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Acupoint transplantation versus non-acupoint transplantation using autologous peripheral blood mononuclear cells in treating peripheral arterial disease

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Abstract

Numerous studies have discussed the therapeutic outcomes of using cell therapy or acupuncture to treat peripheral artery disease (PAD). However, there are no long-term studies on the safety and efficacy of transplanting peripheral blood mononuclear cells (PBMNCs) via acupoints to treat PAD. We first reviewed the short-term and long-term clinical results of PAD patients treated with PBMNCs through intramuscular non-acupoint transplantation (control group; n = 45) or intramuscular acupoint transplantation (acupoint group; n = 45) at a single university hospital general medical center between December 2002 and September 2022. Pain intensity (assessed with the verbal rating scale [VRS] score) in the acupoint group was considerably lower than that in the control group at month 1 (mean ± standard deviation [SD]: 1.29 ± 0.96 vs 1.76 ± 0.82; $P = 0.016$) and month 3 (mean ± SD: 1.27 ± 0.90 vs 1.61 ± 0.86; $P = 0.042$). We observed significant improvement of VRS score ($P < .001$ for all) and ankle-brachial index (ABI; $P < .001$ for all) from baseline in both groups at months 1, 3, 6, 12, 36, and 60. The 10-year cumulative rate of major amputation–free survival (MAFS) was higher in the acupoint group as compared to the control group (81.9%, 95% confidence interval [CI]: 71.3%–94.1% vs 78.5%, 95% CI: 66.7%–92.3%; $P = 0.768$). Compared with the routine injection method, intramuscular transplantation of PBMNCs via selected acupoints could significantly decrease the short-term pain intensity in patients with PAD, which remains an option for consideration.

Key Words: Acupoint; Ischemic limb; Peripheral arterial disease; Peripheral blood mononuclear cells; Traditional Chinese Medicine

1. INTRODUCTION

Peripheral artery disease (PAD) is commonly referred to as lower-extremity PAD (LE-PAD).[1] It is characterized by atherosclerotic blockage of the arteries feeding the legs and is prevalent worldwide.[2] From 2000 to 2010, the number of patients aged ≥25 years with PAD increased from 163.60 million cases to 202.06 million, an increase of 23.51% within a decade.[3] In 2015, 236.62 million adults aged ≥25 years were reported to have PAD, an increase of 17.10% over the past 5 years.[4] It is a widely held view that PAD can lead to the deterioration of lower limb function and even necessitate limb amputation.[3,5] Medical therapy (advancements include more efficient antiplatelet drugs and even the association of anticoagulants), structured exercise program, and revascularization (including balloon dilation [angioplasty], stents, and atherectomy) are the currently acknowledged therapies for PAD.[4,6] Therapeutic efficacy is generally insufficient in patients with poor distal circulation without collateral circulation or older patients with severe cardiovascular disease, leading to compulsory amputation.

A reasonable approach for addressing this issue could be to employ cell therapy as a surrogate treatment.[7,8] In prior short-term studies, we revealed that transplantation of autologous peripheral blood mononuclear cells (PBMNCs) relieved limb ischemia in patients with lower limb arteriosclerosis obliterans (LASO) and with diabetic critical limb ischemia (CLI).[9–11] Additionally, PBMNCs should be more practicable than bone marrow mononuclear cells (BMMNCs) in the treatment of LASO.[11] Based on Traditional Chinese Medicine (TCM) theory, acupuncture therapy can alleviate gangrene and minimize pain by stimulating the appropriate acupoints.[12] The activation of meridians and collaterals is achieved through the precise stimulation of acupoints, facilitating the tonification and promotion of qi.[13] Acupoint injection involves the targeted administration

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of medication into specific acupoints to achieve therapeutic benefits through the synergistic interaction between drugs, acupoints, and acupuncture techniques.[14] This approach effectively harnesses these elements to dredge the meridians, activate blood circulation to alleviate blood stasis, optimize the circulation of both qi and blood, and maximize the therapeutic potential of the injected drug itself.[15] As a result, it promotes increased blood flow in the lower limbs, surpassing the efficacy of traditional acupuncture or simple intramuscular injections.[16] Furthermore, the findings from prior animal experiment support the hypothesis that acupoints may serve as a promising avenue for the delivery of stem cells.[17] However, to our knowledge, no long-term studies combine acupoint injection with cell therapy for PAD. Therefore, we aim to compare the clinical outcomes of acupoint and non-acupoint transplantation in the treatment of PAD.

2. MATERIALS AND METHODS

2.1. Study population

Participants who underwent PBMNCs transplantation in treating significant PAD (ankle-brachial index [ABI] <0.90) at our institute between December 2002 and September 2022 were consecutively enrolled in this retrospective study.[2] Eligible patients who matched the selection criteria were enrolled: unfit for surgical or endovascular intervention and without improvement for ≥3 months after surgical or endovascular intervention or experienced unsatisfactory treatment outcomes following ≥3 months of conservative management (includes smoking cessation, drug therapy, dietary changes, and physical training). The exclusion criteria were as follows: severe health issues existed <3 months prior to admission, such as severe cardiocerebrovascular diseases, pulmonary embolism, severe liver failure, severe renal failure; acute lower limb ischemia; gangrene above the ankle joint; proliferative retinopathy; a malignancy diagnosis or suspect <5 years prior to admission; incapable of tolerating the transplant.

2.2. Procedures

Recombinant human granulocyte-colony stimulating factor (rhG-CSF; Kirin Pharmaceuticals, Tokyo, Japan) was administered subcutaneously at a dose of 300 μg twice daily for 5 days to mobilize bone marrow stem cells. Additionally, intravenous heparin calcium (10,000 units/d) was administered for 5 days to prevent a hypercoagulable state. When the white blood cell (WBC) count increased to $>40\times10^9$/L or on day 5, PBMNCs suspension was obtained using a blood cell separator. Approximately 300 mL of the standard harvest volume was condensed to 1×10^8 cells/mL. Cell implantation for the lower limbs was achieved via intramuscular injection under general anesthesia (Fig. 1A). Three hours later, in the non-acupoint transplantation (control) group, each diseased lower leg was intramuscularly and evenly injected (60 sites, roughly 3 × 3 cm grid, 1–1.5 cm deep, 1×10^8 PBMNCs per site) in the muscles below the knee and above the ankle (Fig. 1B). In the acupoint transplantation (acupoint) group, a total of 60 acupoints located within the muscles (below the knee and above the ankle) were carefully chosen (Supplementary Table S1, http://links.lww.com/BS/A75). These acupoints traverse 6 specific meridians, namely, the stomach meridian of Foot-Yangming, gallbladder meridian of Foot-Shaoyang, bladder meridian of Foot-Taiyang, spleen meridian of Foot-Taiyin, liver meridian of Foot-Jueyin, and kidney meridian of Foot-Shaoyin (Fig. 1B). The control group did not deliberately avoid acupoints during injection, however, there was barely any overlap with the acupoint injection locations; moreover, both groups purposefully avoided blood vessels and bony landmarks. The quantity of cells injected at each site and the injection depth remained consistent in both groups. An extra transplant of the same amounts of cells preserved in liquid nitrogen was administered every 40 days to the lower extremities with severe lesions. Debridement, local wound care, and anti-infection treatment were performed if necessary.

2.3. Outcome variables and definitions

The primary endpoint was major amputation–free survival (MAFS). The secondary endpoints were total amputation–free survival (TAFS), new lesion–free survival (NLFS), pain intensity, and ABI. MAFS was defined as the number of days from the day of PBMNCs injection to the day of major amputation or death, whichever occurred first. TAFS was defined as the number of days from the day of PBMNCs transplantation to the day of total amputation or death, whichever came first. In this study, a major amputation refers to amputation at the ankle or higher, and a minor amputation refers to any procedure

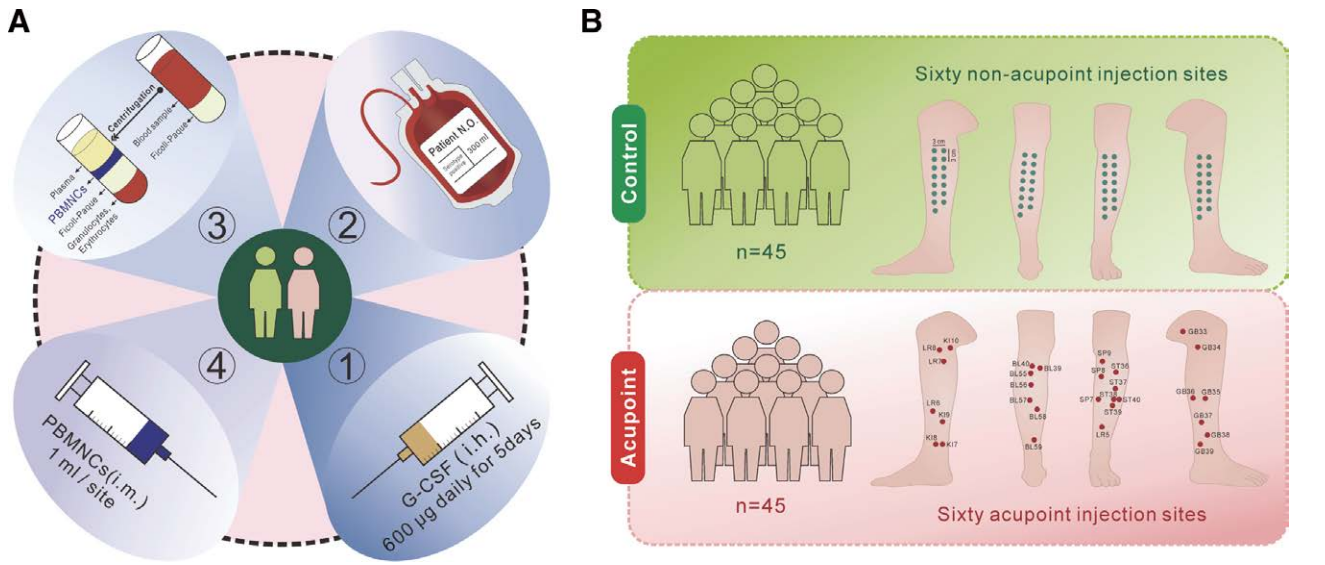

Figure 1. Operation procedure and injection sites. (A) Operation procedure. (B) Injection sites in the acupoint group and control group. Control refers to non-acupoint transplantation. The distance in (B) represented by the black line segments applies to all adjacent 2 points of the control group, in both vertical and horizontal dimensions. The detailed names and locations of the acupoints of (B) are shown in Supplementary Table S1, http://links.lww.com/BS/A75. PBMNCs = peripheral blood mononuclear cells, G-CSF = granulocyte-colony stimulating factor, i.h. = subcutaneous injection, i.m. = intramuscular injection.

involving amputation under the ankle, including that of the foot or toe(s). Total amputation included major and minor amputations.[5] NLFS was defined as the number of days from the day of PBMNCs injection to the day of new lesion or death, whichever occurred first. A new lesion refers to the appearance of severe ischemic symptoms that were not previously present and limited to ulcer or gangrene. The degree of pain experienced by the patients was scored using a verbal rating scale (VRS) with 5 levels, with a score of 0 indicating no pain and 4 indicating unbearable pain. ABI was evaluated by medacord personal vascular laboratory (PVL; MEDASONICS/®, Mountain View, CA).

Major adverse events recorded in our study encompassed all-cause mortality, cardiovascular events, cerebrovascular events, injection site pain, and mobilization-related adverse events. Mobilization-related adverse events were characterized by mild fevers, transient headaches, backaches, and pruritus.

In addition to outpatient and inpatient evaluations, the patients were followed up over the phone. Apart from pain intensity and ABI, which were evaluated at the first 6 time points, they were carried out postoperative evaluations of all other endpoint events in months 1, 3, 6, 12, 36, 60, and 120.

2.4. Statistical analysis

The quantitative data are displayed as the mean ± standard deviation (SD), or median with interquartile range (IQR), depending on their distribution. Categorical data are shown as numbers with percentages. The 2 groups were compared for adverse events using Pearson chi-squared test or Yate continuity correction. Changes in variables from baseline to each of the follow-up time points and differences between the 2 groups were examined by the *t* test. The MAFS, TAFS, and NLFS were plotted by Kaplan-Meier curves and evaluated by the log-rank test. R version 4.1.3 (R Foundation for Statistical Computing, Vienna, Austria) was used for all statistical analyses. A bilateral *P* value of 0.050 was considered significant.

3. RESULTS

3.1. Characteristics of patients

Between December 2002 and September 2022, we screened 104 patients with significant PAD who underwent PBMNCs transplantation at our center. Among them, 14 were excluded who met the exclusion criteria (Fig. 2). Thus, in all, 90 patients were enrolled and received acupoint transplantation (n = 45) or non-acupoint transplantation (n = 45). Baseline information about the patients is displayed in Table 1. The majority (53/90, 58.9%) of enrolled patients' cases of limb ischemia were caused by arteriosclerosis obliterans (ASO). At enrollment, VRS 3 to 4 patients accounted for 63.3%. Prior to PBMNCs transplantation, each patient had conservative treatment and, in some cases, at least 1 form of revascularization. The baseline characteristics were similar between the 2 groups. The median (IQR) age was 65.5 (51.5–72.3) years; 17 patients (18.9%) were female, and 73 (81.8%) were male (Table 1). The procedure (Approval No. QTJC2023008-EC-1) was approved by the ethics committee at our center.

3.2. Survival analysis

The mean follow-up period was 171.6 months (range 1–240 months), with the mean follow-up time in the acupoint and control groups being 162.3 and 180.9 months, respectively (*P* = .66). Of the 26 deaths, no death was identified as related to the study interventions. There were 5 of 26 (19.2%) of the patients who died because of PAD, including 4 of 26 (15.4%) from ASO and 1 of 26 (3.8%) from diabetes mellitus. Moreover, 7 of 26 (26.9%) of the patients experienced fatal strokes, while 5 of 26 (19.2%) of the patients succumbed to acute myocardial infarction (AMI). Furthermore, 2 of 26 patients (7.7%) succumbed to heart failure, while an additional 2 of 26 patients (7.7%) fell victim to severe pulmonary infection. The remaining 5 patients died from cancer, septic shock, sudden death (possible myocardial infarction), renal failure, and heart failure complicated by stroke, respectively. There were 35 (38.9%) total amputations, including 13 (14.4%) major amputations. In all, 58 (64.4%) patients were followed up for more than 10 years.

During the follow-up period, there were 7 (15.6%) major amputations in the control group: this event manifested within the first 2 years in 5 of 7 patients (71.4%), while the other 2 patients occurred at months 66 and 102, respectively. In the acupoint group, 6 (13.3%) major amputations occurred: 4 of 6 (66.7%) of the patients experienced this event within the first 2 years, while the remaining 2 patients encountered it in the third and fourth years, respectively. A decade after the transplant, the cumulative MAFS rate was 80.3% (95% confidence interval [CI]: 72.3%–89.2%; Fig. 3A). In the 2 cell groups, the

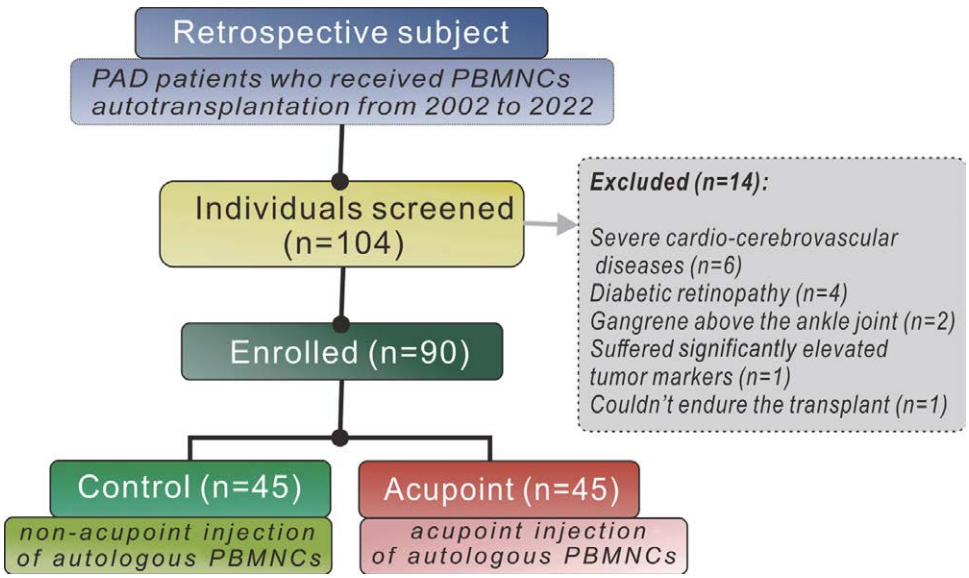

Figure 2. Study flowchart. PAD = peripheral arterial disease, PBMNCs = peripheral blood mononuclear cells.

Table 1

Patient characteristics.

Variable	No. (%) Control group* (n = 45)	Acupoint group (n = 45)	P†
Age, median (IQR), y	65.0 (51.0–70.0)	67.0 (48.0–74.5)	0.990
Male	37 (82.2)	36 (80.0)	>0.999
Etiology			
ASO	28 (62.2)	25 (55.6)	0.160
TAO	9 (20.0)	10 (22.2)	
DF	0	5 (11.1)	
Takayasu arteritis	1 (2.2)	1 (2.2)	
ASO and DF	7 (15.6)	4 (8.9)	
Hypertension	19 (42.2)	17 (37.8)	0.830
Diabetes mellitus	12 (26.7)	21 (46.7)	0.080
Hyperlipidemia	7 (15.6)	4 (8.9)	0.520
Cerebrovascular disorder	11 (24.4)	8 (17.8)	0.605
Coronary artery disease	17 (37.8)	11 (24.4)	0.255
Smoker	29 (64.4)	27 (60.0)	0.828
Rutherford classification			
1–3	10 (22.2)	11 (24.4)	>0.999
4–6	35 (77.8)	34 (75.6)	
Vascular surgery‡	9 (20.0)	5 (11.1)	0.383
Course of transplant			
<3	22 (48.9)	20 (44.4)	0.833
≥3	23 (51.1)	25 (55.6)	
VRS			
0–2	16 (35.6)	17 (37.8)	>0.999
3–4	29 (64.4)	28 (62.2)	
Gangrene‡	7 (15.6)	8 (17.8)	>0.999
Infection‡	11 (24.4)	11 (24.4)	>0.999
Total amputation§	3 (6.7)	7 (15.6)	0.315
Major amputation§	3 (6.7)	3 (6.7)	>0.999

ASO = arteriosclerosis obliterans, DF = diabetic foot, IQR = interquartile range, PBMNCs = peripheral blood mononuclear cells, TAO = thromboangiitis obliterans, VRS = verbal pain rating scale.

*Control refers to non-acupoint transplantation.

†All tests were 2-sided. $P < 0.050$ was considered significant.

‡All variables refer to a procedure or condition for the lower extremities.

§Total amputation includes major and minor amputations. Major amputation refers to amputation at the ankle or higher, and minor amputation refers to that below the ankle.

cumulative MAFS rate at the 10-year mark was slightly higher in the acupoint group (81.9%, 95% CI: 71.3%–94.1%) compared to the control group (78.5%, 95% CI: 66.7%–92.3%; $P = 0.768$; Fig. 3B).

In the control group, there were 14 (31.1%) total amputations with 12 of 14 (85.7%) taking place within the first year. Among the remaining patients, 1 (7.1%) underwent amputation in the 19th month, while another patient (7.1%) underwent amputation in the 66th month. In the acupoint group, a total of 21 amputations (46.7%) were recorded, with 17 (81.0%) occurring within the first year. Among these, 9 of 17 amputations (52.9%) took place during the last 6 months of the first year. Over intervals of 20, 36, 40, and 48 months, respectively, the remaining 4 patients underwent amputations. The cumulative 10-year TAFS rate after transplantation was over half of those surveyed (56.2%, 95% CI: 46.8%–67.6%; Fig. 3C). Additionally, compared with the patients in the control group, the individuals in the acupoint group who received PBMNCs had a similar 10-year cumulative TAFS rate (48.5%, 95% CI: 35.8%–65.6% vs 64.0%, 95% CI: 51.3%–79.9%; $P = 0.147$; Fig. 3D).

New lesions were detected in 4 (8.9%) patients in the control group, including 3 (75.0%) cases of gangrene in the left lower leg and 1 (25%) of ulcer on the right lower limb. After receiving appropriate conservative care and 2 rounds of transplantation, this 1 patient's ulcer resolved. The 3 patients with gangrene underwent 4 transplants and at 6, 19, and 102 months, respectively, they underwent major amputations because of the quick progression of ischemia. Five (11.1%) patients in the acupoint group (including 3/5 [60.0%] with ulcers and 2/5 [40.0%] with gangrene) developed new lesions. All 5 individuals showed symptom alleviation after standard treatment, underwent transplantation 2 to 5 courses, and received varying degrees of amputation. Among these patients, there were major amputations in months 7, 24, and 48, respectively, in 3 of 5 (60.0%) of the patients; a minor amputation in month 2 in 1 (20.0%) patient; and 3 minor amputations in months 6, 12, and 58 in the remaining 1 patient. The 10-year cumulative NLFS rate was 85.4% (95% CI: 78.4%–93.1%) in those assessed (Fig. 3E). In terms of 10-year cumulative NLFS, the acupoint and control group were comparable (84.3%, 95% CI: 77.1%–97.2% vs 86.6%, 95% CI: 74.3%–95.7%; $P = 0.822$; Fig. 3F).

3.3. Pain intensity and ABI

Compared with the control group, the acupoint group experienced obviously reduced pain intensity after 1 month (mean ± SD: 1.29 ± 0.96 vs 1.76 ± 0.82; $P = 0.016$) and after 3 months (mean ± SD: 1.27 ± 0.90 vs 1.61 ± 0.86; $P = 0.042$), whereas, in the subsequent months, there was no statistical difference between the groups (Fig. 4A). The VRS scores in the acupoint group significantly alleviated at 6 time points compared with that at baseline (mean ± SD: 1 month, 1.29 ± 0.96; 3 months, 1.27 ± 0.90; 6 months, 1.18 ± 0.85; 1 year, 1.11 ± 0.77; 3 years, 1.07 ± 0.95; 5 years, 1.05 ± 0.68 vs baseline, 2.73 ± 1.36; $P <$ .001 for all; Table 2). In fact, 1 month after transplantation, significant improvements in the VRS scores were noted in both groups, and these persisted throughout the subsequent 5 years (Table 2).

In comparison to the baseline, the ABI in the acupoint group significantly improved at each of the first 6 time periods (mean ± SD: 1 month, 0.73 ± 0.23; 3 months, 0.75 ± 0.29; 6 months, 0.75 ± 0.28; 1 year, 0.75 ± 0.28; 3 years, 0.78 ± 0.30; 5 years, 0.76 ± 0.30; vs baseline, 0.44 ± 0.21; $P <$ .001 for all; Fig. 4B). The control group experienced the same phenomenon in ABI (mean ± SD: 1 month, 0.77 ± 0.28; 3 months, 0.75 ± 0.29; 6 months, 0.74 ± 0.31; 1 year, 0.74 ± 0.31; 3 years, 0.73 ± 0.33; 5 years, 0.70 ± 0.30 vs baseline, 0.42 ± 0.20; $P <$ .001 for all; Fig. 4B). However, there was no significant difference in ABI between the 2 cell groups of patients in months 1, 3, 6, 12, 36, and 60 (Table 3).

3.4. Safety evaluation

No instances of severe acute adverse effects were detected among the patients who underwent autologous PBMNCs implantation. Safety results for the past 10 years are reported in Table 4. Nearly a third of the participants (27 patients, 30%) complained of pain at the injection sites (well-tolerated and disappeared after 1–2 days without intervention) as the most frequent adverse event after cell implantation, followed by mobilization-related adverse events (15 patients, 16.7%), cardiovascular events (7 patients, 7.8%), and cerebrovascular events (7 patients, 7.8%). In the acupoint group, 3 (6.7%) patients developed a mild fever, 2 (4.4%) experienced back pain, and 2 (4.4%) experienced temporary headaches. In the control group, 3 (6.7%) patients developed a mild fever, 3 (6.7%) experienced backache, and 2 (4.4%) developed pruritus; all these patients made a full recovery within a week after transplantation.

4. DISCUSSION

Our study aimed to assess the safety and efficacy of acupoint transplantation of PBMNCs for the treatment of PAD during a comprehensive 10-year follow-up. Within this study, the acupoint group exhibited substantial reductions in pain intensity, as evidenced by significant improvements in patients' VRS scores in comparison to the control group (within the first 3 months)

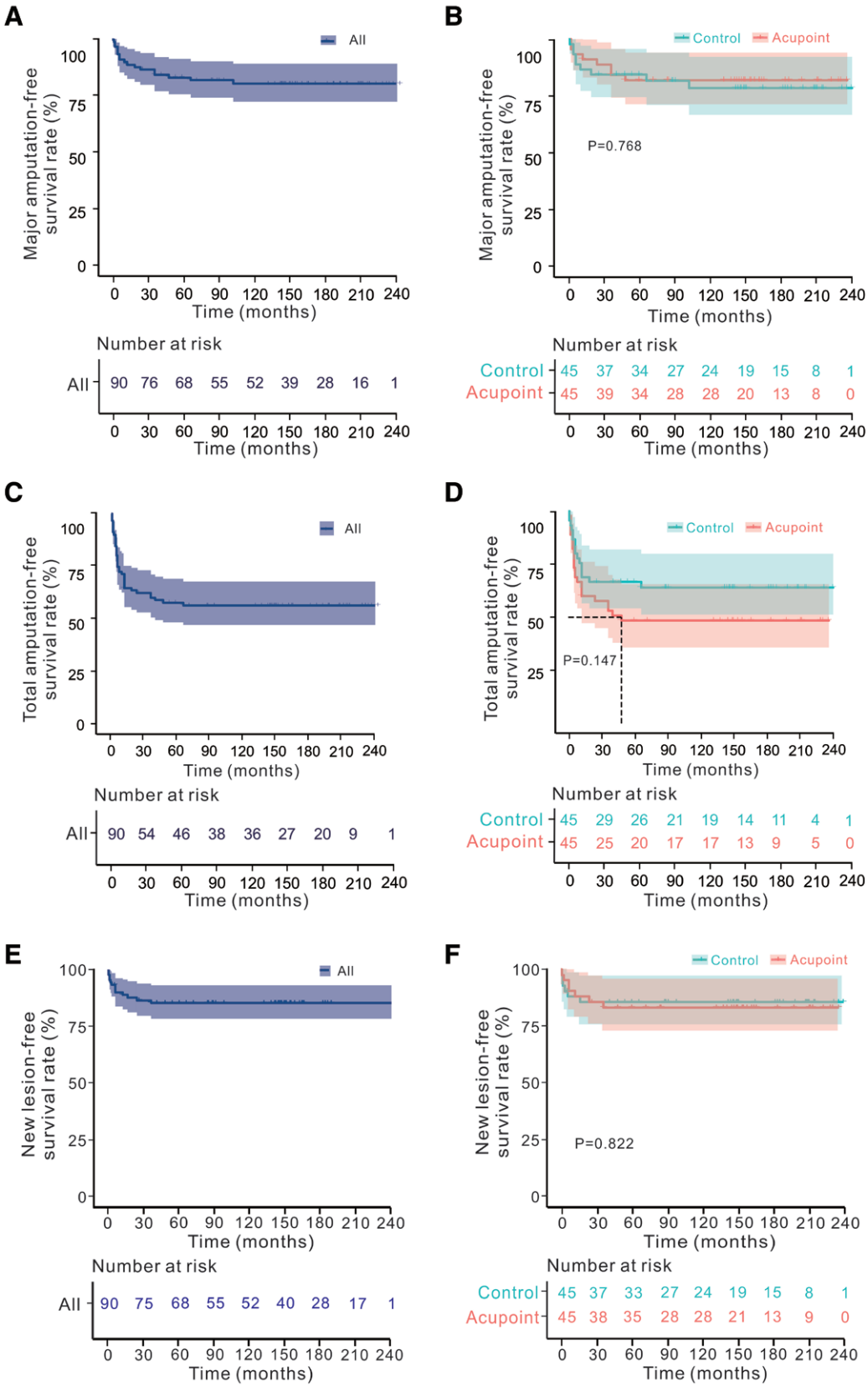

Figure 3. Kaplan-Meier analysis of MAFS rate, TAFS rate, and NLFS rate after PBMNCs transplantation. (A) MAFS in all participants (not grouped). (B) MAFS in both groups. (C) TAFS in all participants (not grouped). (D) TAFS in both groups. (E) NLFS in all participants (not grouped). (F) NLFS in both groups. Shaded areas indicate 95% CI in (A–F), and the dashed line indicates the median survival timeline in (D). CI = confidence interval, MAFS = major amputation–free survival, NLFS = new lesion–free survival, PBMNCs = peripheral blood mononuclear cells, TAFS = total amputation–free survival.

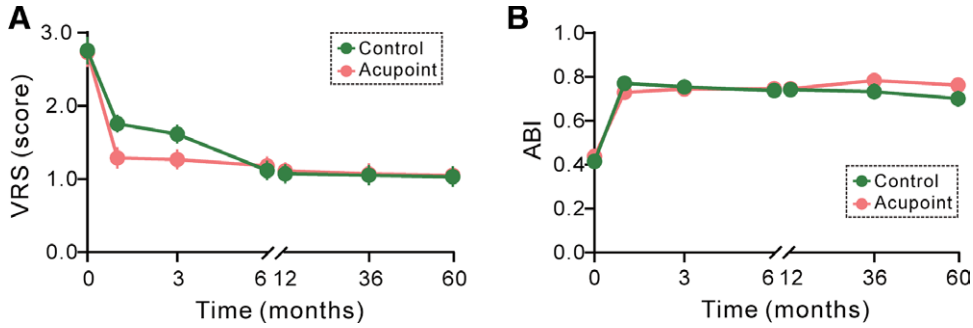

Figure 4. Serial changes in VRS and ABI. (A) VRS. (B) ABI. There was a significant difference in VRS scores between the 2 groups in both the first and third month ($P < 0.050$). During the first 6 follow-up assessments, both the acupoint and control groups displayed significant reductions in VRS scores and increases in ABI compared to baseline (all $P < .001$ vs baseline). ABI = ankle-brachial index, VRS = verbal rating scale.

Table 2

Pain score over time.

	Mean (SD)		
VRS score	Control group (n = 45)	Acupoint group (n = 45)	*P**
Baseline	2.76 (1.25)	2.73 (1.36)	0.936
1 mo	1.76 (0.82)	1.29 (0.96)	0.016
3 mo	1.61 (0.86)	1.27 (0.90)	0.042
6 mo	1.12 (0.84)	1.18 (0.85)	0.737
1 y	1.07 (0.83)	1.11 (0.77)	0.819
3 y	1.05 (0.86)	1.07 (0.95)	0.922
5 y	1.03 (0.80)	1.05 (0.68)	0.906

SD = standard deviation, VRS = verbal pain rating scale.
**P* values are based on the *t* test.

and baseline values (during the 5 years). These findings are likely the result of a synergistic effect arising from cell therapy and acupoint stimulation, each facilitating therapeutic benefits through distinct mechanisms.

According to the available data, acupuncture, a branch of complementary and alternative medicine, might be an affordable approach with minor risks and minimal side effects.[18] Adenosine, calcium, glutamate, and opioid peptides are just a few of the signal molecules that acupuncture has been demonstrated to produce and convey to particular pain-relieving regions.[19] Additionally, acupuncture helps lessen the firing of pain pathways via the enhancement of immune cell harmony and suppression of inflammatory reactions.[20] Available evidence has indicated an increase in nitric oxide (NO) levels at acupoints and meridians, leading to vasodilation and an augmented local blood supply, which is enriched with substances that contribute to pain relief.[21] Moreover, it has been postulated that the enhanced blood perfusion caused by acupuncture stimulation could be attributed to the suppression of sympathetic nerve activity, subsequently resulting in vasodilation within local microvascular networks.[22] This vasodilatory response may be one of the reasons for the reduced pain intensity in patients with acupoint transplantation. Some scholars have incorporated acupuncture point injection therapy into PAD treatment.[15,23,24] Through clinical investigations, it has been observed that patients exhibited noticeable improvements in short-term follow-ups (1–4 weeks) when compared to the control group. These improvements were manifested in reduced symptoms such as lower limb numbness, intermittent claudication (IC), and resting pain. These suggested the feasibility of acupuncture point injection of stem cells as a treatment for PAD.

Stem cells can differentiate into blood vessels and secrete certain pro-angiogenic growth factors and cytokines, such as vascular endothelial growth factor, angiopoietin-1 (Ang-1), hepatic growth factor, insulin-like growth factor (IGF)-1, stroma-derived factor-1 alpha (SDF-1α), and endothelial nitric oxide synthase (eNOS)/inducible nitric oxide synthase (iNOS),[25,26] which are the fundamental components of stem cell therapy. These factors stimulate the proliferation and migration of pre-existing endothelial cells, activate angiogenesis, and indirectly support vascular regeneration and tissue homeostasis.[27] Adult vasculogenesis is the process of circulation of cells with angiogenic potential that are discharged from the bone marrow and placed in the bloodstream by substances such as G-CSF.[28] Multiple types of cells, namely those derived from the bone marrow, peripheral blood-derived cells, progenitor or stem cells that are separated using unique surface markers from the bone marrow or blood, adipose vascular stromal cells, or mesenchymal stem cells, have been investigated in PAD since 2002 when the initial cell therapy pilot study for PAD was implemented.[29,30] Among the cell types that have been most probed are PBMNCs.[31] Ultimately, we designed the present study with an aim to treat ischemia of the lower limb by using a combination of TCM and PBMNCs treatment and to obtain further in-depth information on PAD.

We found that the combination of acupuncture and PBMNCs treatment had satisfactory therapeutic efficacy. Acupuncture therapy regulates a variety of organs and tissues, particularly in pathological circumstances. In tissue affected by ischemia, acupuncture has also been proven to encourage cellular proliferation.[32] It is interesting to note that stem cell compartments like CD133 + 34− cells have been found to be mobilized by acupuncture.[33] The expression of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) considerably enhanced following acupoint injection with cell therapy owing to therapeutic angiogenesis.[34] It can be attributed to the following 2 reasons. Firstly, the acupoint injection may aid in dredging the meridians, enhancing blood flow, and relieving blood stasis. The 3 Yin meridians and the 3 Yang meridians of the foot can achieve the objective of "balancing yin and yang"

Table 3

ABI over time.

	Mean (SD)		
ABI	Control group (n = 45)	Acupoint group (n = 45)	*P**
Baseline	0.42 (0.20)	0.44 (0.21)	0.452
1 mo	0.77 (0.28)	0.73 (0.23)	0.263
3 mo	0.75 (0.29)	0.75 (0.29)	0.844
6 mo	0.74 (0.31)	0.75 (0.28)	0.854
1 y	0.74 (0.31)	0.75 (0.28)	0.914
3 y	0.73 (0.33)	0.78 (0.30)	0.297
5 y	0.70 (0.30)	0.76 (0.30)	0.215

ABI = ankle-brachial index, SD = standard deviation.
**P* values are based on the *t* test.

Table 4

Safety results over the past 10 y.

Safety events	No. (%) Control group (n = 45)	Acupoint group (n = 45)	*P**
Mobilization-related adverse events†	8 (17.8)	7 (15.6)	0.781
Pain at the injection sites	17 (37.8)	10 (22.2)	0.114
All-cause mortality	15 (33.3)	11 (24.5)	0.353
Cardiovascular events	4 (8.9)	3 (6.7)	>0.999
Cerebrovascular events	2 (4.5)	5 (11.1)	0.434

**P* values are based on Pearson chi-squared test or Yate continuity correction.

†Mobilization-related adverse events included mild fevers, temporary headaches, backaches, and pruritus.

by regulating the dysfunction of the internal environment after ischemia. This improves stem cell survival after injection by fostering a favorable environment for them.[34] Secondly, acupoint injection has a quick and potent pharmacological effect. The medication delivery route's evident directionality allows it to directly target the ailment along the meridians, accelerating drug absorption and minimizing superfluous "consumption" in the medication action process.[34]

Arteriosclerosis leads to arterial narrowing and obstruction, causing insufficient blood supply and disrupting the balance between oxygen demand and supply. The key to treating PAD lies in ensuring vascular patency, promoting collateral circulation, and improving microcirculation in peripheral tissues.[35] The TCM theory of "obstruction leads to pain" aligns well with the pathophysiological mechanisms of PAD. Among the selected acupoints, 20 (Zusanli [ST-36], Shangjuxu [ST-37], Tiaokou [ST-38], Xiajuxu [ST-39], Fenglong [ST-40], Xiyangguan [GB-33], Yanglingquan [GB-34], Yangjiao [GB-35], Waiqiu [GB-36], Guangming [GB-37], Yangfu [GB-38], Xuanzhong [GB-39], Weizhong [BL-40], Heyang [BL-55], Feiyang [BL-58], Fuyang [BL-59], Zhongdu [LR-6], Xiguan [LR-7], Zhubin [KI-9], and Yingu [KI-10]) exhibit analgesic properties.[36] Additionally, 17 acupoints (ST-36, ST-38, ST-39, ST-40, GB-33, GB-34, GB-35, GB-36, GB-37, GB-38, GB-39, BL-40, BL-55, Chengjin [BL-56], Chengshan [BL-57], BL-58, and BL-59) activate the meridians.[36] Thus, we assumed that the utilization of the acupoints above can alleviate the clinical symptoms in PAD patients, particularly in terms of reducing pain. Compared to the other acupoints we selected, ST-36, renowned for its effectiveness in fortifying the body's immune system to combat pathogenic factors, additionally has the ability to promote neurogenesis,[37,38] act as a neuroprotective barrier against oxidative damage,[39] inhibit apoptosis,[40] and enhance the expression of several anti-inflammatory mediators.[41] Guided by the holistic view of TCM, we leave no meridian along the lower limbs unexplored.[42] Our hope is that these acupoints spanning 6 meridians can collaboratively harmonize the functions of various body systems, thereby optimizing the efficacy of acupoint therapy. It is noteworthy that a universally accepted standard combination of acupoints for PAD remains absent. In many cases, researchers tend to devise acupoint combinations based on their individual clinical experience. Consequently, there is a pressing need for further investigation into the optimal acupoint combination in future studies.

The current findings demonstrate a significant impact of acupoint injection with PBMNCs on pain reduction, notably enhancing their quality of life (QoL). However, no notable differences were observed between the 2 groups concerning ABI, adverse events, MAFS, TAFS, and NLFS. Acupoint injection of PBMNCs exerts a dual effect, combining cell therapy with acupoint stimulation, facilitating both localized and systemic regulation. In theory, this should yield more favorable therapeutic outcomes. The current results may be attributed to certain aspects of our experimental design that require further refinement. This may involve increasing the depth of needle penetration during acupoint stimulation and employing specific techniques to induce the De Qi sensation in patients, thereby optimizing the benefits of acupoint stimulation. Prolonging the duration of acupoint stimulation might further enhance the therapeutic potential of stem cells. Our research is still in its preliminary stages. Large-scale, multicenter studies are crucial for a comprehensive evaluation of treatment efficacy.

Multiple studies, including our own, have statistically classified disorders with various etiologies, such as ASO and thromboangiitis obliterans (TAO), as a class of peripheral vascular diseases.[43–45] According to the Intraarterial Progenitor Cell Transplantation of Bone Marrow Mononuclear Cells for Induction of Neovascularization in Patients With Peripheral Arterial Occlusive Disease (PROVASA) study, mononuclear cell treatment appeared to benefit TAO patients more than ASO patients, with a better likelihood of ulcer repair and rest pain relief.[46] However, researchers of one study hypothesized that the relation of etiology to prognosis is at least partly explained by aging and aging-related deficits in cellular functions as the individuals with TAO had substantially younger beginning ages than those suffering from ASO.[47] As a result, scientists believe that age affects the effectiveness of PBMNCs transplants more directly than etiology does.[47] Because the etiology of ASO predominated in our research population (53 patients, 58.8%), it is possible that our therapy would be more effective in a population where TAO predominated.

Our investigation identified several adverse events, including cardiovascular (7 patients, 7.8%) and cerebrovascular (7 patients, 7.8%) incidents. Given the underlying pathological condition, these occurrences can be anticipated, and no apparent causal relationship has been established between them and PBMNCs transplantation therapy. In comparison to recent studies,[48] our study observed a relatively higher incidence of cardio-cerebral events, which aligns with earlier findings.[49] This divergence could be attributed to the extended duration covered by our research, which was characterized by limited advancements in novel antiplatelet, anticoagulant, and lipid-modifying medications available in the early years, unlike the current medical landscape. Therefore, it is crucial to meticulously evaluate the association between cell therapy and the aforementioned adverse events in future prospective clinical trials.

However, this research has several limitations. Our study had a small sample size and some heterogeneity. This is because we use alternative methods in a somewhat innovative way. We should be conscious, nevertheless, that prior cellular treatments have demonstrated that treatment outcomes might differ from disease to disease. Unfortunately, a comparison of therapy outcomes by underlying illness is not possible due to the study's small sample size. Prospective, randomized, controlled studies involving a large number of participants are needed to increase the statistical power, corroborate the findings of this study, and contrast the results of therapy for various etiologies. Furthermore, we mainly studied "real" hard endpoints such as survival and amputation. Further clinical parameters, including QoL, pain-free walking time (PFWT), ulcer healing rate, toe-brachial index (TBI), transcutaneous oxygen pressure ($TcPO_2$), return-to-work rates, and CLI-free ratio will be tested in subsequent research. These parameters will support the development of more accurate and consistent conclusions about PBMNCs transplantation in patients with PAD. Two of the most difficult questions in cell treatment are whether stem cells will convert into various tissues rather than blood vessels and the precise process of stem cell differentiation remains unclear. It is crucial to acknowledge the relevance of the ischemia environment in the context of neovascularization, and

how to prevent harm to new cells and blood vessels in the ischemic microenvironment needs to be explored.

In conclusion, this study showed that acupoint injection of PBMNCs may be a potential and promising complementary and alternative therapy in the treatment of PAD; especially, this novel treatment decreased the short-term pain intensity significantly.

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AUTHOR CONTRIBUTIONS

W.G., L.P., and R.Y. performed data collection, analysis, and writing the manuscript. W.G., L.P., J.S., and Q.H. were involved in the study design and revised the manuscript. P.H. was involved in the critical revision of the manuscript. All authors were involved in the final approval of the article.

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