Long-term follow-up of patients with Buerger’s disease after autologous stem cell therapy

Çağdaş Baran, Serkan Durdu, Evren Özçınar, Mehmet Çakıcı, Ali İhsan Hasde, Bahadir İnan, Mustafa Şırlak, Rüçhan Akar

Department of Cardiovascular Surgery, Faculty of Medicine, Ankara University; Ankara-Turkey

Objective: We investigated the long-term results of autologous bone marrow mononuclear cells (ABMMNCs) implantation in patients with Buerger’s disease (BD).

Methods: Twenty-eight patients (25 males and 3 females) who had BD and critical unilateral limb ischemia were investigated between April 2003 and August 2005. The patients were administered multiple injections of CD34+ and CD45+ positive ABMMNCs into the gastrocnemius muscle, the intermetatarsal region, and the dorsum of the foot (n=26) or forearm (n=2) and saline injection into the contralateral limb.

Results: The mean follow-up time was 139.6±10.5 months. No complication related to stem cell therapy was observed during the follow-up. The ankle–brachial pressure index evaluated at 6 months and 120 months was compared to the baseline scores (p<0.001 and p=0.021, respectively). Digital subtraction angiography (DSA) was performed for all patients at baseline, 6 months, and 120 months. The angiographic improvement was 78.5% and 57.1% at 6 and 120 months, respectively. Patients demonstrated a significant improvement in the quality of life parameters at 6 months compared to baseline (p=0.008) and 120 months compared to the baseline (p=0.009). The 10-year amputation-free rate was 96% (95% CI=0.71–1) in ABMMNC-implanted limbs and 93% (95% CI=0.33–0.94) in saline-injected limbs (p=1).

Conclusion: Autologous stem cell therapy could be an alternative therapeutic method for BD at long-term follow-up. (Anatol J Cardiol 2019; 21: 155-62)

Keywords: Buerger’s disease, stem cell therapy, autologous bone marrow mononuclear cell

Introduction

Buerger’s disease (BD) is a non-atherosclerotic, progressive vasculitis of the small and medium arteries. As there is a distally localized diffuse segmental involvement, revascularization is not frequently feasible. Instead, therapeutic angiogenesis by cellular strategies may be an option for patients with BD (1).

It has been reported that stem cell therapy leading to new collateral vessel development relieves ischemic symptoms in patients with BD (2-5). In contrast, previous studies have stated clinical limitations to stem cell therapy due to limited number of patients and short follow-up times (6).

In the first study on stem cell therapy that was published in 2002 by Tateishi-Yuyama et al. (6), 22 patients (44 limbs) with arteriosclerosis obliterans (ASO) were administered autologous bone marrow mononuclear cells (ABMMNCs). Further, it was reported that increased collateral vessel formation developed in ischemic limbs 6 months after implantation, and decrease in rest pain and increase in ankle-brachial pressure index (ABPI) and transcutaneous oxygen pressure were observed.

Furthermore, recent studies have shown that the implantation of ABMMNCs and autologous peripheral blood mononuclear cells (APBMNC) improve critical limb ischemia (CLI) (7-11).

In the present study, we aimed to evaluate the feasibility, safety, reproducibility, and efficacy of ABMMNC implantation in patients with CLI due to BD.

Methods

Study design

Twenty-eight patients were diagnosed with grade II or III BD between April 2003 and August 2005; according to the recom-
mended classification by Rutherford et al. (12), none of the patients were suitable for conventional revascularization therapies. Informed written consent was obtained from all the patients, and the study was approved by our institution’s Local Ethics Committee. The study protocol has been published previously and is also available with the full text of this article at https://www.jvascsurg.org/issue/S0741-5214(06)X0188-X. The 2-year results have also been published previously (3). The clinical characteristics of the patients are shown in Table 1.

Baseline evaluation
All patients underwent complete blood count; biochemical analysis, including liver and kidney functions, and fasting blood glucose; urine analysis; and serologic profile, including erythrocyte sedimentation rate, C-reactive protein, antinuclear antibody, rheumatoid factor, anticitrullinated antibody, antiphospholipid antibody, Scl-70, fibrinogen, complement measurements, serum homocysteine, and hypercoagulability screen (protein C, protein S, and antithrombin III plasma levels, factor V Leiden, and prothrombin 20210A gene mutation analysis); chest radiograph; electrocardiogram; transthoracic echocardiography; and ophthalmologic examinations. All patients also underwent a routine preharvest examination by an experienced hematologist from the Stem Cell Transplantation Unit. For patients who used aspirin and clopidogrel, medication was discontinued 5–7 days before ABMMNCs administration.

Standard vascular examination, including ankle–brachial pressure index (ABPI) measurement was assessed using duplex ultrasonography.

All patients underwent a validated progressive treadmill protocol with reduced initial speed (1.6 km/h); patients continued walking until they felt claudication pain or any other clinical condition that required the test to stop (13). The claudication onset time, claudication distance, and peak walking time and distance were recorded. Patients who were previously amputated below their knee were asked to walk with a prosthesis for an increasing distance at a self-selected velocity. A 10-cm visual analog scale (VAS) was recorded monthly by the patients for both the ischemic and control limbs, where a score of 0 implied no pain and a score of 10 implied most severe pain ever experienced.

Systemic antibiotic therapy was prescribed for patients with trophic ulcers that were determined using microbiologic quantitative tissue cultures and sensitivity results. Digital color photography was used for the documentation of ischemic ulcers. All patients underwent local wound care and surgical debridement until three cultures revealed negative microbiologic results. Thereafter, the patients were accepted for cellular therapy administration.

Intra-arterial digital subtraction angiography
Digital subtraction angiography (DSA) (Multistar Plus/ T.O.P, Siemens AG, Forchheim, Germany) of both upper and lower extremities was performed for all patients at baseline, 6 months, and 120 months of follow-up. The amount and the force of low-osmolarity nonionic contrast injection and the position of the catheter tip (5f; Weinberg, pigtail, Digiflex, Boston Scientific Corp, Watertown, Mass) was strictly fixed for DSA studies to obtain identical imaging conditions.

Angiographic mapping of both lower limbs and comparison of all the evaluated segments were reviewed by two independent radiologists blinded to the treated extremity. The angiographic scores for the formation of new collateral vessel formation were assessed as 0 (no collateral development), 1 (slight), 2 (moderate), and 3 (rich), as described previously.

Vascular Quality of Life Questionnaire
The King’s College Hospital Vascular Quality of Life Questionnaire (VascuQol) was used to assess quality of life (QoL) at baseline and 3, 6, and 120 months after the procedure (14). The 25-item questionnaire included parameters to investigate pain and other symptoms as well as patient’s activity and social and emotional status. The total VascuQol score was between 1 and 7 (all item scores were divided by 25) for each evaluation.

Bone marrow harvest and isolation of mononuclear cells
After the patients were stabilized in a prone position, the stem cell transplantation team collected the bone marrow from bilateral spina iliaca posterior superior under general anesthesia, and this procedure was completed approximately 2 hours before the ABMMNCs were implanted. The collected bone marrow (mean amount, 653.2±77.3 mL) was immediately transferred to the Apheresis Unit. During the implantation process, a total mononuclear cell amount of 1±10e8–9/mL was targeted and unnecessary red blood cell (RBC) implantation and higher harvest volumes were avoided. The harvest material was processed on COBE® Spectra (Gambro BCT, Lakewood, Colo) using the bone marrow processing program and software version 5.1.

After the RBCs were depleted and the total volume was reduced using a continuous flow cell separator in a closed system (15), 91±2% RBC depletion was achieved and ABMMNCs and total mononuclear cells were concentrated to a final volume of 59.9±9.2 mL and 1.69±0.89±10e9/mL, respectively. The total number of implanted CD34+ cells was 53.1±35.9±10e6. A cytfluorometric analysis showed that 99% of implanted stem cells were viable and 97.5±2.2% of CD45+ cells were included. Cultures of cell preparations were negative for bacterial and fungal contamination.

ABMMNCs Implantation
The implantation of ABMMNCs was performed within 2 hours after bone marrow was collected, and multiple intramuscular injections were administered into the gastrocnemius muscle, intermetatarsal region of the limb with CLI, and around the trophic lesions. The implantation of ABMMNCs started 4–5 cm proximal to the obstructive lesion and continued distally. A 22-gage spinal needle and a 7-cm grid were used to implant approximately 1 mL of ABMMNCs into each injection site (3–4 cm deep and oblique) for a median total of 54±7 sites (range, 41–70). For patients who
required debridement of ischemic ulcers, general anesthesia was performed, whereas mild sedation was preferred for other patients. During each implantation, saline solution was also injected into the contralateral limb in eligible patients.

Follow-up
Patients were seen in the outpatient department at 1 month, 3 months, 6 months, and thereafter annually for postoperative evaluation.

Short-term follow-up
The clinical operative and follow-up data were prospectively recorded in a computerized database. Three (10.7%) patients were on anti-lipid drugs at their initial evaluation in the outpatient clinic (Table 1). Aspirin (300 mg/day), statin (in case of total cholesterol >150 mg/dL), and L-arginine (500 mg/day) were started in all patients at the first visit, and patients were required to take this medication for 6 months prior to the study start.

The patients were examined in the outpatient clinic every 2 weeks postoperatively for trophic lesions and evaluated for pain scores and QoL using the VascuQol questionnaire. DSA was performed every 6 months.

Long-term follow-up
All patients were called after 10 years and were evaluated for ABPI and VascuQol scores as well as whether they had amputation. Twelve (42.8%) patients maintained strict smoking cessations for at least 10 years; 15 (53.5%) patients were taking aspirin/clopidogrel, and 13 (46.5%) patients were taking cilostazol with aspirin/clopidogrel. DSA was performed every 120 months.

End points
The primary end points were total healing of the most prominent lesion while avoiding major or minor amputation, the relief of rest pain without the need for analgesics, and the safety and feasibility of the treatment. Secondary end points were the alterations in the ABPI, angiographic evidence of collateral vessel formation or remodeling, QoL, and amputation-free survival.

Statistical analysis
Because of the small sample size and abnormal distribution of the variables, non-parametric tests were used to measure the difference. We used the Friedman’s test to calculate the difference between the groups and the Wilcoxon signed-rank test to determine which group created this difference. The Bonferroni adjustment was also used for the significance level. The amputation-free rate was calculated using the Kaplan–Meier method. The 95% confidence interval (CI) was calculated for each test. Data were analyzed using The Statistical Package for Social Sciences (SPSS) version 22.0 (SPSS Inc, Chicago, Illinois, USA) for Windows (Microsoft Corp, Redmond, Washington, USA). A two-tailed p value of <0.05 was considered statistically significant.

Table 1. Demographic characteristics of the study group

| Variable                                              | n  | %    |
|-------------------------------------------------------|----|------|
| Mean age (years, mean±SD)                             | 42.6±7.9 (25-56) |
| Male                                                  | 25 | 89.2 |
| Duration between diagnosis and ABMMNCs implantation   | 9.5±4.8 (1-23) |
| Hypertension                                          | 3  | 10.7 |
| Hyperlipidemia                                        | 3  | 10.7 |
| Previous smoking                                      | 28 | 100  |
| History of intermittent claudication                  | 26 | 92.8 |
| Pain at rest (narcotic requirement)                   | 23 | 82.1 |
| Ischemic non-healing ulcer                            | 18 | 64.3 |
| Thrombophlebitis                                      | 18 | 64.3 |
| Raynaud’s phenomenon                                  | 11 | 39.2 |
| Sensorial findings                                    | 21 | 75   |
| Abnormal Allen’s test result                          | 14 | 50   |
| Upper extremity involvement by DSA                    | 9  | 32.1 |
| Previous ethernet abuse                               | 8  | 28.6 |
| Previous hyperbaric oxygen                            | 13 | 46.4 |
| Previous sympathectomy                                | 17 | 60.7 |
| Previous sympathetic nerve block                      | 9  | 32.1 |
| Previous amputation                                   | 12 | 42.8 |
| Major/minor                                           | 2/10 | 7.1/35.7 |
| Distal bypass graft                                    | 2  | 7.1  |
| Below knee/crural arteries                            | 1/1 | 3.6/3.6 |
| Previous infusion of iloprost                          | 5  | 17.8 |
| Medications                                           |    |      |
| ACE-inhibitor/ARB                                      | 1  | 3.6  |
| Statin                                                | 3  | 10.7 |
| ASA/clopidogrel                                       | 22 | 78.6 |
| Pentoxifylline                                        | 26 | 92.8 |
| Calcium channel blocker                               | 19 | 67.8 |
| Cilostazol                                            | 1  | 3.6  |
| NSAID                                                 | 20 | 71.4 |
| Morphine                                              | 14 | 50   |

Data presented as n (%) or mean±SD. ABMMNCs - autologous bone marrow mononuclear cells; DSA - digital subtraction angiography; ACE - angiotensin converting enzyme; ARB - angiotensin receptor blocker; ASA - acetylsalicylic acid; NSAID - nonsteroidal anti-inflammatory drug; SD - standard deviation

Results
The ratio of patients diagnosed with BD to those with ASO has been 1:4 at our outpatient clinic since the year 2000. During this study period, 52 patients with BD (46 males and 6 females) were admitted to the Heart Center at Ankara University. Thirty-two pa-
tients were Rutherford grade II–III, and 28 patients were included in the present study (Fig. 1).

Twenty patients who were Rutherford grade I disease were excluded. Four patients were not included in the study, as they refused to undergo cellular therapy after they received detailed information about the possible side effects. All patients had a history of smoking and applied with ischemic rest pain and/or trophic lesions. The mean age at disease onset was 33 years (range, 20–45 years), whereas the mean age at initial evaluation was 42 years (range, 25–54 years). The infrapopliteal artery and upper extremities were involved in 28 (100%) and 9 patients (32%), respectively (Table 1).

**Procedural data**

The same surgical team performed all procedures. The mean duration for total bone marrow harvest was 35.7±6.1 min (range, 27–50 min) and the mean duration for ABMMNCs implantation was 32.5±4.4 min (range, 22–39 min). Approximately 2 million cells were implanted in a volume of 1 mL during each injection. To eradicate necrotic and devitalized tissues, surgical debridement was performed in 18 patients (64.2%) who had ischemic ulcers.

**Safety data**

No complications due to ABMMNCs implantation occurred. A blood transfusion was performed following a bone marrow harvest; three (10.7%) patients were diagnosed with iron deficiency anemia and were treated with oral iron replacement therapy for 3 months. All patients who had rest pain were hospitalized and discharged on the second day according to the protocol. The duration of hospitalization after ABMMNCs implantation was 26.4±14.6 days (range, 7–52 days) for patients with trophic lesions. No patient had retinopathy or teratoma in funduscopic examinations. The levels of C-reactive protein, white blood cells, and fibrinogen at baseline and follow-up were similar.

---

**Figure 1.** Study diagram showing the flow of participants through each stage

**Figure 2.** Resting ankle–brachial pressure index (ABPI) was measured three times in the treated and control groups (mean±standard deviation, 95% confidence interval data). Autologous bone marrow-mononuclear cell (ABMMNC) implanted limbs and saline-injected contralateral limbs at baseline and 6 and 120 months after the procedure

**Figure 3.** Pre and postoperative angiograms of the lower extremities. (a, d, g) The baseline digital subtraction angiographic (DSA) studies. (b, e, h) Lower extremity DSA study confirms new collateral vessel formation in the right limb at the 6 month follow-up after the implantation of autologous bone marrow-derived mononuclear cells (ABMMNC). (c, f, i) Lower extremity DSA study confirms new collateral vessel formation in the right limb at the 120-month follow-up after implantation of autologous bone marrow mononuclear cells.

*ABMMNC-implanted limb.

∆Saline-injected control limb.

Arrows indicate new collateral vessel formations that were visible at ABMMNC injection sites compared with the baseline angiography.
Follow-up evaluation
No patient was lost to follow-up, and the mean follow-up duration after ABMMNCs implantation was 139.6±10.5 months.

Ankle–brachial pressure index
The ABPI increased from 0.52±0.09 at the baseline to 0.67±0.13 after 6 months (p<0.001) and to 0.58±0.10 after 120 months (p=0.021) of follow-up (Fig. 2). However, ABPI did not change significantly at 6 and 120 months in the contralateral saline-injected leg.

Digital subtraction angiography
New vessel formation was observed in 22 (78.5%) patients at 6 months and 16 (57.1%) patients at 120 months (Fig. 3).

Quality of life
The VascuQol scores showed significant improvement in QoL at 6 months after ABMMNC implantation (p=0.008) and further improvement was achieved at 120 months (p=0.009). Additional subgroup analyzes for activity, pain, and other symptoms as well as emotional and social items are presented in Figure 4.

Figure 4. Vascular Quality of Life Questionnaire (VascuQol) subgroup values at baseline change from 6 months and at 6 months change from 120 months of follow-up (mean±standard deviation)
Ulcer status

Ischemic ulcers healed completely in 17 patients (94%) after 120 months; especially, three patients had perfect healing after 10 years (Fig. 5).

Amputation rate and survival

One patient in the ABMMNCs implantation arm and 2 patients in the saline injection arm had toe amputation; however, the difference was not statistically significant (p=1) (Fig. 6). Patients had a history of smoking.

A 62-year-old male patient was diagnosed with Alzheimer disease 9 years after stem cell therapy. This patient consulted to a neurology department.

Discussion

Stem cell therapy has been recently performed in patients with BD and ASO. This procedure that aims the development of new vessels has gained popularity during the last 10 years. In 2002, Tateishi–Yuyama et al. (6) implanted stem cells from a pa-
tient’s bone marrow to his ischemic leg and reported new col-
lateral development on angiography after 6 months, and rest pain
and the duration of maximum walking improved in patients at the
24-month follow-up.

In 2005, Ishida et al. (4) performed peripheral autologous stem
cell transplant on patients with peripheral arterial disease (PAD)
and observed decreased ischemia. In the first part of the present
study published in 2006, we injected CD34+ and CD45+ positive
ABMMNCs that were obtained from the bone marrow of patients
with BD into their legs, and we showed the development of new
vessel formation during a mean follow-up of 16.6 months (3).

In previous studies, it has been shown that autologous bone
marrow stem cell transplant decreased the rate of amputation
in patients with CLI (16, 17). No significant difference was found be-
tween the effect of treatments with bone marrow mononuclear
cells and granulocyte colony stimulating factor (G-CSF)-mobi-
ized peripheral blood mononuclear cells on long term morbidity
(amputation) and mortality in patients with CLI (18). In this study,
it was emphasized that CD34+ cells were important indicators for
vascular therapy.

Smoking and tobacco consumption are major factors associ-
ated with the development of BD; since tobacco may trigger an
immune response or unmask a clotting defect, tobacco can incite
an inflammatory reaction in the vessel wall (19). Also, lower ex-
treme exercise training contributes to improve maximum walk-
ing time, peak oxygen uptake, and QoL in patients with intermit-
tent claudication (20). Guo et al. (21) reported that modest clinical
improvements in patients who stop smoking but are not treated
with ABMMNCs might not cure ischemia alone but that smoking
cessation is a critical factor in providing appropriate stem cell
function. In our study, we have seen the long-term positive ef-
effects of ABMMNCs treatment.

A significant decrease in the amputation rate was detected
in patients with BD following autologous bone marrow stem cell
CD34+ and CD133+ therapy (22). The effects of mesenchymal
and embryonic stem cells on the beginning of angiogenesis are also
a popular issue (23, 24).

Treatment with in vitro-expanded, peripheral blood-derived,
autologous stem cells was found efficient and safe in patients
with ASO at the long-term follow-up (25, 26).

In a meta-analysis by Wang et al. (27), the randomized and
nonrandomized studies on ABMMNCs implantation in patients
with PAD were evaluated, and it was reported that cell therapy
is advantageous in patients with no scope for revascularization.

In the present study, we evaluated patients with BD with a
mean follow-up time of 139.6 months. We observed that a sig-
nificant increase in ABPI continued in the legs implanted with
ABMMNCs. However, there was a significant decrease in the
legs injected saline solution. When the QoL was evaluated, we
observed that improvement in activity, pain, other symptoms,
and social and emotional parameters also continued. The am-
putation rate was higher in the control group without significant
difference.

Study limitation
The major limitation of this study is the small number of pa-
patients.

Conclusion

The present study in which we reported our long-term results
shows that ABMMNCs implantation did not cause possible side
effects, such as out-of-control cell reproduction, intra-arterial
occlusion, and dysrythmia in patients with BD. After a 10-year
follow-up, we can say that the cell therapy procedure is safe. We
observed that ischemic ulcer wounds in patients healed com-
pletely, and patients had better QoL during the follow-up.

Larger and randomized series of stem cell therapy studies
together with new cellular modalities are needed particularly in
patients with BD and PAD.

Funding: This work was supported Biotechnology Institute Research
Fund and Ankara University School of Medicine Research Council, Tur-
key (EA-TUBA-GBIP/2004-1-1).

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept – Ç.B., S.D., E.Ö., R.A.; Design –
Ç.B., S.D., M.Ç., M.Ş., R.A.; Supervision – Ç.B., S.D., E.Ö., B.I., R.A.; Matri-
als – Ç.B., S.D., E.Ö., M.Ç., R.A.; Data collection &/or processing – Ç.B.,
S.D., E.Ö., M.Ç.; Analysis &/or interpretation – Ç.B., M.Ç., A.I.H., R.A.; Lit-
erature search – Ç.B., S.D., M.Ç., A.I.H.; Writing – Ç.B., S.D., A.I.H., B.I.,
R.A.; Critical review – Ç.B., S.D., M.Ş., R.A.

References

1. Akar AR, Durdu S, Corapcioglu T, Ozyurda U. Regenerative medicine
for cardiovascular disorders. New milestones: adult stem cells. Ar-
tif Organs 2006; 30: 213-32.
2. Kim DI, Kim MJ, Joh JH, Shin SW, Do YS, Moon JY, et al. Angiogen-
esis facilitated by autologous whole bone marrow stem cell trans-
plantation for Buerger’s disease. Stem Cells 2006; 24: 1194-200.
3. Durdu S, Akar AR, Arat M, Sancak T, Eren NT, Ozyurda U. Auto-
logical bone-marrow mononuclear cell implantation for patients with
Rutherford grade II-III thromboangiitis obliterans. J Vasc Surg 2006;
44: 732-9.
4. Ishida A, Ohya Y, Sakuda H, Ohshiro K, Higashiuesato Y, Nakaema
M, et al. Autologous peripheral blood mononuclear cell implanta-
tion for patients with peripheral arterial disease improves limb isch-
emia. Circ J 2005; 69: 1260-5.
5. Motukuru V, Suresh KR, Vivekanand V, Raj S, Girija KR. Therapeu-
tic angiogenesis in Buerger’s disease (thromboangiitis obliterans)
patients with critical limb ischemia by autologous transplantation
of bone marrow mononuclear cells. J Vasc Surg 2008; 48(6 Suppl): 53S-60S.
6. Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, et al.; Therapeutic Angiogenesis using Cell Transplantation (TACT) Study Investigators. Therapeutic angiogenesis for patients with limb ischemia by autologous transplantation of bone-marrow cells: a pilot study and a randomized controlled trial. Lancet 2002; 360: 427-35.

7. Higashi Y, Kimura M, Hara K, Noma K, Jitsuiki D, Nakagawa K, et al. Autologous bone-marrow mononuclear cell implantation improves endothelium dependent vasodilation in patients with limb ischemia. Circulation 2004; 109: 1215-8.

8. Saigawa T, Kato K, Ozawa T, Toba K, Makiyama Y, Minagawa S, et al. Clinical application of bone marrow implantation in patients with arteriosclerosis obliterans, and the association between efficacy and the number of implanted bone marrow cells. Circ J 2004; 68: 1189-93.

9. Huang P, Li S, Han M, Xiao Z, Yang R, Han ZC. Autologous transplantation of granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells improves critical limb ischemia in diabetes. Diabetes Care 2005; 28: 2155-60.

10. Huang PP, Li SZ, Han MZ, Xiao ZJ, Yang RC, Qiu LG, et al. Autologous transplantation of peripheral blood stem cells as an effective therapeutic approach for severe arteriosclerosis obliterans of lower extremities. Thromb Haemost 2004; 91: 606-9.

11. Kawamura A, Horie T, Tsuda I, Ikeda A, Egawa H, Imamura E, et al. Prevention of limb amputation in patients with limbs ulcers by autologous peripheral blood mononuclear cell implantation. Ther Apher Dial 2005; 9: 59-63.

12. Rutherford RB, Baker JD, Ernst C, Johnston KW, Porter JM, Ahn S, et al. Recommended standards for reports dealing with lower extremity ischemia: revised version. J Vasc Surg 1997; 26: 517-38.

13. Arslan O, Arat M, Ciftci A, Ayyildiz E, Ilhan O. Depletion of donors erythrocytes using cell separator in major ABO mismatched allogeneic bone marrow transplantation. Blood 2000; 96 (Suppl Part 2): 5152.

14. Morgan MB, Crayford T, Murrin B, Fraser SC. Developing the Vascular Quality of Life Questionnaire: a new disease-specific quality of life measure for use in lower limb ischemia. J Vasc Surg 2001; 33: 679-87.

15. Davey R, Amin F, Amin M, Gill R, O’Donnell C, Rees B. The effect of bone marrow transplantation on limb salvage in patients with severe peripheral vascular disease. Vase Med 2001; 6: 215-21.

16. Liang TW, Jester A, Motaganahalli RL, Wilson MG, G’Sell P, Akingba GA, et al. Autologous bone marrow mononuclear cell therapy for critical limb ischemia is effective and durable. J Vasc Surg 2016; 63: 1541-5.

17. Matoba S, Tatsumi T, Murohara T, Imaizumi T, Katsuda Y, Ito M, et al.; TACT Follow-up Study Investigators. Long-term clinical outcome after intramuscular implantation of bone marrow mononuclear cells (Therapeutic Angiogenesis by Cell Transplantation [TACT] trial) in patients with chronic limb ischemia. Am Heart J 2008; 156: 1010-8.

18. Onodera R, Teramukai S, Tanaka S, Kojima S, Horie T, Matoba S, et al.; BMMNC Follow-Up Study Investigators; M-PBMNC Follow-Up Study Investigators. Bone marrow mononuclear cells versus G-CSF-mobilized peripheral blood mononuclear cells for treatment of lower limb ASO: pooled analysis for long-term prognosis. Bone Marrow Transplant 2011; 46: 278-84.

19. Tanaka K. Pathology and pathogenesis of Buerger’s disease. Int J Cardiol 1998; 66 Suppl 1: S237-42.

20. Fokkenrood HJ, Bendermacher BL, Lauret GJ, Willigendael EM, Prins MH, Teijink JA. Supervised exercise therapy versus non-supervised exercise therapy for intermittent claudication. Cochrane Database Syst Rev 2013; 8: CD005263.

21. Guo J, Guo L, Cui S, Tong Z, Dardik A, Gu Y. Autologous bone marrow-derived mononuclear cell therapy in Chinese patients with critical limb ischemia due to thromboangiitis obliterans: 10-year results. Stem Cell Res Ther 2018; 9: 43.

22. Lee KB, Kang ES, Kim AK, Kim MH, Do YS, Park KB, et al. Stem cell therapy in patients with thromboangiitis obliterans: assessment of the long-term clinical outcome and analysis of the prognostic factors. Int J Stem Cells 2011; 4: 88-98.

23. Descamps B, Emanuelli C. Vascular differentiation from embryonic stem cells: novel technologies and therapeutic promises. Vascul Pharmacol 2012; 56: 267-79.

24. Watt SM, Gullo F, van der Garde M, Markeson D, Camicia R, Khoo CP, et al. The angiogenic properties of mesenchymal stem/stromal cells and their therapeutic potential. Br Med Bull 2013; 108: 25-53.

25. Smadja DM, Duong-van-Huyen JP, Dal Cortivo L, Blanchard A, Bruneval P, Emmerich J, et al. Early endothelial progenitor cells in bone marrow are a biomarker of cell therapy success in patients with critical limb ischemia. Cytotherapy 2012; 14: 232-9.

26. Szabo GV, Köves Z, Cserepes J, Daróczy J, Belkin M, Acsády G. Peripheral blood-derived autologous stem cell therapy for the treatment of patients with late-stage peripheral artery disease—results of the short- and long-term follow-up. Cytotherapy 2013; 15: 1245-52.

27. Wang ZX, Li D, Cao JX, Liu YS, Wang M, Zhang XY, et al. Efficacy of autologous bone marrow mononuclear cell therapy in patients with peripheral arterial disease. J Atheroscler Thromb 2014; 21: 1183-96.