Received: 24 January 2020 | Accepted: 12 November 2020

DOI: 10.1002/sctm.20-0033

HUMAN CLINICAL ARTICLE

STEM CELLS TRANSLATIONAL MEDICINE

Three-year outcomes of peripheral blood mononuclear cells vs purified CD34$^+$ cells in the treatment of angiitis-induced no-option critical limb ischemia and a cost-effectiveness assessment: A randomized single-blinded noninferiority trial

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Funding information

Zhongshan Funds for the Institute of Vascular Surgery, Fudan University; Fudan University "Star of Tomorrow" Famous Doctor Training Project; Shanghai Excellent Academic Leader, Grant/Award Number: 19XD1401200; Excellent Core Member Training Program at Zhongshan Hospital, Fudan University, China, Grant/Award Number: 2015ZSYXGG02; China National Natural Science, Grant/Award Numbers: 81900426, 81970407, 30801122; Training Program for Outstanding Academic Leaders of the Shanghai Health and Family Planning System, Grant/Award Number: 2018BR40

Abstract

For patients with angiitis-induced critical limb ischemia (AICLI), cell transplantation, such as purified CD34$^+$ cells (PCCs) and peripheral blood mononuclear cells (PBMNCs), is gradually being used as a promising treatment. This was the first randomized single-blinded noninferiority trial (number: NCT 02089828) specifically designed to evaluate the therapeutic efficacies of the transplantation of PCCs vs those of PBMNCs for the treatment of AICLI. We aimed to compare the mid-term safety and efficacy between the two groups and determine their respective advantages. From April 2014 to September 2019, 50 patients with AICLI were equally allocated to the two groups, except for 1 lost patient, 1 amputee, and 1 patient who died of heart disease. The other 47 patients completed the 36-month follow-up. The endpoints were as follows: major amputation-free survival and total amputation-free survival at 6 months, which were 96.0% and 84.0% in the PBMNCs group and 96.0% and 72.0% in the PCCs group, respectively. These rates remained stable at 12, 24, and 36 months. The PCCs group had a significant higher probability of rest pain relief than the PBMNCs group, whereas earlier significant improvements in the Rutherford classification were observed in the PBMNCs group. Accordingly, PCCs would be preferred for patients with significant pain, whereas PBMNCs may be a good option for patients with two or more critically ischemic limbs. Concerning cost-effectiveness, PCCs are not more cost-effective than PBMNCs. These outcomes require verification from long-term trials involving larger numbers of patients.

KEYWORDS

adult stem cells, angiogenesis, CD34, cellular therapy

Hao Liu and Tianyue Pan contributed equally.

1 | INTRODUCTION

As the end stage of peripheral artery disease, critical limb ischemia (CLI) is characterized by rest pain and tissue loss with a 6-month mortality rate of approximately 20%.[1,2] Owing to a high postoperative reocclusion rate and poor anatomical conditions, 20%-50% of patients with CLI are not suitable for either surgical or endovascular treatment and are also called no-option CLI (NO-CLI) patients.[3] For these patients, both the 6-month major limb amputation rate and 1-year mortality rate reached 10%-40%.[2,4,5] Angiitis-induced critical limb ischemia (AICLI), defined as ischemia caused by thromboangiitis obliterans (TAO) or other arteritis-related autoimmunological diseases such as systemic lupus erythematosus (SLE), erythema nodosum, Crohn's disease, psoriasis, and scleroderma, constitutes a substantial proportion of NO-CLI patients. AICLI usually has a common feature of involving the distal or even micro vessels, and consequently underlies the inefficacy of conventional revascularization. Some studies and meta-analyses informed that stem cell therapies, such as transplantation involving bone marrow mononuclear cells (BMMNCs), peripheral blood mononuclear cells (PBMNCs), and purified $CD34^+$ cells (PCCs), have shown encouraging results in ischemia improvement among patients with AICLI. The long-term efficacies of PBMNCs and BMMNCs are similar,[6] whereas BMMNCs might be less feasible than PBMNCs because of the tedious and risky steps required, such as bone marrow aspiration and general anesthesia. However, there are few studies comparing PBMNCs with PCCs in the treatment of AICLI. PCCs have potential advantages over PBMNCs in terms of the higher purities of $CD34^+$ cells, which might enhance angiogenesis and lessen the inflammatory reaction.[7,8] On the other hand, PCCs have the following possible disadvantages: (a) loss of >50% of $CD34^+$ cells during isolation; and (b) removal of $CD34^-$ cells and thus loss of their paracrine effect, which is considered helpful for angiogenesis. Therefore, in 2014, we launched a randomized single-blinded parallel-group controlled trial (ClinicalTrials.gov: NCT 02089828) to compare PBMNCs and PCCs for the treatment of AICLI. The 12-month outcomes demonstrated an overall equivalent efficacy between the two autoimplants, except for earlier ischemia relief and less pain at injection sites in the PCC group.[9] The present study aims to compare the 3-year outcomes between PBMNC and PCC treatment. Given the comparable 12-month results between these two treatments and the additional cost for the purification of PCCs, a cost-effectiveness analysis was performed.

Lessons learned

- This study demonstrated the satisfactory mid-term outcomes of transplantation of both peripheral blood mononuclear cells (PBMNCs) and purified $CD34^+$ cells (PCCs) in the treatment of no-option angiitis-induced critical limb ischemia.
- PBMNC transplantation was more cost-effective in most conditions and may be preferred for patients with ≥2 limbs needing transplantation at one stage.
- PCC transplantation was advantageous in its ability to achieve earlier pain relief and thus was preferred for patients with severe pain.
- In patients with obvious inflammation, PCCs could be chosen to avoid aggravation of inflammation for its lower injected volume and removal of most $CD34^-$ cells.

Significance statement

In 2014, the first clinical trial specifically designed to evaluate the therapeutic efficacy and safety of transplantation of peripheral blood mononuclear cells (mixed cell types) with those of purified $CD34^+$ cells (special cell type) for the treatment of angiitis-induced no-option critical limb ischemia was initiated in the authors' center. The mid-term outcomes of the study showed similar satisfactory efficacy and safety of the two therapies and provided evidence for more precise application of cell therapy under different conditions.

2 | MATERIALS AND METHODS

2.1 | Design and participants

Fifty patients were enrolled in this randomized single-blinded parallel-group controlled trial from April 2014 to September 2019, and the study was approved by the Ethics Committee of Fudan University Affiliated Zhongshan Hospital. All the participants signed informed consent before enrollment. The study protocol was detailed previously.[9] In brief, the inclusion criteria were as follows: patients aged 18-80 years; the presence of stenotic or occlusive lesions in the limb arteries, as confirmed by magnetic resonance angiography, computed tomography angiography, or digital subtraction angiography; CLI with a Rutherford classification of 4-5 that was unsuitable for or not improved ≥3 months following surgery or an endovascular intervention; rest pain that was not alleviated after ≥1 month of conservative treatments; and an area of tissue loss that had not diminished in size after ≥1 month of these treatments. The exclusion criteria were cardiac-cerebral vascular events ≤3 months before admission; proliferative retinopathy; a life expectancy of ≤1 year; a diagnosis or suspicion of cancer ≤5 years before admission; or contraindications for the administration of recombinant human granulocyte colony-stimulating factor (rhG-CSF).

2.2 | Randomization and masking

As we had described,[9] participants' demographic data, CLI etiologies, Rutherford classifications, comorbidities, other disease histories, and medical and surgical histories were recorded by the investigators before randomization. Then, the eligible patients were allocated 1:1 to the

PBMNCs or PCCs groups at random using a computer-generated randomization schedule (SAS, Proc Mixed, version 8.2; SAS Institute, Cary, North Carolina). All of the patients were masked before and after the interventions. The cell transplantations, the independent decisions about minor or major amputations after the transplantations and the patients' follow-up assessments, and data collection and analysis were completed by three separate groups of surgeons who were blinded to the work of other groups. The masking was removed if a serious adverse event occurred that was related to the trial, death, or loss to follow-up occurred or if an emergency required unmasking. When the 36-month follow-up period ended, the database was locked.

2.3 | Procedures

RhG-CSF (Neupogen; Amgen, Thousand Oaks, California; 5-10 μg/kg per day) and enoxaparin (4000 IU/day) were subcutaneously administered in all patients for 4 days. Apheresis (COM.TEC; Fresenius Hemocare GmbH, Bad Homburg, Germany) was performed on the fifth day. Then, for the patients in the PBMNCs group, cells separated by apheresis were washed three times and resuspended in an ethylenediaminetetraacetic acid-phosphate buffered saline solution (200 mL) that contained 0.5% human albumin. For the patients in the PCCs group, $CD34^+$ cells were purified using a magnetic cell sorting system (MiltenyiBiotec GmbH, Bergisch Gladbach, Germany) immediately after leukapheresis. The final cell products were assessed by flow cytometry and leukocyte counting. The surgeons implanted the cells into the calves/arms and feet/hands of the ischemic limbs via equidistant intramuscular injections (0.5 mL/site) under general anesthesia. Severely infected wounds were debrided. Additional details about the procedure have been previously described.[10]

2.4 | Outcomes

All of the adverse events and all-cause mortality from mobilization to 2 weeks after injection, the leukocyte counts during hospitalization and at 1, 2, 3, 6, 12, 24, and 36 months during the follow-up, and pathological retinal angiogenesis were included in the safety outcomes. The primary efficacy outcomes included minor amputation (below the ankle), major amputation (above the ankle), and total amputation (both). The major amputation-free survival (MAFS) and total amputation-free survival (TAFS) rates were calculated. The secondary efficacy outcomes included complete wound healing, the Wong-Baker Faces Pain Rating Scale (WBFPS; a score of 0 represents no pain and a score of 10 represents the greatest pain), Rutherford classification, pain-free walking time (PFWT; at 2.5 km/h and at a 10% incline on a treadmill), the ankle-brachial index (ABI), the toe-brachial index (TBI), transcutaneous oxygen pressure ($TcPO_2$), and quality of life (QoL).[9,11,12] During the 3-year follow-up, recurrence (transplanted limb had CLI again) and new

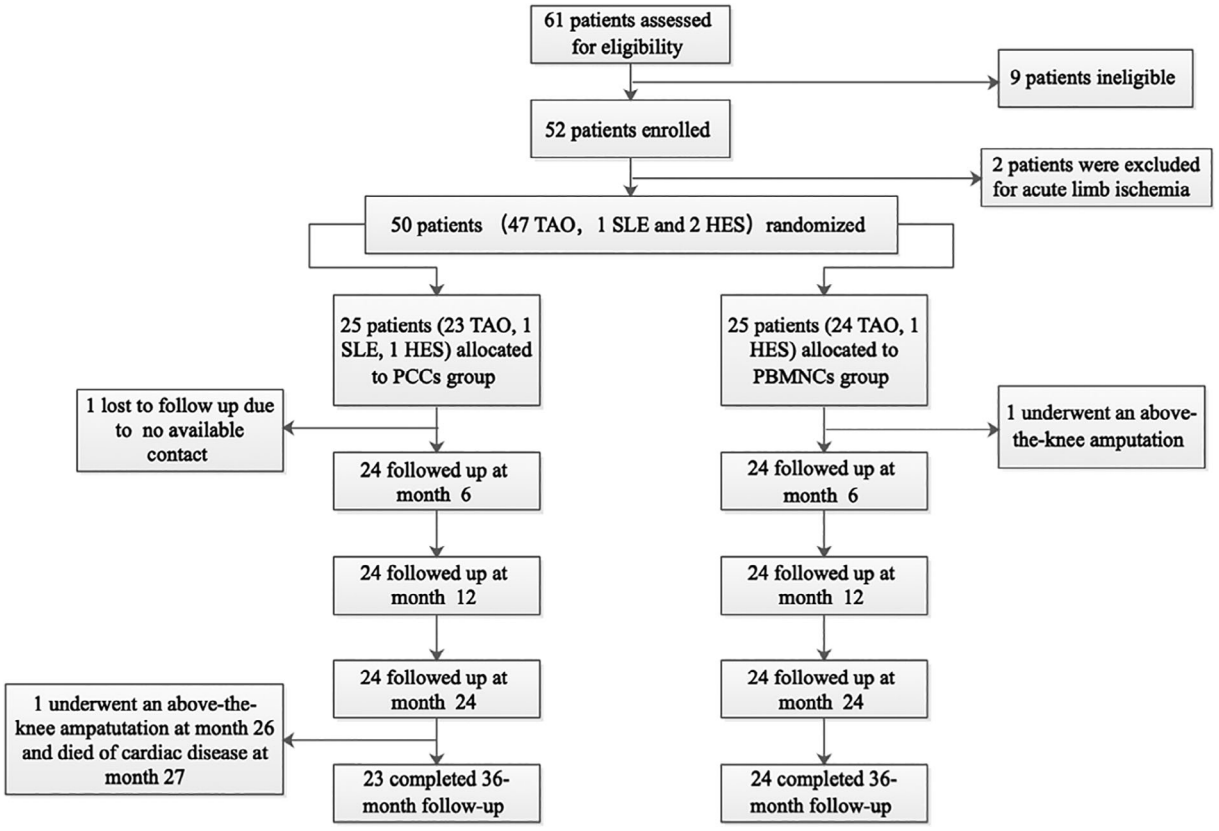

FIGURE 1 Trial design. HES, hypereosinophilic syndrome; PBMNCs, peripheral blood mononuclear cells; PCCs, purified $CD34^+$ cells; SLE, systemic lupus erythematosus; TAO, thromboangiitis obliterans

lesions (untransplanted limb had CLI) were assessed. The recurrence rate (RR) and new lesion rate (NLR) were evaluated. By using incremental cost-effectiveness ratios (ICERs), cost-effectiveness was quantified as the ratio of the difference in cost and the effect between the two strategies. Quality-adjusted life-year (QALY), which ranges from 0 (death) to 1 (health), was used to evaluate the effect of the two strategies. We calculated the QALYs by transferring the scores of the 36-item Short Form Health Survey (version 2; SF-36 v2) to the Short Form 6-Dimension (SF-6D).[13,14] Any patient who was lost to follow-up or dead was considered the worst-case scenario. All outcomes at 24 and 36 months were observational.

2.5 | Statistical analyses

The quantitative data are shown as the estimated margin mean (EMM) ± SE (for comparison between two groups), mean ± SD, or as the median with the interquartile range (IQR), depending on their distribution. The categorical data are presented as numbers with percentages. Pearson's chi-square test with or without Yete's continuity correction or Fisher's exact test was used to compare the groups in relation to all-cause mortality, complete wound healing, rest pain alleviation, Rutherford class, and recurrence/new lesions. A linear mixed model was used to analyze the effects of the cell type on the longitudinal changes in the continuous variables and to determine the presence of any interactions between the individual groups and the time point. The Wilcoxon signed-rank test was used to compare the QoL scores at baseline and at 1 year and 3 years after transplantation. Kaplan-Meier curves and the Breslow-Wilcoxon test were used to depict and compare the MAFS, TAFS, and rest pain relief probabilities. The significance level was set at .05 for all statistical tests.[9] The statistical analyses were performed using PASW software, version 19 (IBM Corporation, Armonk, New York) and GraphPad Prism version 5.0 (La Jolla, California).

3 | RESULTS

3.1 | Baseline clinical characteristics

From April 2014 to December 2016, 50 out of 61 patients with AICLI were enrolled in the trial after screening. All patients were equally allocated to each group at random. Forty-seven patients completed the 36-month follow-up period. One patient (PCCs group) was lost to follow-up at 2 months after transplantation, one patient (PBMNCs group) underwent a major amputation within 6 months after transplantation, and one patient (PCCs group) underwent a major amputation at 26 months and died of cardiac disease at 27 months after transplantation (Figure 1). The baseline characteristics of the patients were detailed in our previous study.[9] The mean age of all patients was 41.46 years, and all patients were male with unilateral ischemic lower limb. In both groups, most patients had a smoking history (92.0% of the PBMNCs group and 84.0% of the PCCs group), whereas only a few patients had risk factors for cardio-cerebrovascular disease, such as hypertension (4.0% of the PBMNCs group and 8.0% of the PCCs group), diabetes mellitus (4.0% and 8.0%, respectively), and hyperlipidemia (8.0% and 8.0%, respectively). All patients were in AICLI condition with a 4-5 Rutherford classification. Forty-seven patients had TAO, two had hypereosinophilic syndrome (HES), and one had SLE. At admission, two patients had only rest pain (Rutherford classification = 4), 18 patients had a nonhealing ulcer, and 30 patients had gangrene (Rutherford classification = 5). The mean ABI, TBI, and $TcPO_2$ were similar in both groups. No significant differences were observed between the groups among all the baseline characteristics.

3.2 | Safety assessment

Nausea, low back pain, and/or low-grade fever were observed in 13 patients (6 in the PBMNCs group, 7 in the PCCs group; $P = .747$) during mobilization and resolved spontaneously within 3 days. No serious adverse events, such as death, cardio-cerebrovascular events, or hepatic or renal dysfunction, were observed during hospitalization. The transplantation volume was significantly higher in the PBMNCs group than in the PCCs group (80 [60-110] mL vs 39 [38-40] mL; $P < .001$). Pain at the

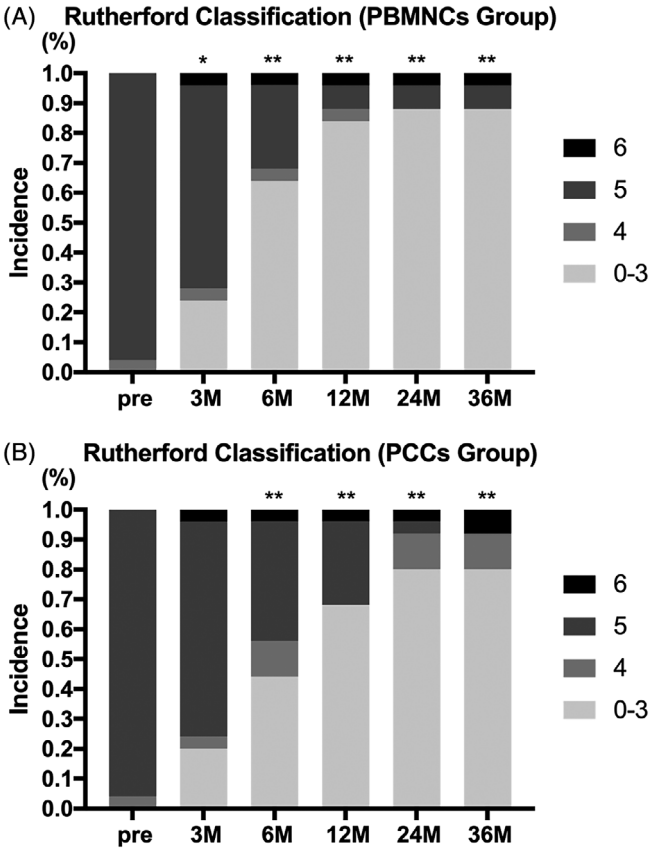

FIGURE 2 The change in Rutherford classification during the 3-year follow-up. Serial changes in Rutherford classification (0-6) proportions of the A, PBMNCs and B, PCCs groups. *$P < .05$ vs baseline; **$P < .01$ vs baseline. PBMNCs, peripheral blood mononuclear cells; PCCs, purified $CD34^+$ cells

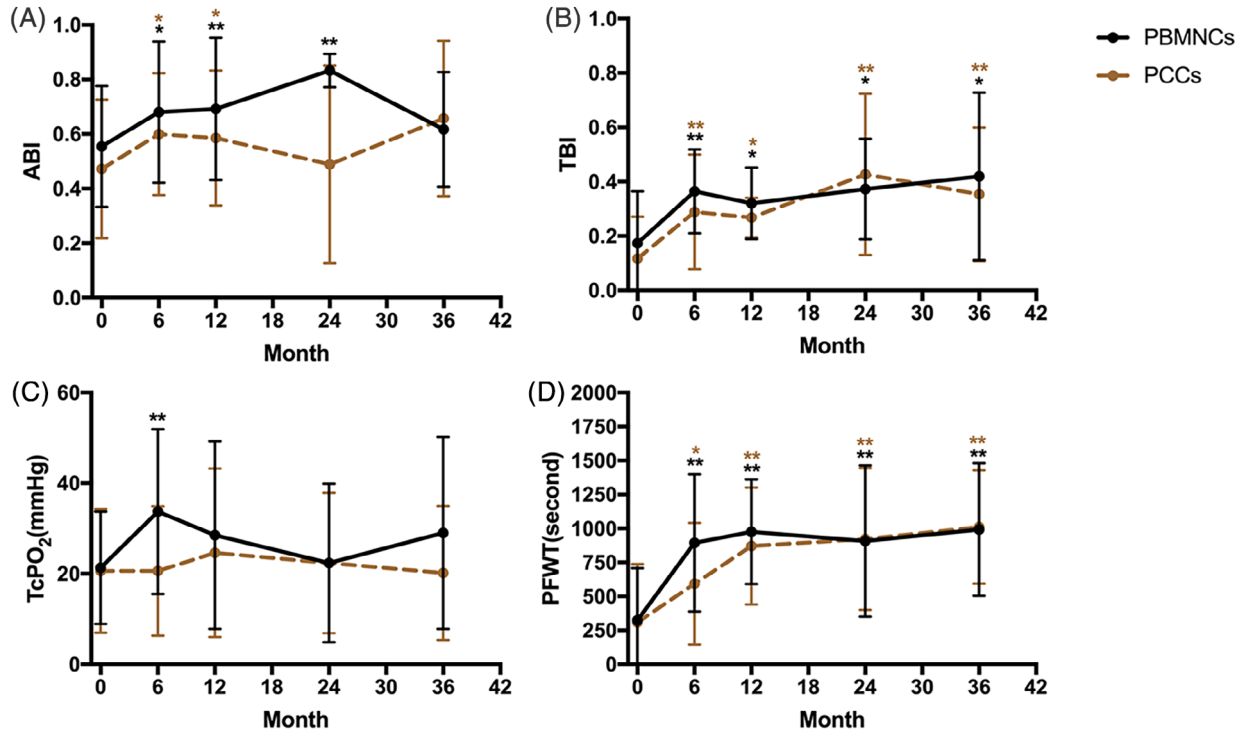

FIGURE 3 Longitudinal changes in blood perfusion restoration and functional improvement. The assessments of blood perfusion restoration included the A, ankle-brachial index, B, toe-brachial index, and C, transcutaneous oxygen pressure, and the functional improvement was assessed based on D, pain-free walking time. The values are presented in linear graphs that show the means and SDs. *P < .05 vs baseline; **P < .01 vs baseline. ABI, ankle-brachial index; PBMNCs, peripheral blood mononuclear cells; PCCs, purified CD34$^+$ cells; PFWT, pain-free walking time; TBI, toe-brachial index; $TcPO_2$, transcutaneous oxygen pressure

injection sites was more frequently observed in the PBMNCs group than in the PCCs group (14/25 vs 2/25; P < .001). No pathological retinal angiogenesis or tumorigenesis was observed during the follow-up. One patient in the PCCs group died of cardiac disease 27 months after transplantation, which was attributed to his long cardiac disease history.

3.3 | Cell product

A CD34$^+$ cell dose of no less than 10^5/kg was included in each cell transplant except for two subjects in the PCCs group (3.54×10^4 cells/kg and 4.56×10^4 cells/kg). The median absolute numbers of CD34$^+$ cells were 7.66×10^7 cells (IQR: 4.40-14.90 $\times 10^7$ cells) in the PBMNCs group and 3.17×10^7 cells in the PCCs group (IQR: 2.34-4.32 $\times 10^7$ cells; P = .003). The median absolute numbers of CD34$^+$ cells per kg were 8.77×10^5 cells/kg (IQR: 5.28-12.50 $\times 10^5$ cells/kg) in the PBMNCs group and 4.86×10^5 cells/kg in the PCCs group (IQR: 3.12-6.60 $\times 10^5$ cells/kg; P = .016).

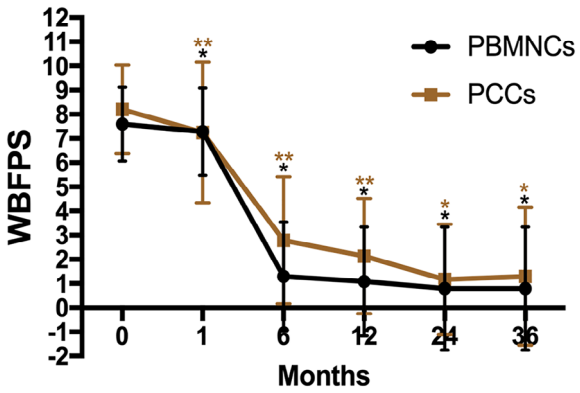

FIGURE 4 Longitudinal changes in the Wong-Baker Faces Pain Rating Scale. The longitudinal changes in the WBFPS in both groups are depicted as linear graphs that show the mean values and the SD bars. *P < .05; **P < .01 (intragroup comparison with baseline, based on a general linear mixed model). PBMNCs, peripheral blood mononuclear cells; PCCs, purified CD34$^+$ cells; WBFPS, Wong-Baker Faces Pain Rating Scale

3.4 | Efficacy evaluation

Preoperatively, 2 patients were categorized as Rutherford classification 4 (1 in the PBMNCs group, 1 in the PCCs group), and 48 were categorized as Rutherford classification 5 (24 in the PBMNCs group and 24 in the PCCs group). During the 36-month follow-up, the Rutherford classification in the PBMNCs group improved significantly by 3 months (P < .05) and was sustained up to 36 months (Figure 2A), whereas in the PCCs group, the Rutherford classification improved significantly by 6 months (P < .001) and was sustained up to 36 months (Figure 2C).

TABLE 1 Comparisons of the groups using the intention-to-treat principle based on worst-case scenarios

	PBMNCs group (*n* = 25)	PCCs group (*n* = 25)[a]	*P* value
Major amputation			
At 6 months	1/25 (4)	1/25 (4)	1.000
At 12 months	1/25 (4)	1/25 (4)	1.000
At 24 months	1/25 (4)	1/25 (4)	1.000
At 36 months	1/25 (4)	2/25 (8)	.552
Minor amputation			
At 6 months	3/25 (12)	6/25 (24)	.462
At 12 months	3/25 (12)	6/25 (24)	.462
At 24 months	3/25 (12)	6/25 (24)	.462
At 36 months	3/25 (12)	6/25 (24)	.462
Total amputation			
At 6 months	4/25 (16.0)	7/25 (28.0)	.306
At 12 months	4/25 (16.0)	7/25 (28.0)	.306
At 24 months	4/25 (16.0)	7/25 (28.0)	.306
At 36 months	4/25 (16.0)	8/25 (32.0)	.185
All-cause mortality			
At 6 months	0/25 (0)	1/25 (4)	.312
At 12 months	0/25 (0)	1/25 (4)	.312
At 24 months	0/25 (0)	1/25 (4)	.312
At 36 months	0/25 (0)	2/25 (8)	.141
Complete wound healing			
At 3 months	7/24 (29.2)	5/24 (20.8)	.505
At 6 months	17/24 (70.8)	13/24 (54.2)	.233
At 12 months	21/24 (87.5)	16/24 (66.7)	.086
At 24 months	21/24 (87.5)	21/24 (87.5)	1.000
At 36 months	21/24 (87.5)	22/24 (91.7)	.637
Rest pain alleviation			
At 1 week	4/25 (16.0)	6/25 (24.0)	.480
At 2 weeks	8/25 (32.0)	17/25 (68.0)	.011
At 3 weeks	15/25 (60.0)	21/25 (84.0)	.059
At 1 month	21/25 (84.0)	22/25 (88.0)	.684
At 3 months	24/25 (96.0)	23/25 (92.0)	.552
At 6 months	24/25 (96.0)	25/25 (100.0)	.312
At 12 months	24/25 (96.0)	25/25 (100.0)	.312
At 24 months	23/25 (96.0)	24/25 (96.0)	.552
At 36 months	23/25 (92.0)	23/25 (92.0)	1.000
Rutherford class at 6 months			
0-3	16/25 (64.0)	11/25 (44.0)	.156
4	1/25 (4.0)	3/25 (12.0)	.602
5	7/25 (28.0)	10/25 (40.0)	.370
6	1/25 (4.0)	1/25 (4.0)	1.000
Rutherford class at 12 months			
0-3	21/25 (84.0)	17/25 (68.0)	.185
4	1/25 (4.0)	0/25 (0)	0.312
5	2/25 (8.0)	7/25 (28.0)	.141
6	1/25 (4.0)	1/25 (4.0)	1.000

TABLE 1 (Continued)

	PBMNCs group (n = 25)	PCCs group (n = 25)[a]	P value
Rutherford class at 24 months			
0-3	22/25 (88.0)	20/25 (80.0)	.440
4	0/25 (0.0)	3/25 (12.0)	.074
5	2/25 (8.0)	1/25 (4.0)	.552
6	1/25 (4.0)	1/25 (4.0)	1.000
Rutherford class at 36 months			
0-3	22/25 (88.0)	20/25 (80.0)	.440
4	0/25 (0.0)	3/25 (12.0)	.074
5	2/25 (8.0)	0.25 (0.0)	.149
6	1/25 (4.0)	2/25 (8.0)	.552
Recurrence			
At 6 months	0/25 (0.0)	0.25 (0.0)	1.000
At 1 year	1/25 (4.0)	1/25 (4.0)	1.000
At 2 years	1/25 (4.0)	1/25 (4.0)	1.000
At 3 years	2/25 (8.0)	2/25 (8.0)	1.000
New lesion			
At 6 months	0/25 (0.0)	0.25 (0.0)	1.000
At 1 year	0/25 (0.0)	0.25 (0.0)	1.000
At 2 years	1/25 (4.0)	2/25 (8.0)	.552
At 3 years	1/25 (4.0)	2/25 (8.0)	.552
Total deterioration			
At 6 months	0/25 (0.0)	0.25 (0.0)	1.000
At 1 year	1/25 (4.0)	1/25 (4.0)	1.000
At 2 years	2/25 (8.0)	2/25 (8.0)	1.000
At 3 years	3/25 (12.0)	3/25 (12.0)	1.000

Note: Data are presented as n (%).
Abbreviations: PBMNCs, peripheral blood mononuclear cells; PCCs, purified $CD34^+$ cells.
[a]One patient in PCCs group was lost to follow-up at 2 months after transplantation.

By the 36-month follow-up assessment, the EMM ± SE ABI of the PBMNCs group increased significantly (ΔABI: 0.068 [±0.028]; $P < .05$) 1 month after transplantation compared with that at baseline. The increase in the EMM ABI in the PCCs group was significant (ΔABI: 0.082 [±0.042]; $P < .001$) at 2 months after transplantation. These significant improvements were sustained up to 12 months ($P < .001$ and $P = .020$, respectively). At 24 months, the increase in the EMM ABI in the PBMNCs group remained significant (ΔABI: 0.274 [±0.037]; $P < .001$), whereas no significant increase was observed in the PCCs group (ΔABI: 0.121 [±0.106]; $P = .272$). No significant differences were observed at 36 months after transplantation in either group (ΔABI: 0.230 [±0.132]; $P = .095$ for PBMNCs and ΔABI: 0.111 [±0.093]; $P = .248$ for PCCs; Figure 3A).

Significant differences in the increase in the EMM (±SE) TBI were observed in both the PBMNCs group (0.104 [±0.033]; $P = .011$) and the PCCs group (0.112 [±0.043]; $P = .007$) at 2 months after transplantation. The significance was sustained up to 36 months after transplantation (0.347 [±0.038]; $P < .05$ for PBMNCs and 0.237 [±0.036]; $P < .001$ for PCCs; Figure 3B).

The increase in the EMM (±SE) $TcPO_2$ of the PBMNCs group was significant at 2 months after transplantation (10.884 [±4.766] mm Hg; $P = .024$), remained significant until 6 months after transplantation ($P = .007$), and became nonsignificant at 12 months (7.304 [±4.120]; $P = .164$) until 36 months ($P = .170$) after transplantation. In the PCCs group, the increases in the EMM (±SE) $TcPO_2$ were significantly improved only at 2 months (12.428 [±4.736]; $P = .015$) and 3 months ($P = .017$) after transplantation and became nonsignificant at 6 months (0.161 [±4.854]; $P = .901$) until 36 months ($P = .933$) after transplantation (Figure 3C).

We evaluated the functional improvement of the patients by comparing the PFWT before and after transplantation. Preoperatively, five patients (three in the PBMNCs group and two in the PCCs group) were ineligible for the test owing to severe deformities, plantar wounds, or contralateral limb amputation. At baseline, the test was immediately abandoned (PFWT score = 0) because of intolerable pain in 16 patients (6 in the PBMNCs group and 10 in the PCCs group). Significant differences were observed at 1 month after transplantation (ΔPFWT: 177 [±85] seconds; $P = .038$ in the PBMNCs group and

ΔPFWT: 160 [±75] seconds; $P = .029$ in the PCCs group) and were sustained up to 12 months (ΔPFWT: 653 [±80] seconds; $P < .001$ in the PBMNCs group and ΔPFWT: 562 [±82] seconds; $P < .001$ in the PCCs group), 24 months ($P < .001$ in the PBMNCs group and $P < .001$ in the PCCs group), and 36 months (ΔPFWT: 669 [±80] seconds; $P < .001$ in the PBMNCs group and ΔPFWT: 702 [±69] seconds; $P < .001$ in the PCCs group; Figure 3D).

All patients were admitted with moderate to severe rest pain (WBFPS > 4). At 1 week after transplantation, significant differences in the EMM (±SE) WBFPS scores were observed compared with those at baseline in both the PBMNCs group (−0.480 [±0.227]; $P = .041$) and the PCCs group (−0.800 [±0.359]; $P = .039$). The differences remained significant at 12 months ($P < .05$ and $P < .001$, respectively), 24 months ($P < .05$ and $P < .05$, respectively), and 36 months (−6.810 [±0.427]; $P < .05$ for PBMNCs and −6.918 [±0.442]; $P < .05$ for PCCs; Figure 4). At 2 weeks, pain relief (WBFPS ≤ 4) occurred in 8 patients (32.0%) in the PBMNCs group and in 17 patients (68.0%) in the PCCs group ($P = .011$). The pain relief rate gradually increased and peaked at 6 months (24/25 of PBMNCs and 22/25 of PCCs) in both groups, and then the pain relief rate of the PCCs group increased to 92.0% (23/25) at 36 months, whereas that of PBMNCs group dropped to 92.0% (23/25) at 24 months and 36 months.

Ulcer or focal tissue loss (Rutherford classification 5) was present in 96% (24/25) of patients in each group at enrollment. At 6 months, the wounds healed in 17 patients (70.83%, 17/24) in the PBMNCs group and 13 patients (54.17%, 13/24) in the PCCs group ($P = .233$). At 12 months, the ulcer-healing rate reached 87.5% (21/24) in the PBMNCs group and was sustained up to 36 months. In the PCCs group, the ulcer healing rates at 12 months, 24 months, and 36 months after transplantation were 66.67% (16/24), 87.50% (21/24), and 91.70% (22/24), respectively (Table 1). The patient who was admitted without an ulcer to the PBMNCs group developed an ulcer at 1 month after transplantation, which had healed by the 12-month follow-up assessment. Recurrent ulcer was observed in one patient in the PBMNCs group at 21 months and had not healed at 36 months. No significant differences were observed between the groups in relation to the wound-healing rate at any time point.

3.5 | Amputation

Four patients (two in each group) underwent planned minor amputation during transplantation for severe infection. We did not include these events in the statistical analysis. Within 6 months after

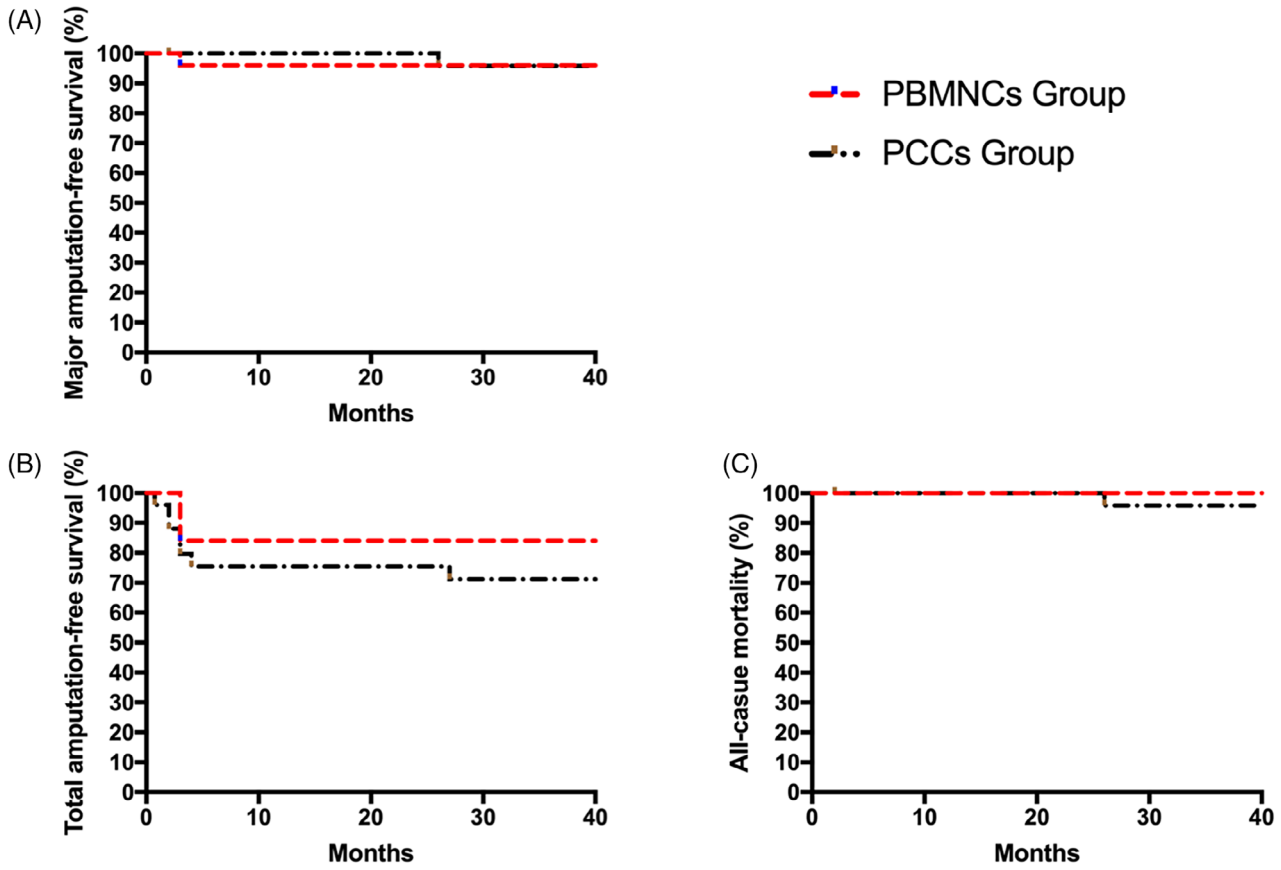

FIGURE 5 Kaplan-Meier curves showing the probabilities of A, major amputation-free survival, B, total amputation-free survival, and C, all-cause mortality in both groups. PBMNCs, peripheral blood mononuclear cells; PCCs, purified $CD34^+$ cells

transplantation, nine patients underwent unplanned minor amputations (three in the PBMNCs group and six in the PCCs group), and one patient in the PBMNCs group underwent major amputations. At 12, 24, and 36 months, only one major amputation was performed in the PCCs group at 26 months. The major amputation rates were 4% (PBMNCs group) and 4% (one patient in the PCCs group was lost to follow-up at 2 months) at 6 months, 12 months, and 24 months and 4% (PBMNCs group) and 8% (PCCs group) at 36 months. The total amputation rate of the PBMNCs group was 16% at 6 months, and this rate was sustained up to 12, 24, and 36 months. In the PCCs group, the total amputation rates were 28% at 6, 12, and 24 months and 32% at 36 months (Table 1). There were no significant differences between the two groups in terms of the probabilities of MAFS and TAFS (Breslow-Wilcoxon test: $P = .161$ and $P = .529$, respectively; Figure 5).

3.6 | Quality of life

The SF-36 v2 was used to estimate the QoL, based on eight dimensions, of patients at admission and 12 months and 36 months after transplantation. Significant improvements could be observed from the

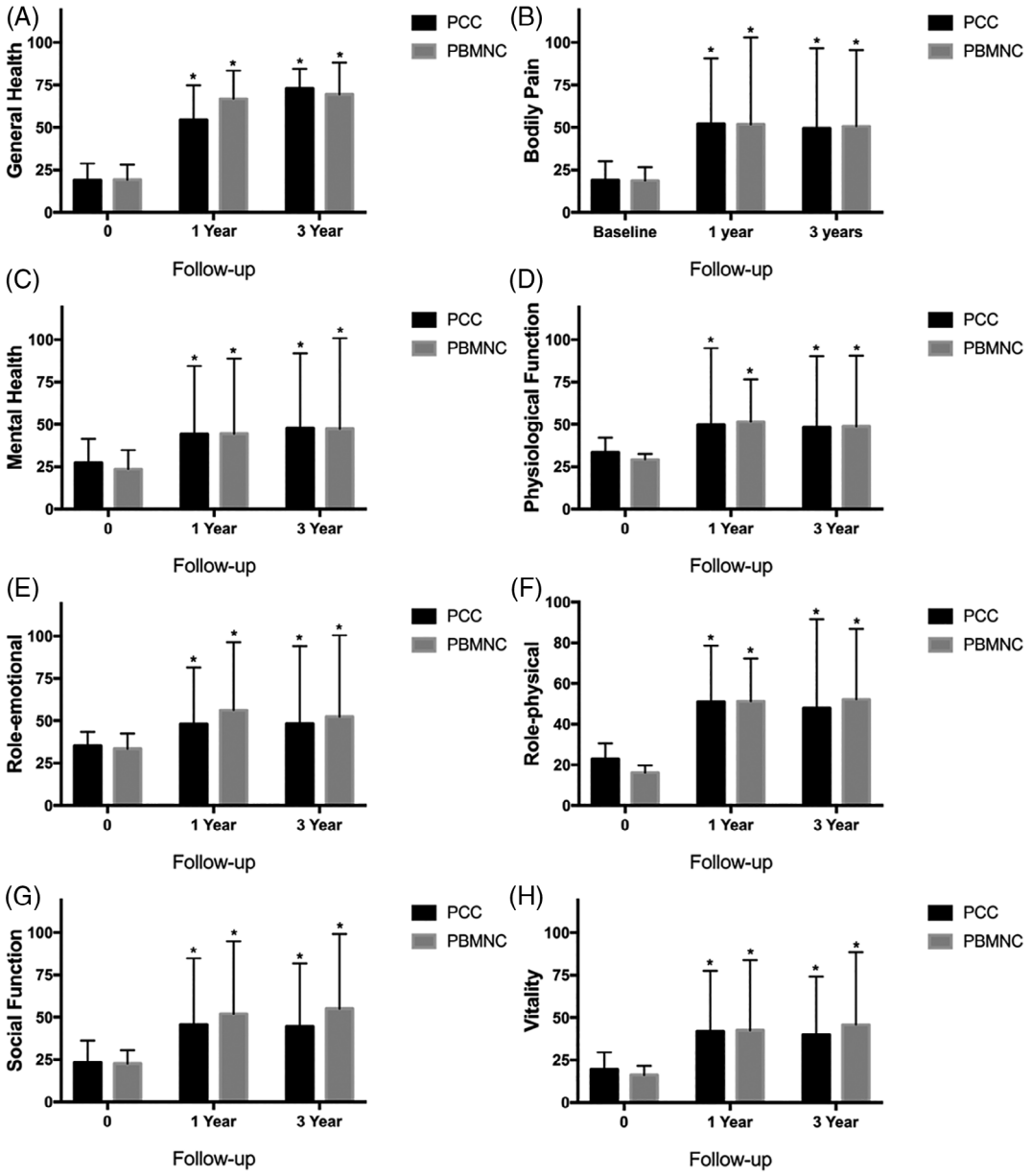

FIGURE 6 Quality of life at baseline and at 1 year after transplantation. Quality of life was assessed using the Short Form-36 scoring system (version 2) in the purified $CD34^+$ cells group and peripheral blood mononuclear cells group, including eight domains: A, general health, B, bodily pain, C, mental health, D, physiological function, E, role-emotional, F, role-physical, G, social function, and H, vitality. $^*P < .05$ (intragroup comparison with baseline, based on the Wilcoxon signed-rank test). PBMNCs, peripheral blood mononuclear cells; PCCs, purified $CD34^+$ cells

12-month data in both groups compared with the baseline data, and these improvements were sustained up to 36 months (Figure 6). All 50 patients were unable to work before transplantation because of the pain and dysfunction of the ischemic limbs. At 12 months, 80% (20/25) of patients in the PBMNCs group and 80% (20/25) of patients in the PCCs group resumed work. At 36 months, 92% of (23/25) patients in the PBMNCs group and 88% (22/25) of patients in the PCCs group were able to work and participate in social activities.

3.7 | Recurrence and new lesions

By the 36-month follow-up assessment, two patients experienced recurrence (one in the PBMNCs group and one in the PCCs group; Table 1). One patient in the PCCs group manifested mild intermittent claudication, which was relieved after conservative treatment. The other patient in the PBMNCs group developed a new ulcer at a different site of the ipsilateral foot, which turned infectious at 40 months (June 2019). A minor amputation was accordingly performed. Five patients developed new lesions during the 36-month follow-up period (Figure S1; Table 1). Among them, four patients (two in the PBMNCs group and two in the PCCs group) had mild intermittent claudication, and one patient (PCCs group) had rest pain in the contralateral lower extremity. The patient in the PCCs group, who had claudication on the untransplanted limb at 23 months after transplantation, was given conservative treatment and hyperbaric oxygen therapy. However, the symptoms were sustained, and he ultimately had a major amputation at 26 months after transplantation and died of cardiac disease at 27 months. The new lesions among the other patients were relieved after conservative treatment. The 3-year RRs were 4% (1/25) in the PBMNCs group and 4% (1/25) in the PCCs group, and the 3-year NLRs were 8% (2/25) in the PBMNCs group and 12% (3/25) in the PCCs group ($P = .637$).

3.8 | Cost-effectiveness

ICERs were used to compare cost-effectiveness between the two groups, and all cost values are reported in RMB. The costs (mean ± SD) in the PBMNCs group and PCCs group were 24 320.80 ± 19 600.39 and 70 156.60 ± 8380.81 ($P < .001$), respectively. QALYs were calculated based on the assumptions that the SF-6D value changed in a linear fashion (Figure S2). The area under the two curves of the two groups showed that the QALYs were 2.051 ± 0.157 (PBMNCs group) and 2.101 ± 0.204 (PCCs group; $P = .971$). Then, according to the formula ICER = △cost/△QALYs, we obtained an ICER of CNY 916 716.00 per QALY. According to the World Health Organization, an ICER that falls within one- to three-times the national gross domestic product (GDP) per capita is considered to be cost-effective,[15] so we set the boundary value of ICER as three-times China's 2016 GDP per capita (8113 USD equals CNY 53 974), which is CNY 161 922 (24 339 USD). Obviously, our ICER was far greater than this limit. This result indicated that PCC treatment was not cost-effective compared with PBMNC treatment.

3.9 | Results of patients with TAO

Most patients had TAO-induced CLI (47/50), and similar efficacy was also observed in patients with TAO among the two groups. The improvements in EMM TBI, EMM WBFPS, and ΔPWFT were significant in TAO patients of the PBMNCs (TPBMNCs) group (all $P < .05$) and TAO patients of the PCCs (TPCCs) group (all $P < .05$) at 12 months after transplantation and remained significant to 24 months (all $P < .05$ in two groups) and 36 months (all $P < .05$ in two groups). The increase in EMM ABI was significant at 12 months ($P < .01$) and 24 months ($P < .01$) after transplantation and became insignificant at 36 months ($P = .130$) in the TPBMNCs group. In TPCCs, no significant increases were observed at 12 months ($P = .194$), 24 months ($P = .643$), and 36 months ($P = .194$). Regarding EMM $TcPO_2$, no significant changes were observed at 12 months ($P = .130$ and .209), 24 months ($P = .092$ and .578), and 36 months ($P = .169$ and .576) in both the TPBMNCs group and the TPCCs group. Among 47 TAO patients, 1 TPBMNCs patient underwent major amputation and 3 TPBMNCs patients and 5 TPCCs patients underwent minor amputation. The 36-month MAFS were 95.83% (23/24) in TPBMNCs and 91.30% (21/23; one patient was lost and one was dead) in TPCCs. The 36-month TAFS were 87.50% (21/24) in TPBMNCs and 73.91% (17/23; one lost patient) in TPCCs. At 36 months after transplantation, 91.67% (22/24) of patients in TPBMNCs and 86.96% (20/23) of patients in TPCCs resumed work with significantly improved QoL ($P < .01$ and $P < .01$, respectively).

4 | DISCUSSION

Stem cell transplantation has been considered a promising treatment for patients with AICLI owing to its effects on vasculogenesis and angiogenesis.[16-19] Both PBMNC and PCC transplantation have been shown to be effective.[10,20,21] Endothelial progenitor cells, of which $CD34^+$ cells are a key component, are responsible for therapeutic angiogenesis.[22] We reported the 12-month follow-up of this study and found that similar results were observed between the PBMNCs and PCCs groups.[9] When we combined the similar 36-month results of the two groups with the significant differences of the absolute number of $CD34^+$ cells and that/body weight between the two groups, we inferred that $CD34^+$ cells play a predominant role in the treatment and that the high purity of $CD34^+$ cells might compensate for the loss of $CD34^-$ cells.

Most patients in the current trial had TAO (24/25 in the PBMNCs group and 23/25 in the PCCs group). Accordingly, the two groups had similar and comparable preoperative demographic characteristics, such as a low rate of cardiovascular and cerebrovascular disease risk factors and a high smoking rate (92% in the PBMNCs group and 84% in the PCCs group). TAO is a kind of disease characterized by non-atherosclerotic, progressive vasculitis of the small and medium arteries. Conventional therapeutic approaches, including surgical and endovascular procedures, have shown poor mid- to long-term efficacy. The major amputation rate of patients with TAO has been reported to be as high as 12%-31%, and approximately 34.8% of

patients lost their ability to work before 42 years of age.[23-25] The poor effect of conventional revascularization methods was ascribed to pathological features, such as involvement of the distal middle and small vessels, preservation of the internal elastic lamina of the involved vessels, superficial phlebitis, and inflammatory nature, which are detrimental to endovascular or surgical treatment.[21,26] The other three patients had other types of angiitis, such as SLE and HES. Although they were not as common as TAO, attention should also be paid to them. A study reported that the prevalence of SLE-induced CLI was 1.4% in a retrospective study, with 71.43% of patients suffering from digit loss,[27] and the prevalence rates of giant cell arteritis-induced upper and lower extremity ischemia were 26% and 18%, respectively.[28]

The data demonstrated 3-year total amputation-free survival rates of 84% (21/25) in the PBMNCs group and 68% (17/25) in the PCCs group ($P = .185$). Only three major amputations (one lost patient was accounted) occurred, and one of these patients died for non-transplantation-related reasons at 27 months. Forty-eight patients had tissue loss or gangrene (24/25 of PBMNCs and 24/25 of PCCs) before transplantation. At 36 months after transplantation, five patients (three in the PBMNCs group and two in the PCCs group) had unhealed wounds. Additionally, the values of EMM PFWT and WBFPS of the two groups both remained significantly improved from baseline, even at 36 months. Compared with the change in ABI and $TcPO_2$, the sustained statistical change in TBI indicated that it can be a relatively more sensitive indicator for the detection of blood perfusion, whether in the short term or long term. All patients lost labor ability before transplantation. Improvements in QoL were continuously significant at 12, 24, and 36 months after transplantation in both groups. Significant increases were observed at 24 months in the results of social functioning ($P = .046$) and at 36 months in the results of vitality ($P = .012$) in the PBMNCs group compared with the results of the PCCs group, indicating that PBMNCs may be superior to PCCs for the improvement of QoL in the medium-term follow-up. We inferred that this is due to the earlier improvement in ischemia associated with PBMNCs after transplantation as a result of a larger number of $CD34^+$ cells and $CD34^-$ cells. The number of patients who re-entered the labor force increased gradually and peaked at 36 months after transplantation (23/25 in the PBMNCs group and 22/25 in the PCCs group). Therefore, when QoL and labor force are considered, PBMNCs may be more meaningful to young patients. Regarding recurrence and new lesions, six patients and seven limbs were involved, and all these patients resumed smoking or were exposed to second-hand smoke after transplantation. Most limbs (5/7) were involved from 18 months to 36 months after transplantation and ended with a 3-year recurrence rate of 8.0% (2/25) in both groups, which was similar to the findings of our previous study.[25] Furthermore, there were no related serious adverse events, such as death, cardio-cerebrovascular events, or hepatic or renal dysfunction, that occurred in any patient. In brief, similar satisfactory mid-term safety and efficacy were observed in the two groups.

In the process of obtaining PCCs, along with $CD34^-$ cells, approximately 73% of $CD34^+$ cells were lost. Therefore, we generally injected part of the PBMNCs transplants and all of the PCCs transplants to control the dosage of $CD34^+$ cells between 10^5/kg and 10^6/kg. Compared with the PBMNCs group, the PCCs group achieved earlier pain relief (at 2 weeks, 68% of the PCCs group vs 32% of the PBMNCs group; $P = .011$). This might be because PCCs lacked $CD34^-$ cells, which could induce the local inflammatory reaction and increase the oxygen consumption of the ischemic area,[8] while the relatively larger volume of the PBMNCs transplant might also lead to late pain relief to a certain degree. However, PBMNCs may induce earlier ischemia improvement (3 months, $P < .05$) than the PCCs group, which is reflected in the Rutherford classification (Figure 2), probably owing to increased angiogenesis as a result of the greater number of $CD34^+$ cells and the paracrine effect of $CD34^-$ cells. Therefore, the use of PCCs would be preferred for patients with severe pain, whereas PBMNCs would be preferred for patients with two or more limbs affected by CLI needing simultaneous cell injection. Considering the high $CD34^+$ loss rate (ratio of the amount of lost $CD34^+$ cells after isolation and the amount of $CD34^+$ cells before isolation; 73%) in the process of obtaining PCCs (12.1×10^7, IQR: [7.94-16.8] $\times 10^7$ $CD34^+$ cells before and 3.21×10^7, IQR: [2.34-4.32] $\times 10^7$ $CD34^+$ cells after the isolation), PBMNCs would be preferred for patients with significant low $CD34^+$ cell count during mobilization. In addition, PCCs might be superior to PBMNCs because of their higher level of potential suitability for future allotransplantation. Concerning the economic perspective, ICER cost-effectiveness evaluation indicated that the PCCs treatment was not more cost-effective than the PBMNCs treatment. As a result, from an economic perspective, PBMNCs may be preferred.

In the present study, of the 47 patients with TAO-induced CLI, similar satisfactory results were observed during the 36-month follow-up, that is, a major amputation rate of 6.38% (3/47) and labor competence rate of 89.36% (42/47). Besides, the 5-year follow-up results of 27 patients with PCCs-transplanted AICLI (23 patients with TAO-induced CLI were included) in our center also demonstrated the efficacy of cell therapy in treating TAO-induced CLI with a 5-year MASF of 88.89% (20/23) and labor competence rate of 73.91% (17/23). Cell therapy shows its significant and long-lasting effect in these studies; therefore, we prefer it as the first-line treatment for patients with TAO-induced CLI.

The present study had some limitations. First, without a placebo-treated group, the placebo effect of the cell therapy might be neglected. However, we considered placebo use to be unethical because the enrolled patients were at the CLI stage and had barely benefited from previous conservative treatments (smoking cessation and drug treatment for at least 1 month before transplantation). On the other hand, the unsatisfactory results of medical, surgical, or endovascular treatment before transplantation could somehow be regarded as self-control to reflect the limited effect of the placebo effect of cell therapy. Second, although the sample size was calculated by the noninferiority test, the lack of power calculation cannot be ignored owing to the relatively small number of patients in our trial. Third, 92% of patients (23/25) in the PBMNCs group and 84% of patients (21/25) in the PCCs group had a smoking history before transplantation. Because of the high

frequencies of smoking history and a high smoking cessation rate of more than 80% at 12 months and 70% at 36 months, the observed CLI improvement could partly result from the effect of smoking cessation. In a recent report,[29] patients were divided into a BMMNCs group and a smoking cessation group, and the results showed that smoking cessation alone may not improve ischemia, but smoking cessation might be a critical factor enabling proper stem cell function. Fourth, because 47 patients of the study had TAO and only 3 patients had SLE/HES, larger trials with more patients without TAO will be needed to further evaluate the clinical outcomes of patients with AICLI treated with PBMNCs or PCCs. Finally, the relative high cell loss rate (73%) during the purification of $CD34^+$ cells may result from some procedures before connecting to the magnetic cell sorting system. For example, the supernatant of centrifugation after leukapheresis still contained an amount of white blood cells because the transfer bag was not originally designed for centrifugation and was prone to deforming during the process.

5 | CONCLUSION

The results of this study indicated that the use of two types of cells seemed to result in similar mid-term efficacy in treating AICLI, and each kind of cell had its own unique advantages. Although PCCs seemed to result in earlier pain relief, it is not a cost-effective treatment compared with PBMNCs. PBMNCs may be a better choice when two or more limbs require transplantation at one stage. Validation of the conclusions is pending more evidence on the basis of the long-term outcomes of a larger number of patients.

ACKNOWLEDGMENTS

This study was supported by the Training Program for Outstanding Academic Leaders of the Shanghai Health and Family Planning System (Hundred Talent Program, Grant No. 2018BR40); China National Natural Science funds (Grant No. 30801122; 81970407; 81900426); the Excellent Core Member Training Program at Zhongshan Hospital, Fudan University, China (Grant No. 2015ZSYXGG02); grants from Shanghai Excellent Academic Leader (No. 19XD1401200) and Fudan University "Star of Tomorrow" Famous Doctor Training Project (2019), and Zhongshan Funds for the Institute of Vascular Surgery, Fudan University, China. This study is registered with ClinicalTrials.gov (NCT 02089828).

CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

H.L., T.P.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing; Y.F., G.F., Y.L., X.J.: collection and/or assembly of data, data analysis and interpretation; B.C.: administrative support, provision of study material or patients; W.Z.: collection and/or assembly of data; S.G.: provision of study material or patients, collection and/or assembly of data; P.L.: administrative support; Z.D., W.F.: conception and design, final approval of manuscript.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Liu H, Pan T, Fang Y, et al. Three-year outcomes of peripheral blood mononuclear cells vs purified $CD34^+$ cells in the treatment of angiitis-induced no-option critical limb ischemia and a cost-effectiveness assessment: A randomized single-blinded noninferiority trial. *STEM CELLS Transl Med*. 2021;10:647–659. https://doi.org/10.1002/sctm.20-0033