010 and Epi Info version 7. Two sided $P < 0.05$ will be regarded as statistically significant. Expected survival rate will be estimated using Kaplan-Meier method. Log rank test will be doen to test association between patient characterictics and amputation free survival. To identify the prognostic factors independently associated with the primary endpoints and to estimate the hazard ratios, Cox proportional hazards model with stepwise selection will be applied. Factors affecting the primary endpoints will be evaluated by uni- and multivariate analysis.



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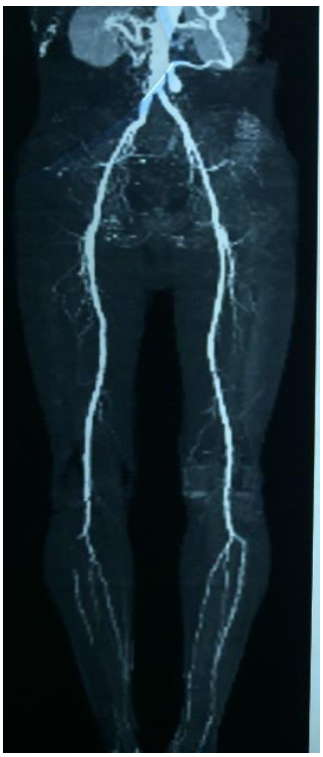

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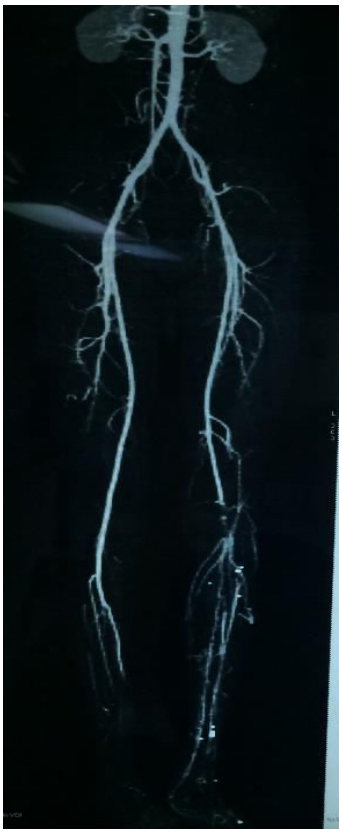

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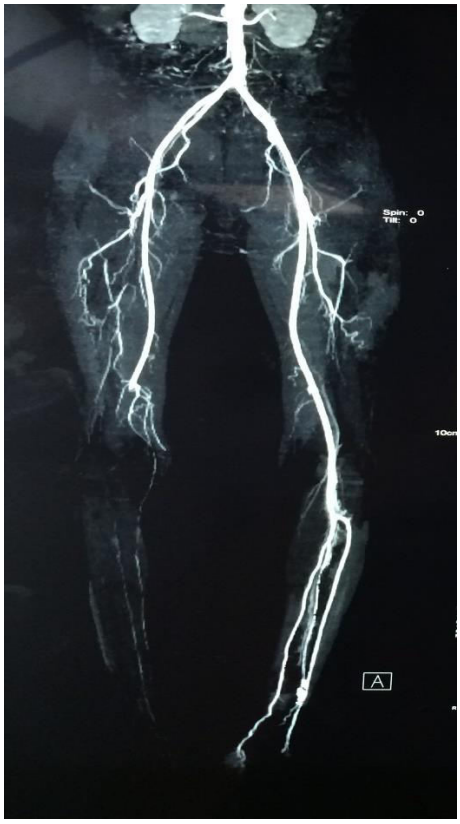

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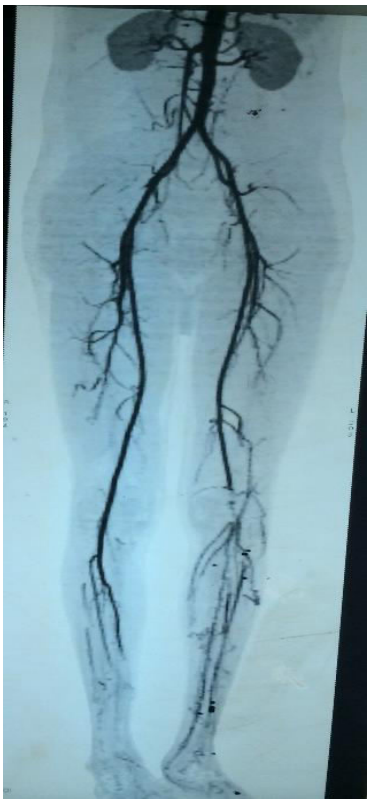

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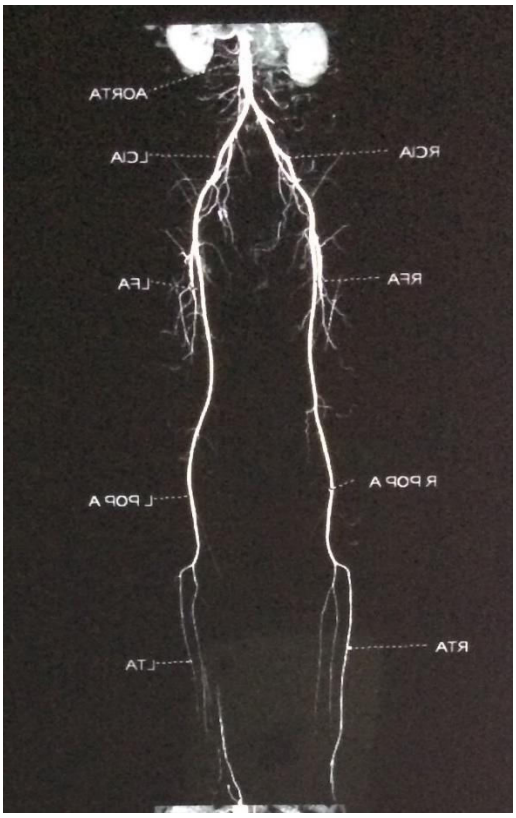

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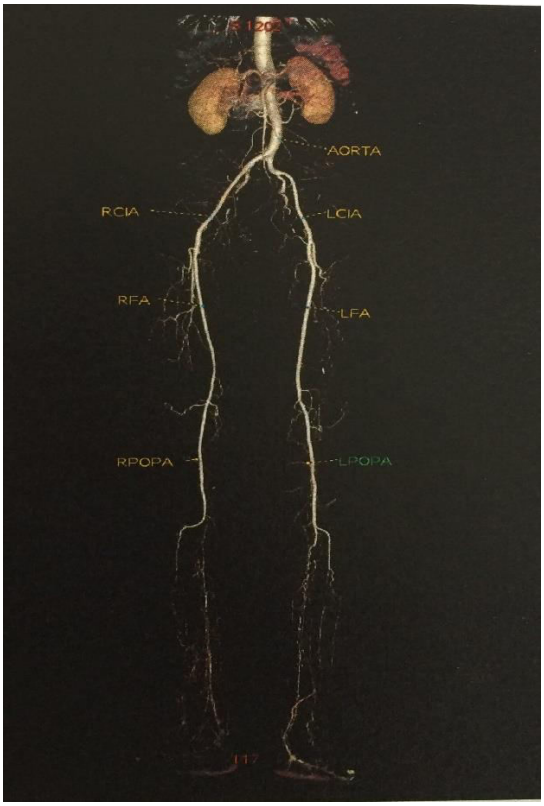

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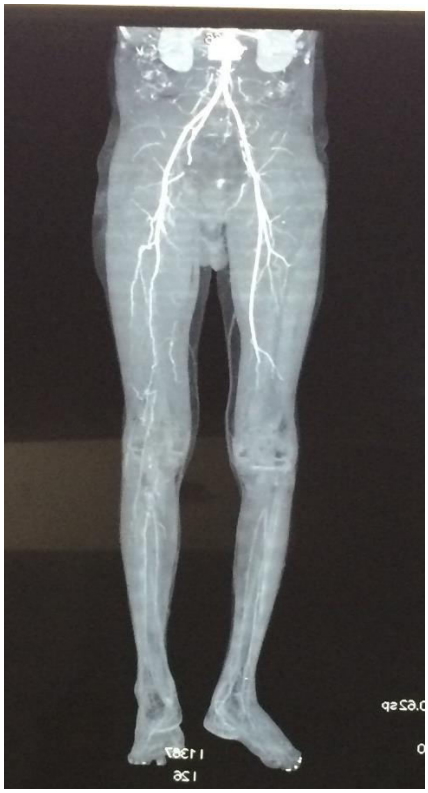

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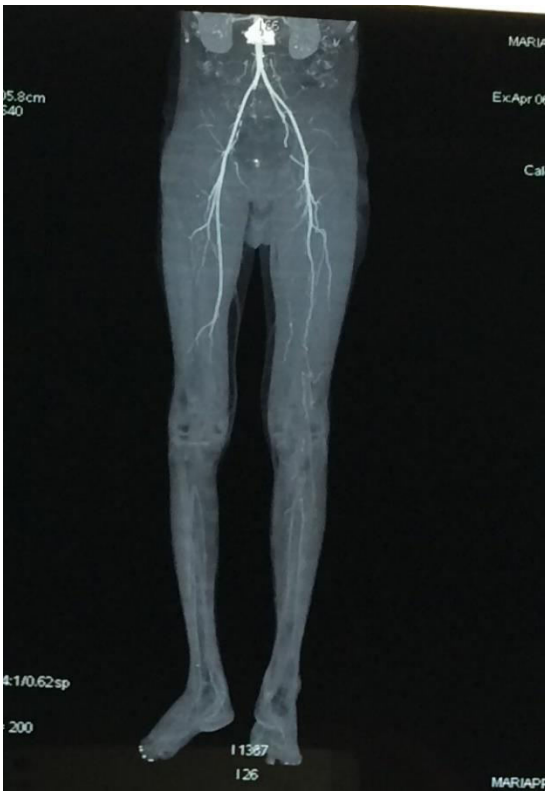

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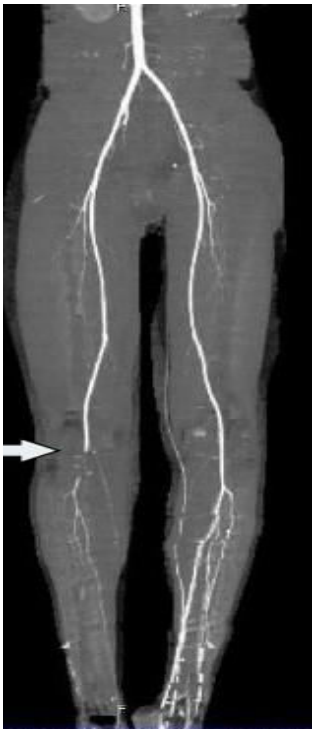

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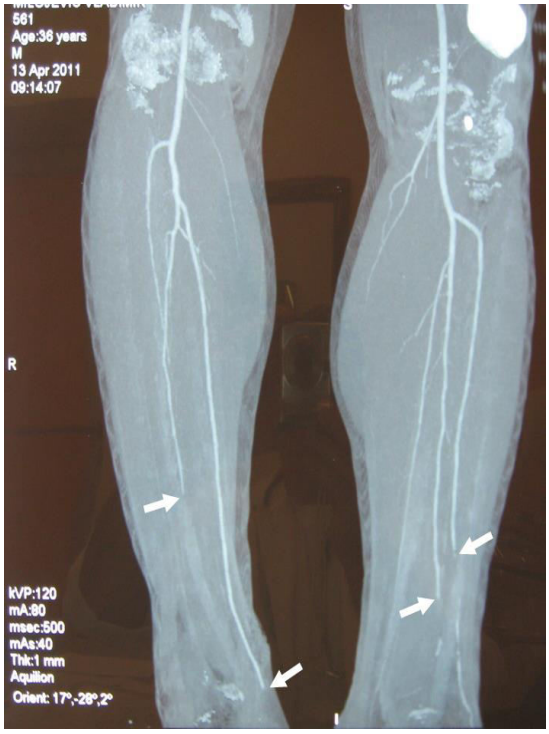

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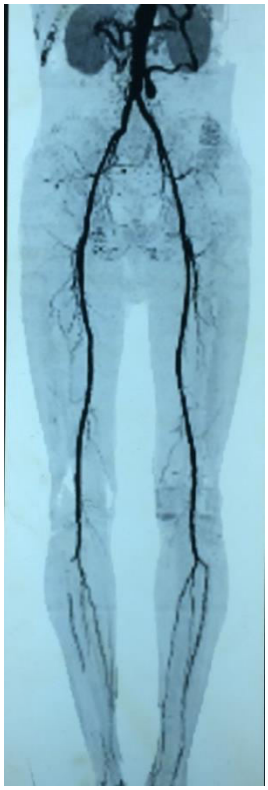

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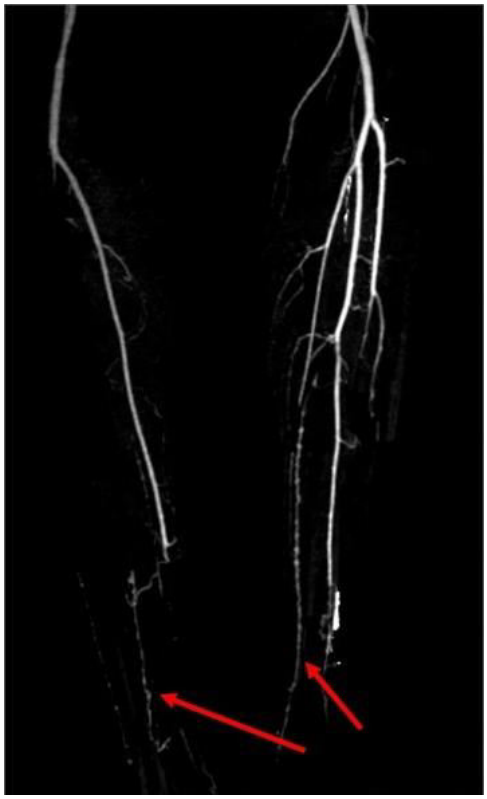

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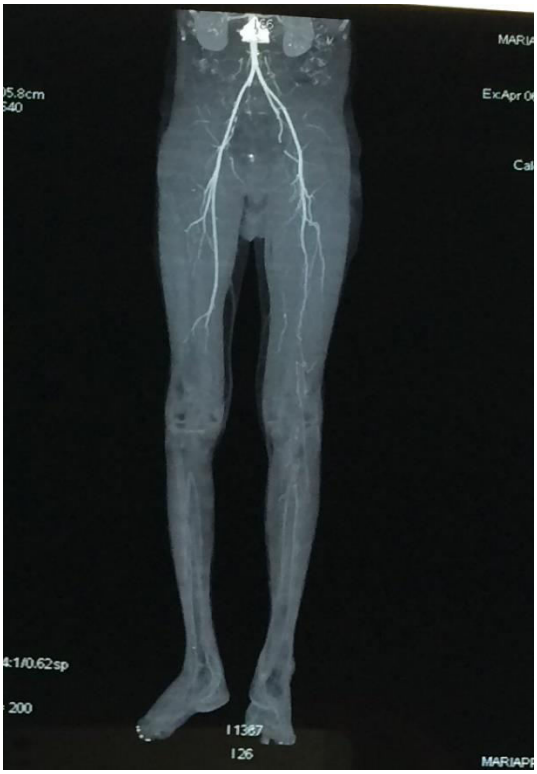

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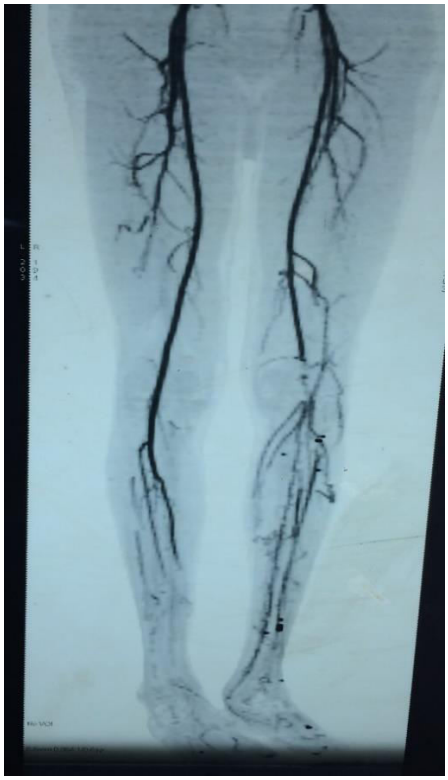

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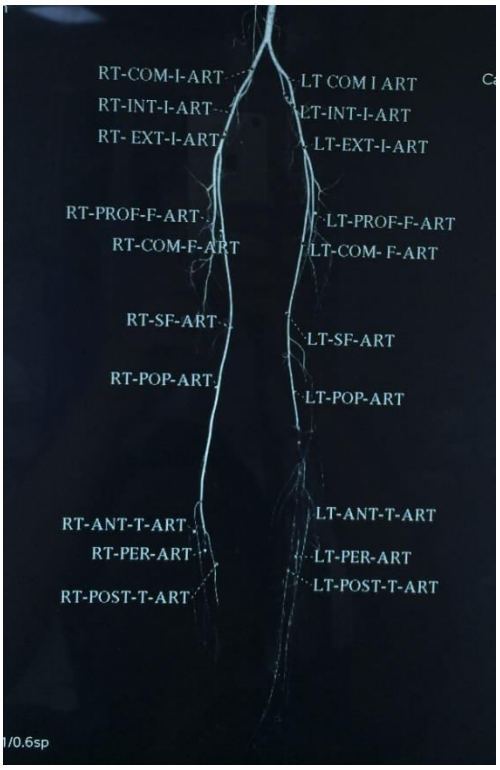

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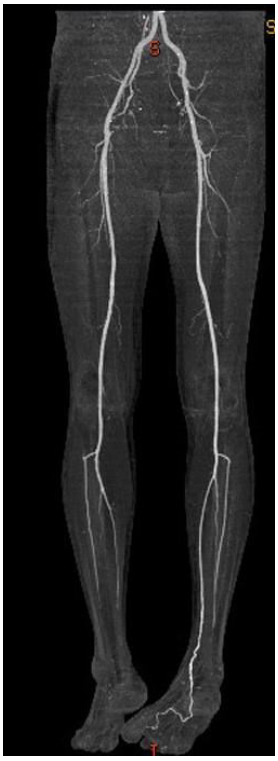

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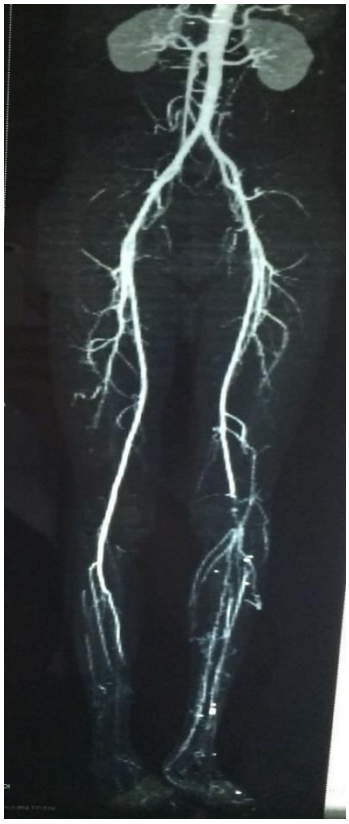

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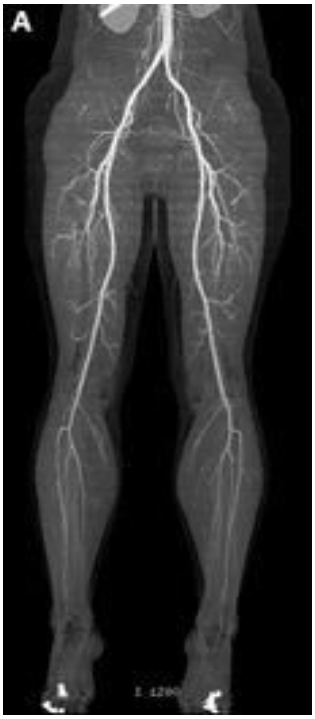

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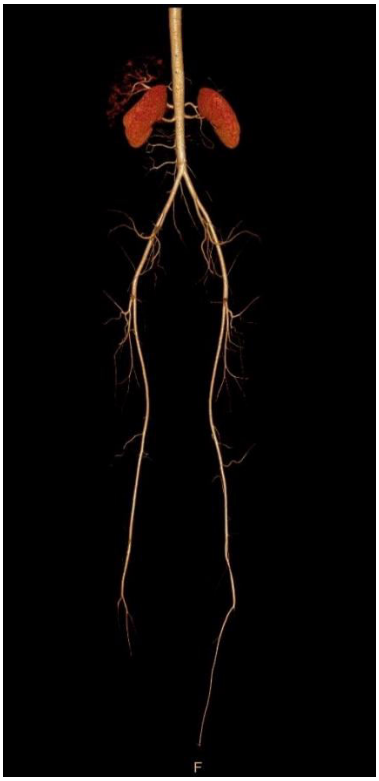

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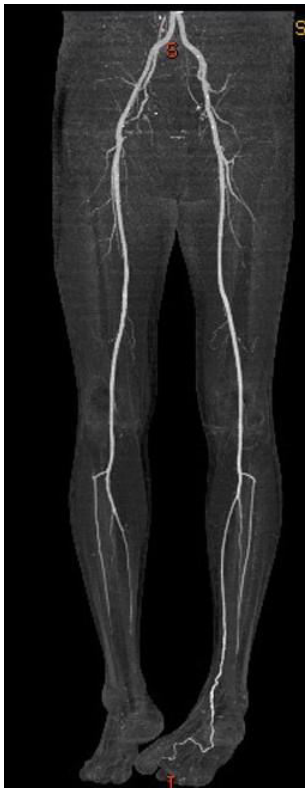

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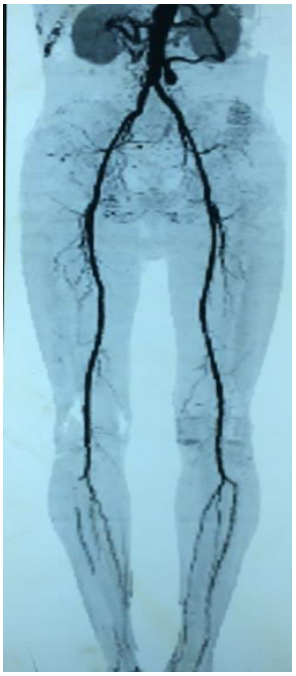

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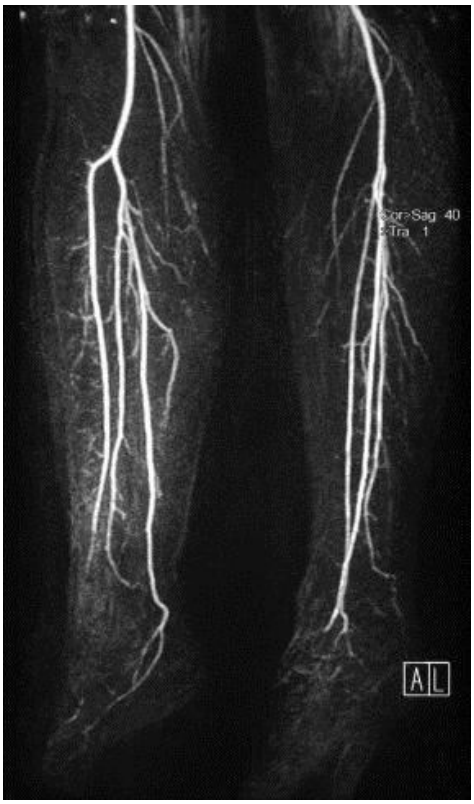

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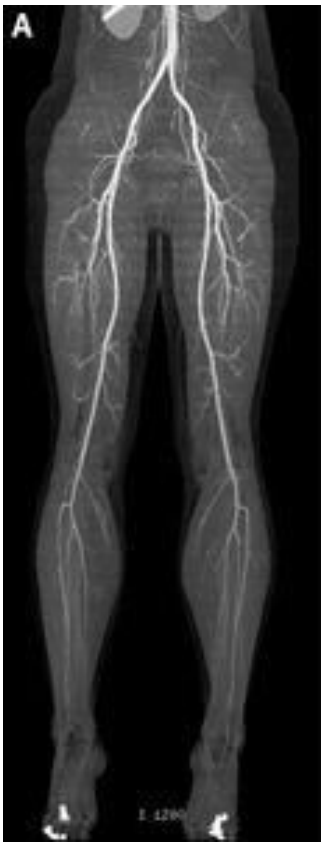

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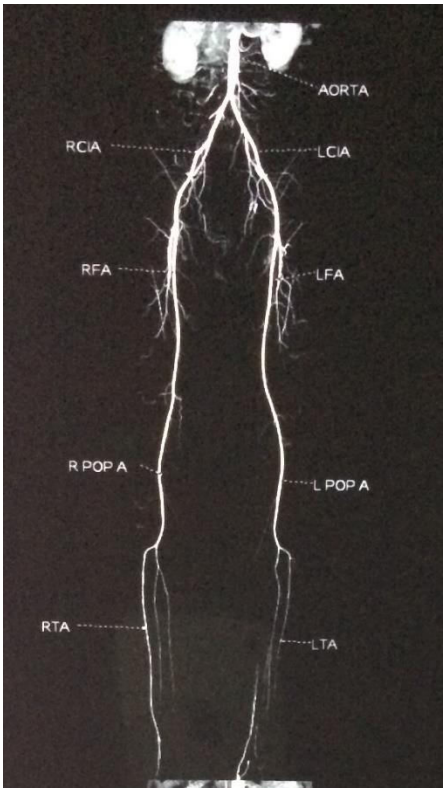

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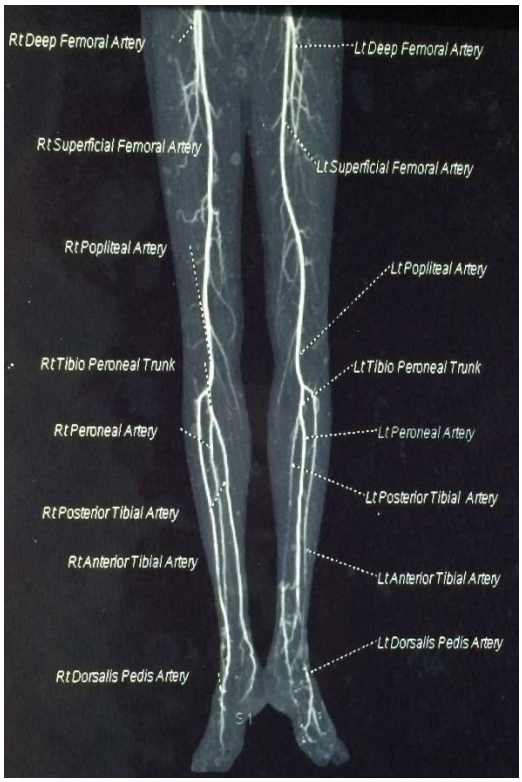

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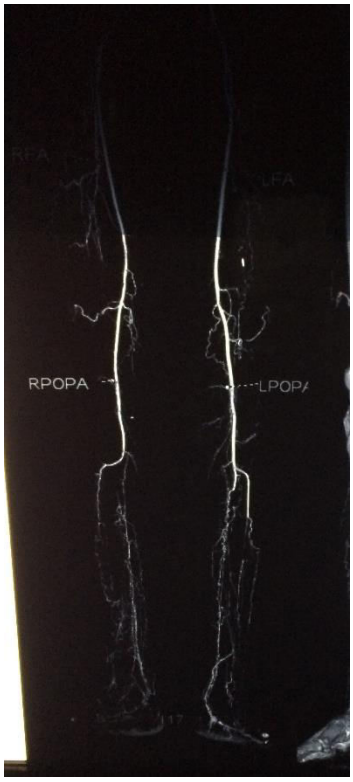

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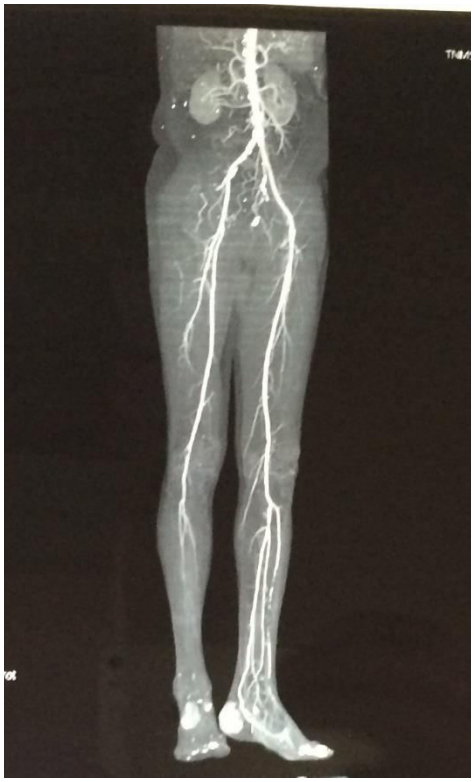

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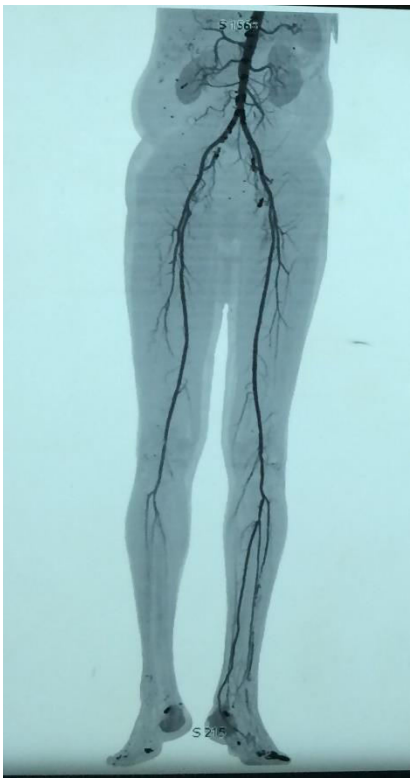

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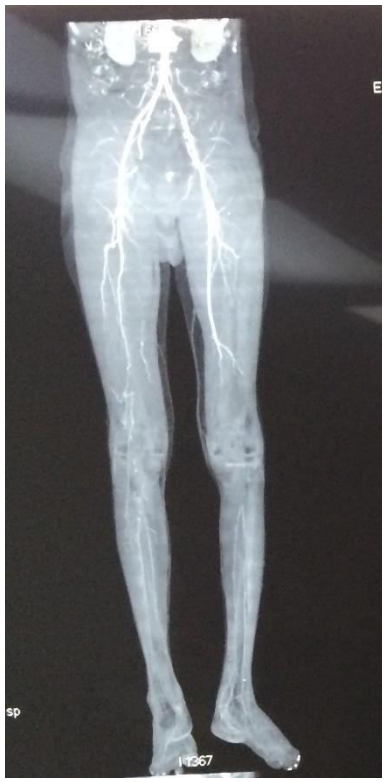

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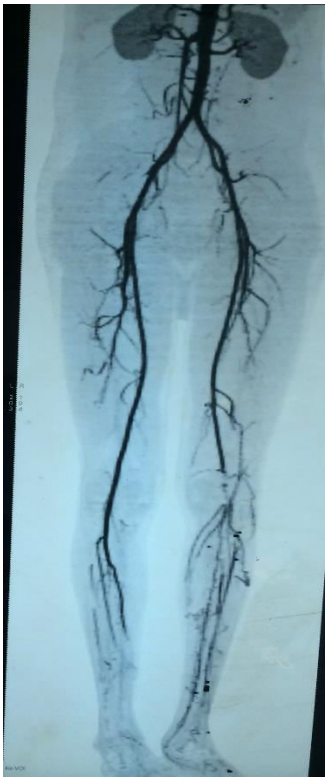

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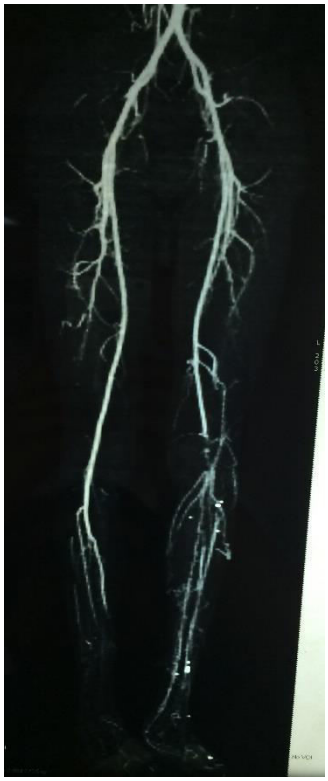

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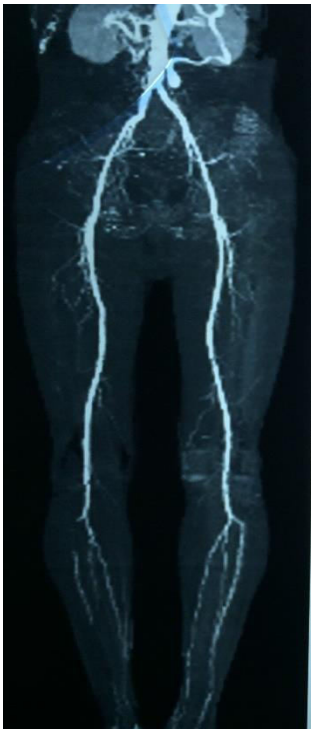

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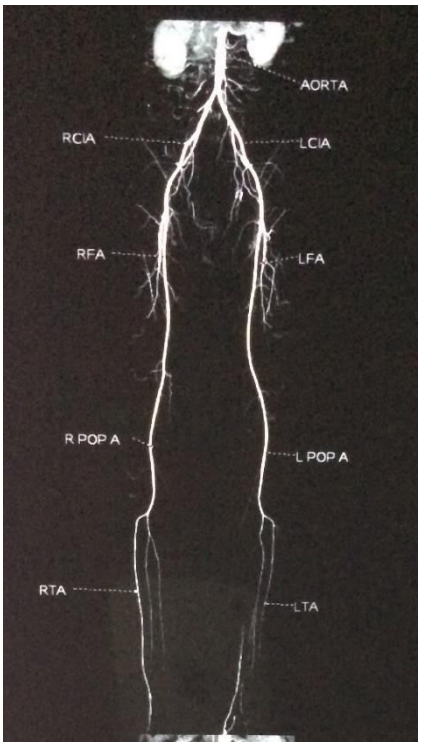

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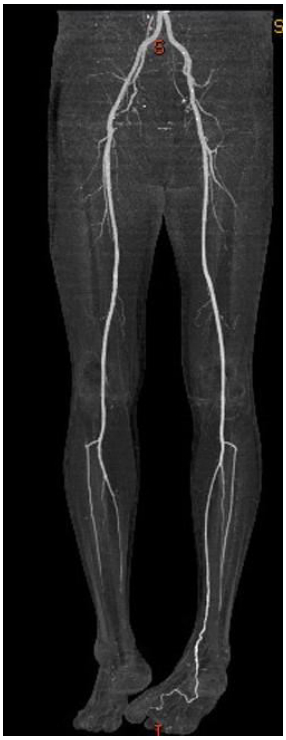

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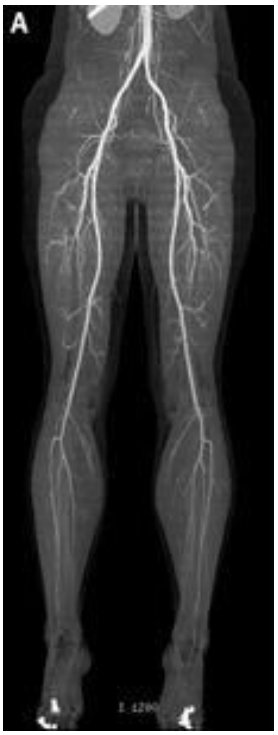
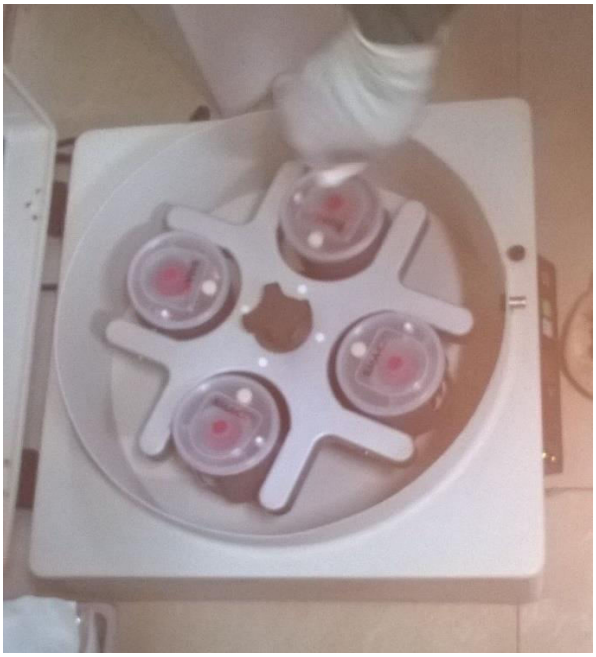
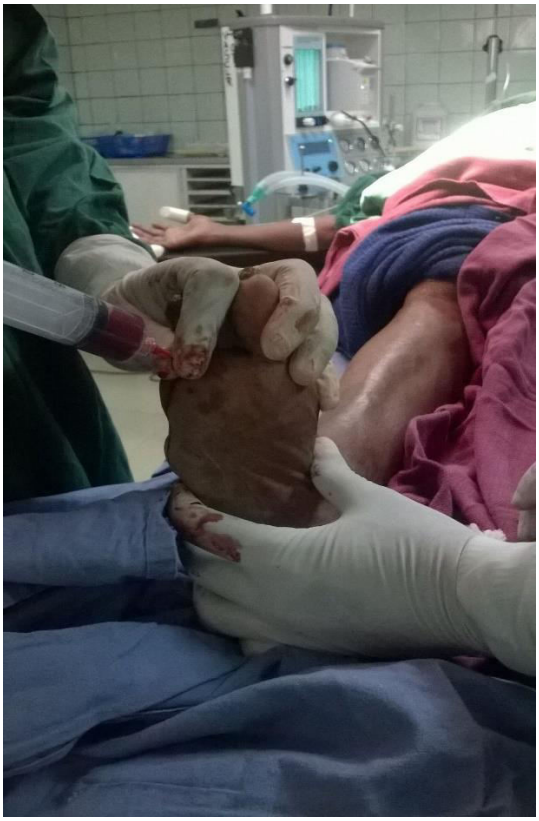
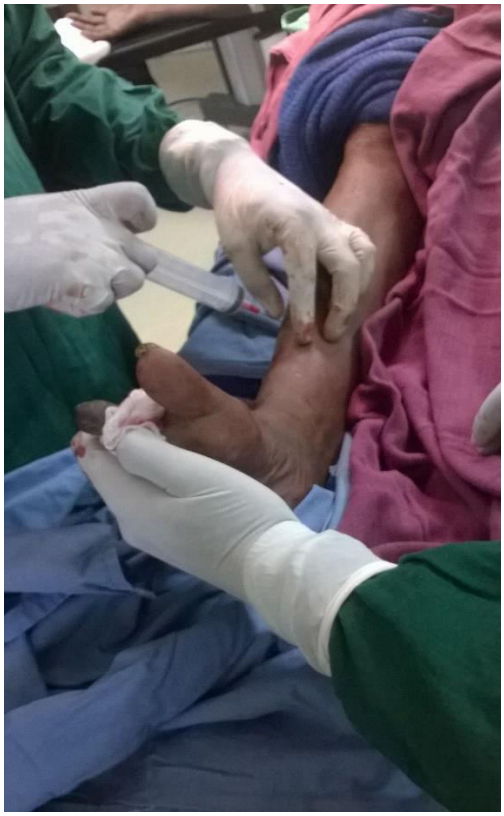


VENKATESH. ID- C120

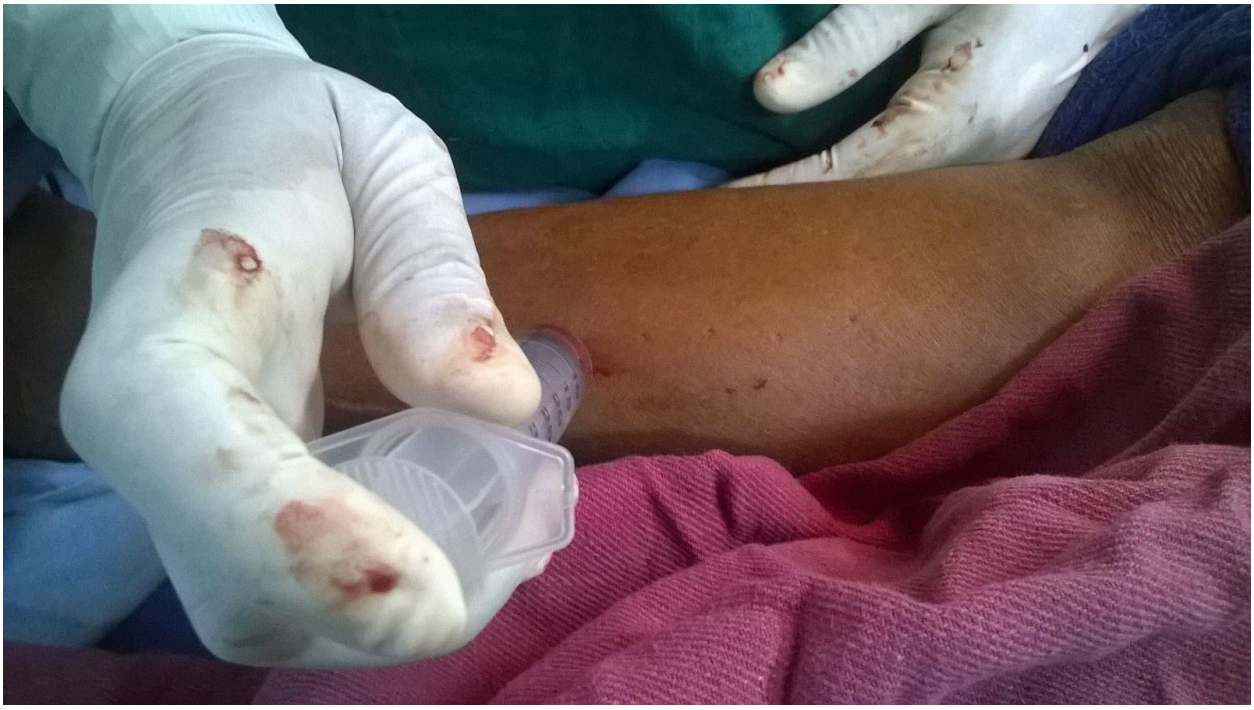
PREPARATION OF THE BONE MARROW DERIVED STEM CELL CONCENTRATE







STEM CELL CONCENTRATE INJECTED IM



Results

A total of 40 patients were enrolled for the study and 37 completed 6 months of follow-up as per the protocol. Three patients were lost to follow-up. Eight patients underwent major amputation- one from stem cell group and 7 from control group. Sixteen patients underwent minor amputation- five from stem cell group and eleven from control group.

1) **There was a statistically significant reduction in major amputation rate among stem cell group in comparison to control group (p-value =0.00**
2) **and <0.0001 using paired t-test and Wilcoxon signed rank test respectively)**
3) **There was a statistically significant reduction in persisting CLI in stem cell group in comparison to control group (p-value= 0.00 and <0.001 using paired t-test and Wilcoxon signed rank test respectively)**
4) **There was statistically significant reduction in the subject's pain perception, determined by VAS pain score, at 3 and 6 months as**

5) **compared to baseline in the stem cell group (p-value =0.00 and <0.0001 using paired t-test and Wilcoxon signed rank test respectively)**
6) **There was a statistically significant increase in pain free walking distance at 6 months as compared to the baseline (p-value= 0.00 and <0.001 using paired t-test and Wilcoxon signed rank test)**
7) **There was a statistically significant improvement in $TcpO_2$ of dorsum of foot as compared to baseline in stem cell group (p-value= 0.002 and <0.0001 using the t-test and Wilcoxon rank sum test)**
8) **There was a statistically significant improvement in ABI at 3 and 6 months relative to baseline in stem cell group (p-value= 0.0001 by noth the paired t-test and Wilcoxon signed rank test respectively).**
9) **No such difference could be noted in control group.**
10) **A moderate positive linear relationship was observed between ABI and maximum walking distance. The correlation co-efficient was 0.40 with p-value of 0.000**

11) There was a decrease in requirement of pain medication from screening to 6 months in the stem cell group.

12) Better wound healing rates were noted in stem cell group as compared to control group

DISCUSSION

Arterial occlusion may cause intermittent claudication and, in some cases, will lead to critical limb ischemia and/or limb loss. Interventional therapies such as bypass surgery or percutaneous angioplasty are performed. However, 50% cases of CLI are non-reconstructable and termed as no-option CLI.

Upto 40% of patients with CLI who fall under the non-reconstructable category end up with major amputation in 6 months. Hence the need for alternative treatment strategy to manage such cases.

Therapeutic angiogenesis is a complex process involving cell-mediated factors. There is a symphony action mediated by EPC, monocyte and various growth factors like VEGF, FGF, TGF, PDGF leading to angiogenesis. No single factor can effectively result in angiogenesis to salvage the limb in CLI.

The vascular endothelium is normally inactive or dormant. Hypoxia due to ischemia and change in the stress and fluid shearing force in the vascular tree proximal to a stenosis or occlusion triggers the circulation of EPCs and Various growth factors to activate the vascular endothelium leading to

angiogenesis and/or arteriogenesis. The main steps in angiogenesis are endothelial activation, migration, proliferation and reorganization. A number of pro-angiogenic growth factors have been identified. The most important factors are VEGF, TGF, FGF. This complex cellular activity is impaired in elderly. It is also impaired in diabetics and in those with hyperlipidemia. Common drugs like NSAIDS, statins, ACE inhibitors also impair angiogenesis. BM aspirate concentrate delivers EPC along with monocytes angiogenesis and platelets from the bone marrow, thus making available a good concentration of various growth factors required for angiogenesis. Delivering these cells close to the diseased vessels definitely seems to help therapeutic angiogenesis.

The optimum dose is not yet known. In this study 40 ml of BMAC was injected IM having nearly 80 million cells and it gave the desired result. Major amputation, persisting CLI were the primary endpoints. Relief of pain was also an important end point.

Statistically significant improvement in CLI and reduced major amputations were noted in patients who received BMAC. Pain free walking distance, rest pain, ABI and tissue oxygen pressure also improved 3 and 6 months after treatment. Better wound healing was also noted in comparison to the control group. No adverse effects were noted in upto 6 months follow-up of the patients.

The safety and efficacy are not inferior to the conventional revascularization therapies.

Cell therapy to initiate angiogenesis for CLI has immense potential in reducing major amputations in patients with CLI.

CONCLUSION

1) **Bone marrow aspirate concentrate therapy is safe**
2) **Limb salvage in patients with no-option CLI was achieved in upto 80%.**
3) **Significant pain relief with improved pain free walking distance and wound healing was noted**
4) **Significant improvement was established in ABI, $TcpO_2$**
5) **Patients scored better in quality of life indexes(EuroQoL 5D-5L, SF36V2)**
6) **There is no complication directly related to the therapy.**

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GOVERNENT STANLEY MEDICAL COLLEGE, CHENNAI- 600 001

DISSERTATION TOPIC: "*STEM CELL THERAPY FOR NO-OPTION CRITICAL LIMB ISCHEMIA*"

INFORMED CONSENT

PLACE OF STUDY: GOVERNMENT STANLEY MEDICAL COLLEGE, CHENNAI

Name of patient :
Address :

I, _____ have been informed about the details of the study in my own language.

I have completely understood the details of the study.

I am aware of the possible risks and benefits, while taking part in the study.

I understand that I can withdraw from the study at any point of time and even then, I will continue to receive the medical treatment as usual.

I understand that I will not get any payment for taking part in this study.

I will not object if the results of this study are getting published in any medical journal, provided my personal identity is not revealed.

I know what I am supposed to do by taking part in this study and I assure that I would extend my full co-operation for this study.

Signature/Thumb impression of the Volunteer

Date:

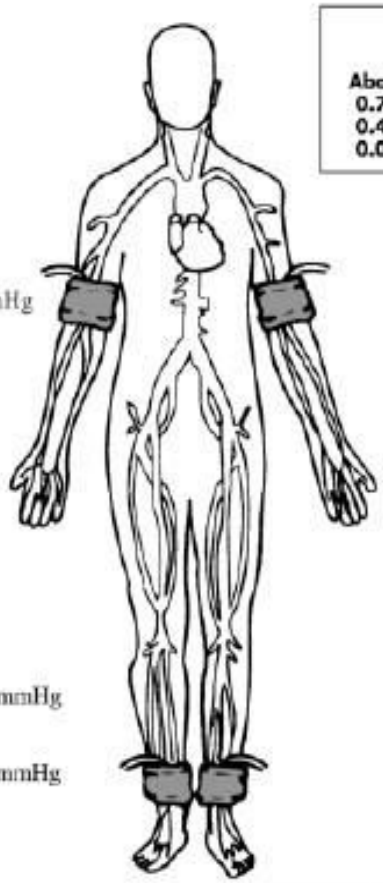
Witnesses:
(Signature, Name & Address)
Date:

Name and signature of investigator:

(DR KITAKA S WOTSA)

ABI WORKSHEET

Ankle-Brachial Index Interpretation
Above 0.90: Normal
0.71 - 0.90: Mild Obstruction
0.41 - 0.70: Moderate Obstruction
0.00 - 0.40: Severe Obstruction



Right Arm:
Systolic Pressure ☐☐☐ mmHg

Left Arm:
Systolic Pressure ☐☐☐ mmHg

Right Ankle:
Systolic Pressure
Posterior Tibial (PT) ☐☐☐ mmHg
Dorsalis Pedis (DP) ☐☐☐ mmHg

Left Ankle:
Systolic Pressure
Posterior Tibial (PT) ☐☐☐ mmHg
Dorsalis Pedis (DP) ☐☐☐ mmHg

Right ABI equals Ratio of:
Higher of the Right Ankle Pressures (PT or DP) ☐☐☐ mmHg
Higher Arm Pressure (right or left arm) ☐☐☐ mmHg
= ☐.☐☐ *

Left ABI equals Ratio of:
Higher of the Left Ankle Pressures (PT or DP) ☐☐☐ mmHg
Higher Arm Pressure (right or left arm) ☐☐☐ mmHg
= ☐.☐☐ *

* The lower of these numbers is the patient's overall ABI.
Overall ABI (lower ABI) = _____

Euro QoL 5D-5L

MOBILITY

I have no problems in walking about ❑

I have slight problems in walking about ❑

I have moderate problems in walking about ❑

I have severe problems in walking about ❑

I am unable to walk about ❑

SELF-CARE

I have no problems in bathing or dressing myself ❑

I have slight problems in bathing or dressing myself ❑

I have moderate problems in bathing or dressing myself ❑

I have severe problems in bathing or dressing myself ❑

I am unable to bathe or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑

I have slight problems doing my usual activities ❑

I have moderate problems doing my usual activities ❑

I have severe problems doing my usual activities ❑

I am unable to do my usual activities ❑

PAIN / DISCOMFORT

I have no pain or discomfort ❑

I have slight pain or discomfort ❑

I have moderate pain or discomfort ❑

I have severe pain or discomfort ❑

I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑

I am slightly anxious or depressed ❑

I am moderately anxious or depressed ❑

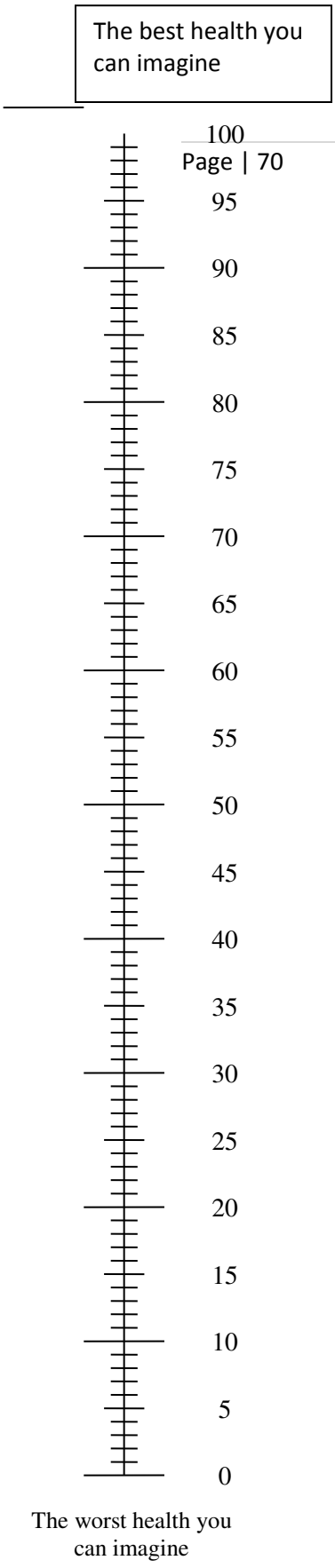
I am severely anxious or depressed ❑

I am extremely anxious or depressed ❑

We would like to know how good or bad your health is TODAY.

- This scale is numbered from 0 to 100.
- 100 means the <u>best</u> health you can imagine.
  0 means the <u>worst</u> health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



SF-36v2™ Health Survey Scoring

1. In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
○	○	○	○	○

2. Compared to one year ago, how would you rate your health in general now?

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
○	○	○	○	○

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
a Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	○	○	○
b Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	○	○	○
c Lifting or carrying groceries	○	○	○
d Climbing several flights of stairs	○	○	○
e Climbing one flight of stairs	○	○	○
f Bending, kneeling, or stooping	○	○	○
g Walking more than a mile	○	○	○

h Walking several hundred yards	○	○	○
i Walking one hundred yards	○	○	○
j Bathing or dressing yourself	○	○	○

4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Cut down on the amount of time you spent on work or other activities	○	○	○	○	○
b Accomplished less than you would like	○	○	○	○	○
c Were limited in the kind of work or other activities	○	○	○	○	○
d Had difficulty performing the work or other activities (for example, it took extra effort)	○	○	○	○	○

5. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Cut down on the amount of time you spent on work or other activities	○	○	○	○	○

b Accomplished less than you would like	○	○	○	○	○
c Did work or activities less carefully than usual	○	○	○	○	○

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

Not at all	Slightly	Moderately	Quite a bit	Extremely
○	○	○	○	○

7. How much bodily pain have you had during the past 4 weeks?

None	Very mild	Mild	Moderate	Severe	Very severe
○	○	○	○	○	○

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8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all	A little bit	Moderately	Quite a bit	Extremely
○	○	○	○	○

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9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the past 4 weeks...

	All of the time	Most of the time	Some of the time	A little of the time	None of the time

a	Did you feel full of life?	○	○	○	○	○
b	Have you been very nervous?	○	○	○	○	○
c	Have you felt so down in the dumps that nothing could cheer you up?	○	○	○	○	○
d	Have you felt calm and peaceful?	○	○	○	○	○
e	Did you have a lot of energy?	○	○	○	○	○
f	Have you felt downhearted and depressed?	○	○	○	○	○
g	Did you feel worn out?	○	○	○	○	○
h	Have you been happy?	○	○	○	○	○
i	Did you feel tired?	○	○	○	○	○

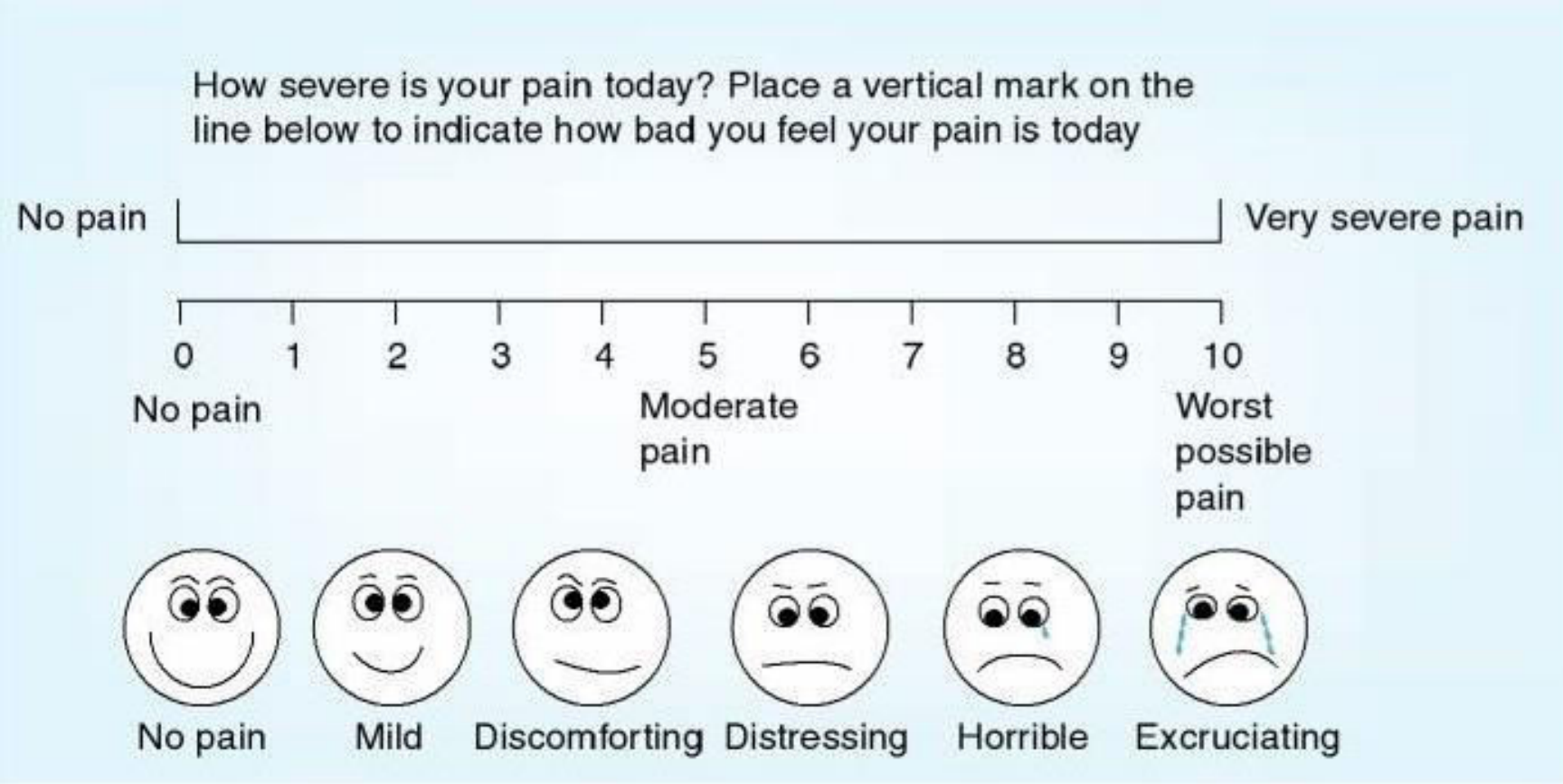
10. During the <u>past 4 weeks</u>, how much of the time has your <u>physical health or emotional problems</u> interfered with your social activities (like visiting friends, relatives, etc.)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time
○	○	○	○	○

11. How TRUE or FALSE is <u>each</u> of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false

A	I seem to get sick a little easier than other people	○	○	○	○	○
B	I am as healthy as anybody I know	○	○	○	○	○
C	I expect my health to get worse	○	○	○	○	○
D	My health is excellent	○	○	○	○	○


How severe is your pain today? Place a vertical mark on the line below to indicate how bad you feel your pain is today
No pain
Very severe pain
0 1 2 3 4 5 6 7 8 9 10
No pain
Moderate pain
Worst possible pain
No pain
Mild
Discomforting
Distressing
Horrible
Excruciating

INSTITUTIONAL ETHICAL COMMITTEE, STANLEY MEDICAL COLLEGE, CHENNAI-1

Title of the Work : Stem Cell Therapy for no option critical limb Inschemia.

Principal Investigator : Dr. Kitaka S Wotsa

Designation : PG in MS (GS)

Department : Department of General Surgery
Government Stanley Medical College,
Chennai-01

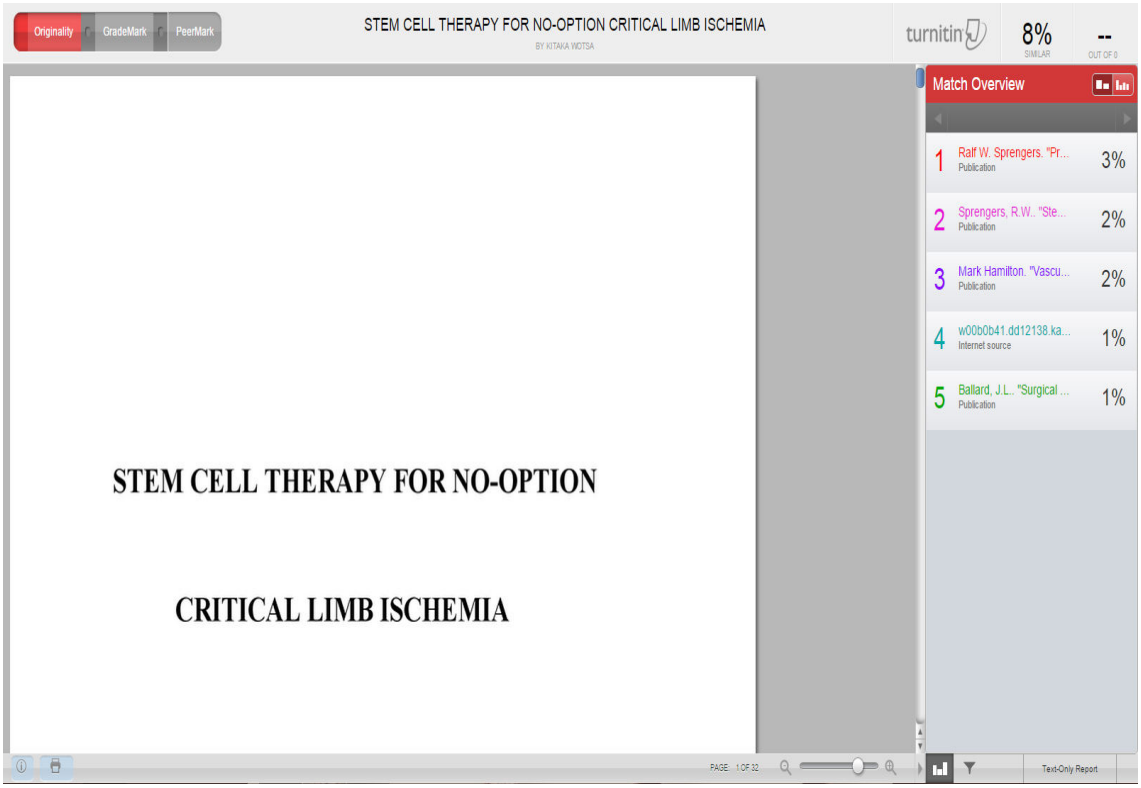
The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 26.11.2014 at the Council Hall, Stanley Medical College, Chennai-1 at 2PM

The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate from the area of the work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reaction.
4. You should abide to the rules and regulation of the institution(s).
5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

MEMBER SECRETARY,
IEC, SMC, CHENNAI


Originality
GradeMark
PeerMark
STEM CELL THERAPY FOR NO-OPTION CRITICAL LIMB ISCHEMIA
BY KITAKA WOTSA
turnitin
8%
SIMILAR
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OUT OF 0
Match Overview
1 Ralf W. Sprengers. "Pr... Publication 3%
2 Sprengers, R.W.. "Ste... Publication 2%
3 Mark Hamilton. "Vascu... Publication 2%
4 w00b0b41.dd12138.ka... Internet source 1%
5 Ballard, J.L.. "Surgical ... Publication 1%
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