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Autologous Stem Cells Transplantation for No-Option Angiitis-Induced Critical Limb Ischemia: Recurrence and New Lesion

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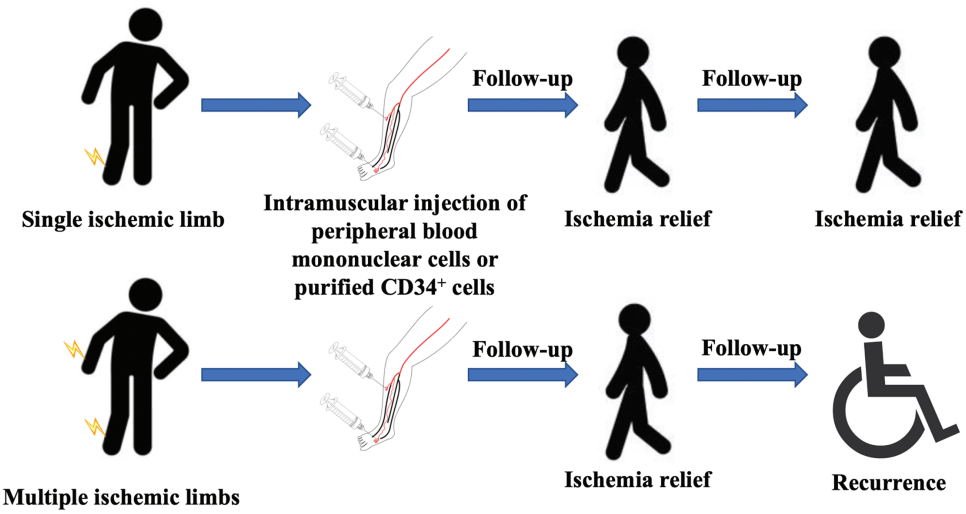
Abstract

Although satisfying outcomes have been demonstrated in terms of autologous stem cell transplantation in the treatment of angiitis-induced critical limb ischemia (AICLI), few studies have systematically reported the recurrence conditions. In the current study, we aimed to investigate recurrence conditions of a relatively large AICLI cohort in our center during a long-term follow-up period. From May 2009 to August 2020, 181 patients with AICLI received peripheral blood mononuclear cells (PBMNCs) or purified CD34+ cells (PCCs) transplantation. The main outcomes included recurrence and new lesions. Patient demographic data, ischemic limb characteristics, interventional characteristics, etc., were identified and analyzed. A logistic multivariable regression was performed to identify the independent risk factors for recurrence by a stepwise selection of variables. One hundred forty-eight patients were enrolled in this study. The mean follow-up period was 62.3 ± 37.4 months (range 12-144 months). The 5- and 10-year recurrence-free rates were 88.5% (95% confidence interval [CI] 3.1%-82.6%) and 71.7% (95% CI 7.6%-58.2%), respectively. The 5- and 10-year new lesion-free rates were 93.2% (95% CI 2.2%-89.0%) and 91.7% (95% CI 2.7%-86.6%), respectively. The finding of multiple limbs involved (OR 1.322 95% CI 1.123-12.549, $P = .036$) and ischemia relief period ≥5 months (OR 3.367 95% CI 1.112-10.192, $P = .032$) were demonstrated to be independent risk factors for recurrence in patients with AICLI who underwent cell transplantation. For patients with AICLI who responded to cell transplantation, the durability of this therapy was satisfactory, with 5- and 10-year recurrence-free rates of 88.5% and 71.7%, respectively. Multiple limbs involved at admission and ischemia relief period ≥5 months were demonstrated to be independent risk factors for recurrence after transplantation.

Key words: cells transplantation; cell therapy; critical limb ischemia; recurrence.

Graphic Abstract



AICLI patients with multiple ischemic limbs seem to be more likely to develop recurrence after cell transplantation. AICLI, angiitis-induced critical limb ischemia.

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Lessons Learned

- Recurrence of angiitis-induced critical limb ischemia (AICLI) was observed in 18 patients during the follow-up (62.3 ± 37.4 months) and the 5- and 10-year recurrence-free rates were 88.5% and 71.7%, respectively,
- This suggests a satisfactory durability of cell therapy in treating AICLI.
- Patients with multiple limbs involved at admission and/or a ≥5 months post-transplantation ischemia relief period seemed more likely to develop recurrence after transplantation.

Significance Statement

Although satisfying efficacy and safety outcomes have been demonstrated in terms of autologous stem cell transplantation in the treatment of angiitis-induced critical limb ischemia in many studies, few studies reported the conditions of patients' recurrence. The current study reported the 5- and 10-year recurrence-free rates of 88.5% and 71.7%, respectively. Multiple ischemic limbs at admission and ischemia relief period ≥5 months was demonstrated to be independent risk factors for recurrence after transplantation.

Introduction

Critical limb ischemia (CLI) is a classic vascular disease caused by various etiologies and is associated with a high major amputation rate and mortality.[1] After surgical and endovascular reconstruction, most patients obtained relief, while 15%-20% of patients with CLI did not.[1] These patients are also called patients with no-option critical limb ischemia (NO-CLI), and NO-CLI is defined as a CLI that unsuitable for either surgical or endovascular treatment owing to a high postoperative reocclusion rate and poor anatomical conditions.[2] Currently, satisfying efficacy and safety outcomes have been demonstrated in terms of autologous stem cell transplantation in the treatment of NO-CLI in many studies.[3-6] Due to the common propensity for affecting and destroying the anatomic run-off necessary for either endovascular or surgical reconstruction, angiitis-induced critical limb ischemia (AICLI), which is defined as CLI caused by thromboangiitis obliterans (TAO) or other arteritis-related autoimmunological diseases (such as systemic lupus erythematosus (SLE), psoriasis, Crohn's disease, etc.), is found to constitute a large proportion of patients with NO-CLI. Our center launched a clinical study of cell transplantation (including peripheral blood mononuclear cells [PBMNCs] and $CD34^+$ cells [PCCs]) in treating AICLI since 2009, and more than 190 patients have so far been treated to the present day.

Although the limb salvage rate of the therapy is promising, a certain number of patients with AICLI still developed recurrent CLI with an extension of the follow-up,[3,6] and only a few studies have reported the recurrence of patients with NO-CLI who received cell transplantation systematically. This might have resulted from the limited numbers of patients in the single studies and the relatively short follow-up. Recurrence, which is defined as transplanted limbs returning to the CLI condition after prior ischemia relief, partly reflects the durability of the cell therapy.

In the current study, we aimed to investigate the recurrence condition of a relatively large AICLI cohort in our center during a long-term follow-up period.

Materials and Methods

The protocol was approved by the Ethics Committee of Zhongshan Hospital affiliated with Fudan University (approval number: No. 2009-016) and was conducted according to the World Medical Association's Declaration of Helsinki. All participants provided written informed consent before enrollment.

Patients

From May 2009 to August 2020, 181 patients with AICLI received PBMNCs or PCCs transplantation. The inclusion and exclusion criteria for cell transplantation are detailed elsewhere.[7] Briefly, patients aged between 18 and 80 years with AICLI (Rutherford class 4-5), which was confirmed by clinical manifestations and computed tomographic angiography (CTA), magnetic resonance angiography, or digital subtraction angiography, were included. The exclusion criteria were (1) serious health events (including but not limited to myocardial infarction, cerebral apoplexy, pulmonary embolism, and severe hepatic and renal dysfunction) that was diagnosed within the last 3 months, (2) a suspicion or a diagnosis of a malignancy at baseline, or (3) a life expectancy of no more than 6 months. In the current study, we also excluded patients who (1) did not achieve CLI relief within 12 months after transplantation (defined as nonresponders to cell therapy), (2) did not complete the 12-month follow-up (lost or died of non-AICLI-related reasons without recurrence/a new lesion) and (3) underwent a second cell transplantation.

Procedures for Cell Transplantation

The procedures were also detailed elsewhere.[7] Subcutaneous injections of rhG-CSF (Neupogen; Amgen, Thousand Oaks, CA, USA) (5-10 μg/kg per day for 4 days) were given to mobilize the bone marrow cells, and enoxaparin (4000 IU/day) was given to prevent hypercoagulable states. On the fifth day, a suspension of PBMNCs was collected via leukapheresis (COM. TEC; Fresenius Hemocare GmbH, Bad Homburg, Germany). Then, after washing 3 times and resuspending the apheresis products in an ethylenediaminetetraacetic acid-phosphate-buffered saline solution (200 mL) that contained 0.5% human albumin, the PBMNCs cell product was obtained. The PCCs were obtained from the PBMNCs by using a magnetic cell sorting system (Miltenyi-Biotec GmbH, Bergisch-Gladbach, Germany). The total cell count of $CD34^+$ cells was determined by leukocyte counting and flow cytometry. With the patients under general anesthesia, the cell products were transplanted into the ischemic limbs via equidistant intramuscular injections (0.5 mL/site).

Data Collection

During hospitalization, the patients' demographic characteristics, characteristics of the autoimplants, critical results of blood examinations, etc., were recorded and analyzed. The baseline features of the patients, such as the numbers of involved limbs, the Rutherford scale, the transcutaneous pressure of oxygen ($TcPO_2$) of the dorsum, the ankle-brachial index (ABI), and the occlusion level of the arteries, were also recorded.

Outcomes and Follow-up

The main outcomes included recurrence and the development of new lesions. Recurrence was defined as transplanted limbs returning to the CLI condition after prior ischemia relief, and a new lesion was defined as untransplanted nonischemic limbs that became CLI during the follow-up. The timepoints of recurrence and the development of new lesions after transplantation were also recorded. Patients were required to return for regular clinical visits at 1, 2, 3, 6, 9, and 12 months and then annually after transplantation. The relief of rest pain, healing of ulcers or gangrene, smoking cessation compliance, and medication compliance were assessed and recorded during the clinical visits. Rest pain was evaluated by Wong-Baker Faces Pain Rating Scale (WBFPS) (a score of 0 represents no pain and a score of 10 represents the greatest pain) and rest pain relief was defined as WBFPS ≤4. Patients' ulcers or gangrene was recorded via taking pictures and compared with prior records. Conditions in terms of smoking cessation and medication compliance were asked at clinical visit or via telephone.

Statistical Analysis

The quantitative data, which were compared using Student's *t*-test, are shown as the mean ± SD or as the median with the interquartile range (IQR), depending on their distribution. Categorical variables, presented as frequencies and percentages, were compared using the χ^2 test or Fisher's exact test. Logistic multivariable regression was performed to identify the independent risk factors for recurrence by a stepwise selection of variables. Factors with a *P*-value <.10 in the univariate analyses were introduced into the multivariate model. The recurrence-free and new lesion-free rates were analyzed by a Kaplan-Meier analysis. All statistical tests were performed using a 2-sided α of 0.05. All tests were performed using PASW software, version 19 (IBM Corporation, Armonk, NY, USA), or R, version 4.0.5.

Results

Baseline Characteristics

Between May 2009 and August 2020, 181 patients with AICLI who underwent cell transplantation for AICLI in our center were identified. After excluding the nonresponders and patients who did not complete the 12-month follow-up, 148 patients were finally enrolled in this study (Fig. 1). The percent of patients who were male was 98.6% (146/148), and the mean age of the patients was 42.0 ± 10.2 years (range 20-68 years). The patients were characterized by low frequencies of cardio-cerebrovascular risk factors except for high frequencies of smoking history (85.1%, 126/148). All patients were admitted with a Rutherford class of 4 (19 patients [12.8%]) or 5 (129 patients [87.2%]), and perioperative infections of ulcers/gangrene were observed in 19 patients (12.8%). Except for 8 patients with nonTAO-related angiitis, the remaining 140 patients (94.6%) were all diagnosed with TAO. One hundred thirty-six patients were admitted with single-limb AICLI, and the remaining 12 patients had 2 or more limbs involved. Sixty-three patients underwent prior interventions, including bypass, endarterectomy, stent grafting balloon angioplasty, thrombolysis and thrombectomy. More details of the baseline characteristics are shown in Table 1.

Interventional Characteristics

The mean duration for autoimplant harvest was 95.9 ± 32.5 minutes (range 50-140 minutes), and the mean duration for cell implantation was 37.4 ± 12.0 minutes (range 20-60 min). There were 69 (46.6%) patients who underwent PCCs

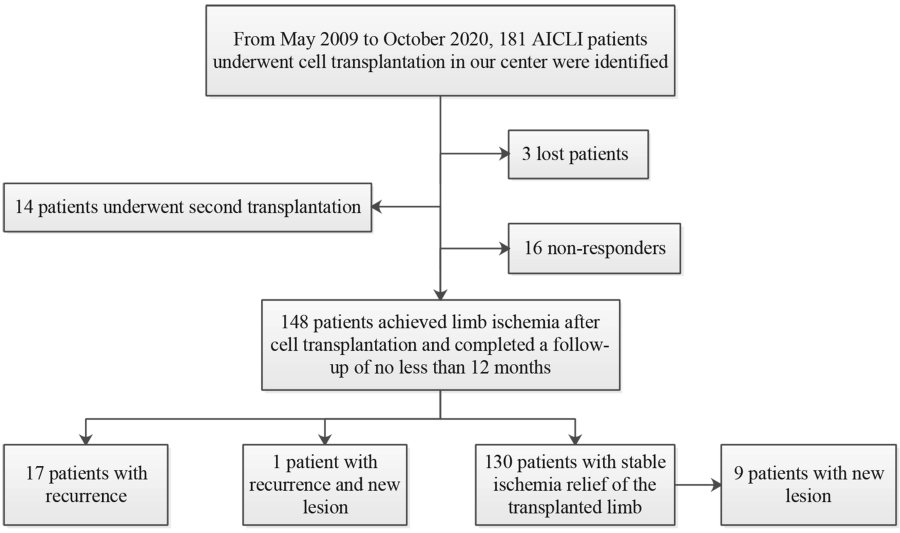

Figure. 1. Protocol of current study. AICLI, angiitis-induced critical limb ischemia.

Table 1. Comparison of baseline characteristics between 2 groups of patients.

	Total (n = 148)	Patients with recurrence (n = 18)	Patients without recurrence (n = 130)	P-value
Age, years, mean ± SD	42.0 ± 10.2	45.7 ± 10.9	41.6 ± 10.4	.121
Gender, (male/female)	146/2	17/1	129/1	.229
Body mass index, (kg/m^2) (mean ± SD)	23.5 ± 3.1	23.6 ± 3.2	23.1 ± 2.7	.556
Cardiovascular risk factors				
Smoking history, n (%)	126 (85.1)	14 (9.5)	112 (75.6)	.312
Hypertension, n (%)	7 (4.7)	2 (1.4)	5 (3.3)	.203
Diabetes mellitus, n (%)	10 (6.8)	2 (1.4)	8 (5.4)	.349
Hyperlipidaemia, n (%)	8 (5.4)	0 (0.0)	8 (5.4)	.596
Etiology				
TAO, n (%)	140 (94.6)	17 (11.5)	123 (83.1)	.976
Other, n (%)	8 (5.4)	1 (0.7)	7 (4.7)	.976
Surgical history				
Bypass, n (%)	4(2.7)	1 (0.7)	3 (2.0)	.408
Endarterectomy, n (%)	1 (0.7)	0 (0.0)	1 (0.7)	.709
Stent grafting, n (%)	6 (4.1)	0 (0.0)	6 (4.1)	.352
Balloon angioplasty, n (%)	20 (13.5)	1 (0.7)	19 (12.8)	.469
Thrombolysis, n (%)	22 (14.9)	1 (0.7)	21 (14.2)	.476
Thrombectomy, n (%)	10 (6.8)	2 (1.4)	8 (5.4)	.349
Rutherford class				
4, n (%)	19 (12.8)	1 (0.7)	18 (12.1)	.470
5, n (%)	129 (87.2)	17 (11.5)	112 (75.7)	.470
Number of ischemic limbs				
1, n (%)	136 (91.9)	14 (9.5)	122 (82.4)	.041
2, n (%)	9 (6.1)	2 (1.4)	7 (4.7)	.300
3, n (%)	2 (1.4)	1 (0.7)	1 (0.7)	.229
4, n (%)	1 (0.7)	1 (0.7)	0 (0.0)	.122
Multiple limbs (≥2), n (%)	12 (8.1)	4 (2.7)	8 (5.4)	.041
Ulcer without gangrene, n (%)	65(43.9)	7 (4.7)	58 (39.2)	.801
Gangrene, n (%)	64(43.2)	10 (6.7)	54 (36.5)	.831
Ulcer or gangrene, n (%)	129 (87.1)	17 (11.4)	112 (75.7)	.324
Ulcer/gangrene with infection	19 (12.8)	6 (4.1)	13 (8.7)	.009
Upper limbs involved, n (%)	11 (7.4)	3 (2.0)	8 (5.4)	.133
Highest level of arterial occlusion				
Iliac artery, n (%)	8 (5.4)	1 (0.7)	7 (4.7)	.976
Femoral/brachial artery, n (%)	61 (41.2)	5(3.4)	56 (37.8)	.216
Popliteal artery, n (%)	21 (14.2)	2 (1.4)	19 (12.8)	.690
Below the knee or elbow, n (%)	58(39.2)	10 (6.7)	48 (32.4)	.129
ABI[a], (median, IQR)	0.52(0.39-0.68)	0.53 (0.38-0.68)	0.52 (0.40-0.66)	.899
$TcPO_2$, mmHg (median, IQR)	21 (13-30)	22.5 (12-33)	20(13-29)	.473
Blood examination				
CRP, mg/L (median, IQR)	4.6 (1.6-11.75)	4.6 (1.8-11.8)	4.4 (1.4-11.6)	.786
ESR, mm/hour (median, IQR)	13 (7-30)	15 (8-32)	13 (6-30)	.374
Fibrinogen, mg/dL (median, IQR)	304 (240-370)	300 (220-390)	306 (240-360)	.704

The data presented are the numbers (%) and the means ± standard deviations or medians and the interquartile ranges.
[a]ABIs of 143 patients with lower limbs treated were included in this analysis, while the other 5 patients with only upper limbs treated were excluded.
Abbreviations: ABI, ankle-brachial index; TAO, thromboangiitis obliterans; IQR, interquartile range.

transplantation, and the remaining 79 (53.4%) patients received PBMNCs transplantation. Concurrent debridement during transplantation was performed in 13 patients owing to the severe infection of patients' ulcers/gangrene. The median number of CD34+ cells transplanted was 41.0 × 10^6 (interquartile range [IQR] 25.2 × 10^6-71.9 × 10^6), and the median number of CD34$^+$ cells transplanted per kg was 6.1 × 105/kg (IQR 3.6 × 10^5-11.7 × 10^5/kg) (Table 2).

Follow-up

The mean follow-up period was 62.3 ± 37.4 months (range 12-144 months). All patients achieved AICLI relief

with a mean relief period of 3.6 ± 2.6 months (range 1-12 months). Two patients died during the follow-up: 1 patient died of heart failure at 27 months, and 1 died of stroke at 25 months. Strict smoking cessation was achieved in 39 (31.0%, 39/126) patients, and 48 (32.4%) patients were compliant with their drug therapy. Recurrence of AICLI was observed in 18 out of 148 patients (Table 3). There were 17 TAO-induced AICLI male patients, but there was only 1 SLE-induced female patient with AICLI. The mean period between the first transplantation and recurrence was 46.0 ± 30.5 months (range 8-106 months). Sixteen patients presented with recurrent ulcers/gangrene (Rutherford class 5), and 2 presented with resting pain (Rutherford class 4). One patient with recurrence was also admitted with a new lesion. Among all 18 patients, 4 patients had ischemia relief after conservative treatment; among the remaining 14 patients, 12 received second transplantations, 1 refused cell transplantation, and 1 patient had an amputation due to rapid progression of ischemia and severe gangrenous infection. Regarding the 12 patients with a second transplantation, ischemia relief was achieved in most of the patients (83.3%, 10/12), and the remaining 2 patients who did not have relief underwent amputation at 4 months and died of stroke at 25 months, respectively. The 5- and 10-year recurrence-free rates were 88.5% (95% confidence interval [CI] 3.1%-82.6%) and 71.7% (95% CI 7.6%-58.2%), respectively (Fig. 2A). New lesions were observed in 10 patients during the follow-up and 1 patient had concurrent recurrence (Table 3). All patients were male TAO patients with a mean new lesion period of 23.0 ± 15.9 months (range 7-61 months). No ischemia relief was observed only in patients with conservative treatment only, and 1 patient underwent an amputation. Seven patients received second cell transplantations, and 6 of them achieved ischemia relief. A significantly higher proportion of patients with prior PCCs transplantation was observed in the group of patients with new lesions (8/69 vs 2/79, P = .028). The 5- and 10-year new lesion-free rates were 93.2% (95% CI 2.2%-89.0%) and 91.7% (95% CI 2.7%-86.6%), respectively (Fig. 2B).

Risk Factors for Recurrence

Compared with the patients without recurrence, the patients with recurrence were characterized by higher proportions of patients with perioperative ulcers/gangrenous infection (6/18 vs 13/130, P = .009) and with multiple limbs involved by AICLI (4/18 vs 8/130, P = .041). No significant differences were observed between the 2 groups in terms of the other demographic characteristics, etiologies, risk factors for cardiovascular disease, or treatment histories (Table 1). Regarding the transplantation procedure, the patients with recurrence seemed more likely to undergo concurrent debridement (5/18 vs. 8/130, P = .011), while there was no significant difference in terms of the number of autoimplants that were given (Table 2). After transplantation, patients with recurrence were characterized by a longer ischemia relief period (5.5 ± 3.1 months vs 3.6 ± 2.3 months, P = .002), and there was a higher proportion of patients who had a ≥5-month ischemia relief period (10/18 vs 31/130, P = .015) in the patients with recurrence group. There were 136 patients (14 patients with recurrence and 126 not) with smoking history and 39 patients who achieved smoking cessation (1 patient with recurrence and 38 not). In addition, 87 patients continued smoking after transplantation (13 patients with recurrence and 74 not).

According to the results of the univariate logistic regression, several variables were screened out: perioperative ulcers/gangrene infection (OR [odds ratio] 4.500, 95% CI [confidence interval] 1.446-14.003, P = .009), multiple limbs involved by AICLI (OR 4.357, 95% CI 1.162-16.335, P = .041), concurrent debridement during cell transplantation (OR 5.865, 95% CI 1.672-20.578, P = .011), an ischemia relief period ≥5 months (OR 3.254, 95% CI 1.261-8.397, P = .015) and the post-transplantation smoking condition (smoking cessation [OR 0.791, 95% CI 0.230-2.714, P = .709], not quit smoking

Table 2. Comparison of intervention and postoperative characteristics between 2 groups of patients.

	Total (n = 148)	Patients with recurrence (n = 18)	Patients without recurrence (n = 130)	P-value
Cell product				
Harvest time, minute, mean ± SD	95.9 ± 32.5	100.3 ± 31.6	95.6 ± 32.2	.562
PBMNCs, n (%)	79 (53.4)	8 (5.4)	71 (48.0)	.418
PCCs, n (%)	69 (46.6)	10 (6.8)	59 (39.8)	.418
$CD34^+$ cells, (10^6) (median, IQR)	41.0 (25.2-79.1)	43.6 (28.3-84.9)	41.0 (25.2-79.0)	.146
$CD34^+$ cells/kg, (10^5/kg) (median, IQR)	6.1 (3.6-11.7)	6.4 (4.6-13.2)	5.8 (3.6-11.0)	.254
Cell viability, %, (median, IQR)	98.6(97.8-99.4)	98.8 (98.0-99.2)	98.6 (97.6-99.4)	.798
Transplantation time, minute, mean ± SD	37.4 ± 12.0	37.3 ± 11.3	37.5 ± 13.2	.951
Concurrent debridement	13 (8.8)	5 (3.4)	8 (5.4)	.011
Ischemia relief period, months, mean ± SD	3.6 ± 2.6	5.5 ± 3.1	3.6 ± 2.3	.002
Ischemia relief period ≥5 months, n (%)	41(27.7)	10 (6.8)	31(20.9)	.015
Persistent drug therapy	48 (32.4)	4 (2.7)	44 (29.7)	.323
Post-transplantation smoking condition				
Smoking cessation	39 (26.4)	1 (0.7)	38 (25.7)	
Not quitting smoking	87 (58.8)	13 (8.8)	74 (50.0)	.094
Without smoking history	22 (14.8)	4 (2.7)	18 (12.1)	

The data presented are the numbers (%) and the means ± standard deviations or medians and the interquartile ranges.
[a]Ischemia relief period was defined as the time period between the first transplantation and postoperative critical limb ischemia relief (Rutherford class <4).
Abbreviations: PBMNCs, peripheral blood mononuclear cells; PCCs, purified $CD34^+$ cells; IQR, interquartile range; CRP, C-reactive protein; GHb, glycosylated hemoglobin; GFR, glomerular filtration rate; ESR, erythrocyte sedimentation rate.

Table 3. Characteristics of patients with recurrence and/or new lesion.

Patient number	Sex	Age	Etiology	Autoimplant type	R or N	Period/ months	Rutherford class	Measures	Outcomes
1	M	34	TAO	PCCs	R	8	5	2nd transplantation	Ischemia relief
2	M	72	TAO	PBMNCs	R	8	4	2nd transplantation	Unrelieved
3	M	32	TAO	PBMNCs	R	19	5	2nd transplantation	Ischemia relief
4	M	50	TAO	PBMNCs	R	23	5	Conservative treatment	Unrelieved
5	M	47	TAO	PCCs	R	25	4	Conservative treatment	Ischemia relief
6	M	42	TAO	PBMNCs	R	26	5	2nd transplantation	Ischemia relief
7	M	30	TAO	PCCs	R	27	5	2nd transplantation	Unrelieved+amputation
8	M	37	TAO	PBMNCs	R	29	5	Amputation[a]	-
9	M	59	TAO	PBMNCs	R	31	5	Conservative treatment	Ischemia relief
10	M	52	TAO	PCCs	R	40	5	Conservative treatment	Ischemia relief
11	M	52	TAO	PBMNCs	R	44	5	2nd transplantation	Ischemia relief
12	M	50	TAO	PCCs	R	58	5	2nd transplantation	Ischemia relief
13	M	51	TAO	PBMNCs	R	69	5	2nd transplantation	Ischemia relief
14	M	41	TAO	PCCs	R	96	5	2nd transplantation	Ischemia relief
15	M	33	TAO	PCCs	R	101	5	2nd transplantation	Ischemia relief
16	M	47	TAO	PCCs	R	106	5	2nd transplantation	Ischemia relief
17	F	45	SLE	PCCs	R	77	5	Conservative treatment	Ischemia relief
18	M	46	TAO	PCCs	R+N	22	5	2nd transplantation	Ischemia relief
19	M	35	TAO	PCCs	N	61	5	2nd transplantation	Ischemia relief
20	M	43	TAO	PCCs	N	14	5	2nd transplantation	Ischemia relief
21	M	34	TAO	PCCs	N	20	5	2nd transplantation	Ischemia relief
22	M	36	TAO	PCCs	N	27	5	2nd transplantation	Ischemia relief
23	M	50	TAO	PCCs	N	7	5	Conservative treatment[b]	Unrelieved+amputation
24	M	53	TAO	PCCs	N	36	5	Conservative treatment	Unrelieved
25	M	36	TAO	PBMNCs	N	19	5	2nd transplantation	Ischemia relief
26	M	49	TAO	PCCs	N	7	5	2nd transplantation	Unrelieved
27	M	27	TAO	PBMNCs	N	17	4	Conservative treatment[b]	Unrelieved

[a]Amputation was performed for this patient because his limb ischemia progressed rapidly and the gangrene was complicated with severe infection.
[b]Conservative treatment was performed in these 2 patients for they were in poor general conditions.
Abbreviations: M, male; F, female; TAO, thromboangiitis obliterans; SLE, systemic lupus erythematosus; PBMNCs, peripheral blood mononuclear cells; PCCs, purified CD34+ cells; R, recurrence; N, new lesion.

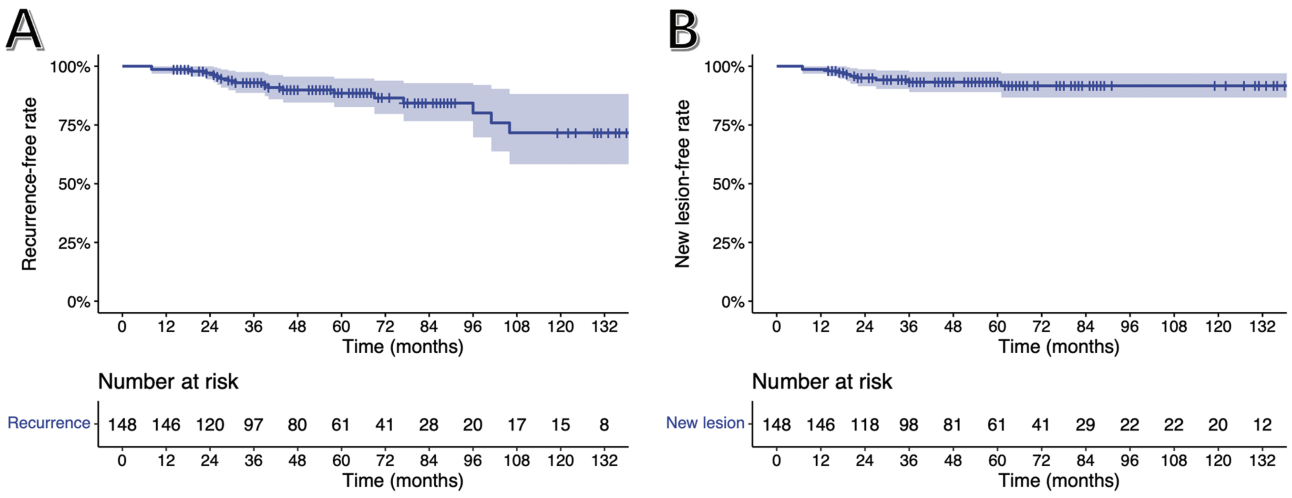

Figure. 2. Kaplan-Meier curves showing the probabilities of (**A**) recurrence-free rate and (**B**) new lesion-free rate.

[P = .164], without smoking history [OR 0.118, 95% CI 0.012-1.137, P = .065]). After the logistic multivariable regression analyses, multiple limbs involved (OR 1.322 95% CI 1.123-12.549, P = .036) and ischemia relief period ≥5 months (OR 3.367 95% CI 1.112-10.192, P = .032) were demonstrated to be independent risk factors for recurrence

Table 4. Univariate and logistic multivariate analysis of independent risk factors.

Candidate variable	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Multiple limbs involved	4.357 (1.162-16.335)	0.041	1.322 (1.113-25.595)	.036
Ulcer/gangrene with infection	4.500 (1.446-14.003)	0.009	—	—
Ischemia relief period ≥5 months	3.254 (1.261-8.397)	0.015	3.367 (1.112-10.192)	.032
Concurrent debridement	5.865 (1.672-20.578)	0.011	—	—
Post-transplantation smoking condition				
Not quit smoking	—	0.164	—	—
Smoking cessation	0.791 (0.230-2.714)[a]	0.709	—	—
Without smoking history	0.118 (0.012-1.137)*	0.065	—	—

[a]The OR (95% CI) values were calculated compared to patients who did not quit smoking after transplantation.
Abbreviations: OR, odds ratio; CI, confidence interval.

in patients with AICLI who underwent cell transplantation (Table 4).

Discussion

As one of the largest centers who perform cell therapy for patients with NO-CLI in China, we have performed cell transplantation in more than 200 patients with AICLI over a 12-year period. The long-term efficacy and safety outcomes of cell therapy have been demonstrated by many studies.[3,7-11] In light of the fact that patients with AICLI are usually males at a relatively young age and who have a long life expectancy, it is important to measure the durability of cell therapy by observing the recurrence in patients. Recurrence is an event that can only occur after the relief of CLI, and the shortest period between the time of transplantation and recurrence was 8 months in our clinical practice. We decided to enroll 148 patients with AICLI who achieved ischemia relief within 1 year after transplantation and completed a follow-up of at least 12-months in the current study.

In 2018, we reported a study concerning the 5-year outcomes of 27 patients with AICLI who received PCCs transplantation. Three patients with recurrence were observed in this study, and there was a 5-year recurrence rate of 11.11%.[3] Likewise, in the current study (with a mean recurrence period of 46.0 ± 30.5 months and a total of 18 patients with recurrence), the 5- and 10-year recurrence-free rates were 88.5% and 71.7%, respectively. These results demonstrated the satisfying durability of cell therapy in treating AICLI. Considering that most recurrence events occurred 24 months posttransplantation (77.8%, 14/18) and that some even occurred after 100 months, and considering the relatively young age of patients with AICLI, a lifelong surveillance of the patients seems to be necessary after cell therapy.

Through univariate analysis and logistic multivariate regression analysis, having multiple limbs involved in AICLI at admission (OR 1.322 95% CI 1.123-12.549, P = .032) was demonstrated to be an independent risk factor for recurrence. We speculate that there are several possible reasons for this. First, considering that most recurrent patients have TAO-induced AICLI (94.4%, 17/18) and that the symptoms of TAO usually begin in the peripheral portion of a single limb, angiitis frequently progresses proximally and involve multiple extremities.[12] The multiple ischemic limbs to some extent reflect greater systemic activity and a longer course of TAO. Second, multiple ischemic limbs require multiple limbs that need transplantation, although each ischemic limb received adequate $CD34^+$ dosage (10^5-10^6/kg) in all patients, the absolute number that each limb received would usually be reduced by half and could even be reduced by three-quarters in patients with all of their limbs affected by ischemia. Finally, for patients with multiple ischemic limbs, especially both of the lower limbs, post-transplantation exercise therapy would be extremely difficult, and the exercise-induced angiogenesis effect would thus be compromised. Additionally, ischemia relief period ≥5 months (OR 3.367 95% CI 1.112-10.192, P = .032) was also demonstrated to be associated with recurrence. We speculate that a long relief period might indicate a slow response to cell therapy and/or a severe ischemic condition, thus posing a great chance of developing recurrence if lesion progresses.

Although smoke exposure is thought to be closely related to the occurrence and development of TAO,[12-16] we did not find smoke cessation to be an independent protective factor, which might be due to the limited number of patients with recurrence and the difficulty in strictly defining smoking cessation (patients with exposure to second-hand smoke in their living or working environment were not classified as smoking cessation). The only female patient who had recurrence in our study, had SLE, which is an incurable systemic autoimmune disease involving multiple systems and organs, and her recurrence was associated with the poor control of her primary disease but was relieved after adequate conservative treatment.

New lesions seemed to be different from recurrence in terms of treatment and prognosis. On the one hand, 3 out of the 18 patients with recurrence achieved ischemia relief via conservative treatment only, and most patients (83.3%, 10/12) who received a second transplantation still achieved ischemia relief (Fig. 3). On the other hand, although the ischemia relief rate after the second transplantation (85.7%, 6/7) was satisfying, patients with new lesions seemed to have just a slight benefit from conservative treatment only. While recurrence seemed to be less severe because it occurred on the basis of prior cell therapy-induced angiogenesis, the new lesions seemed to be associated with poorer prognosis and therefore needed more active intervention. In addition, we found that there was a higher proportion of patients with new lesions who underwent prior PCCs transplantation than prior PBMNCs transplantation (8/69 vs 2/79, P = .028). Compared

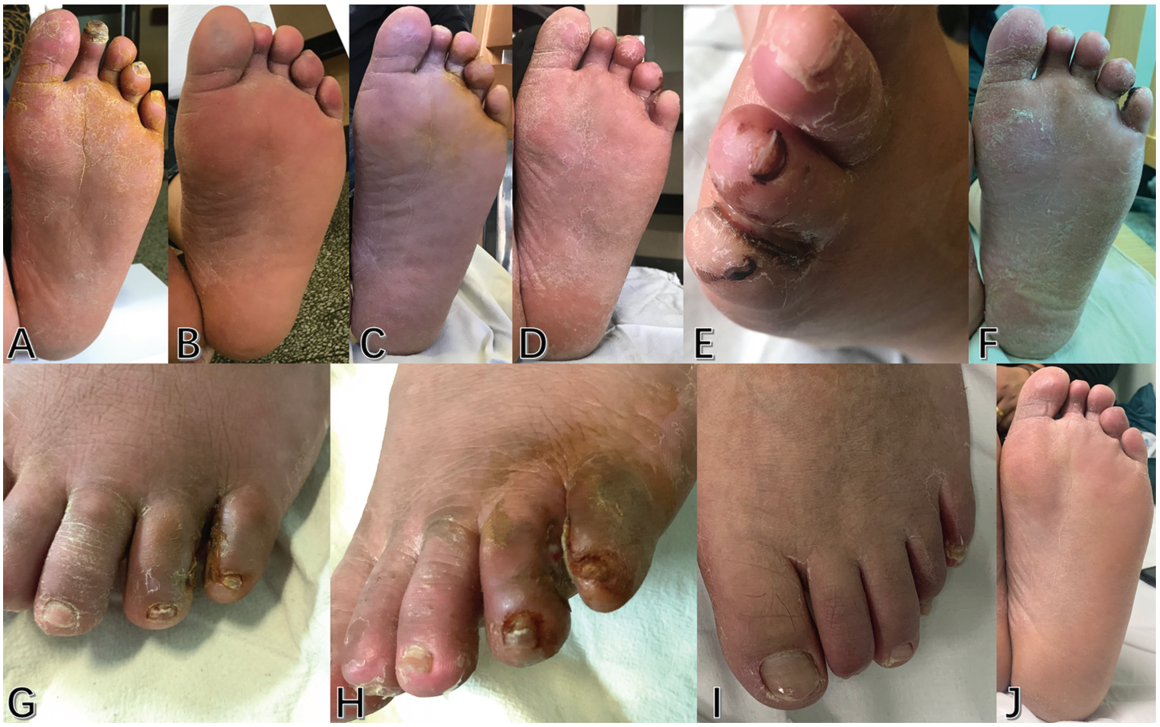

Figure. 3. The treatment process in a patient with recurrence. The patient had gangrene on the second toe of his left foot and dandruff over the plantar surface of his foot before cell therapy (**A**). His pain at rest was significantly alleviated 1 month after transplantation, and his gangrene healed 3 months after transplantation (**B**). At 6 months post-transplantation, he complained of claudication of the left foot without resting pain (**C**), and at 12 months, ulcers and exudation were observed between the fourth and fifth toes of his left foot (**D**, **E**). Despite adequate conservative treatment, including drugs and exercise treatment, he still had no relief, so he underwent a second cell transplantation. The exudation was improved at 1 month (**F**), and the ulcer gradually healed within 2 months (**G**) and 3 months (**H**). At 6 months after the second transplantation (**I**, **J**), his ulcer had completely healed.

with PCCs, PBMNCs were characterized by a larger volume of autoimplants and a larger number of $CD34^+$ and $CD34^-$ cells.[6] Considering that all of the ischemic limbs received CD34+ cells in a certain dosage range (1×10^5-1×10^6 cells/kg) and the volume of autoimplants seemed unlikely to generate efficacy in the untransplanted limb, the new lesion-proof effect might be related to $CD34^-$ cells. $CD34^-$ cells are usually thought to be helpful for angiogenesis by playing the role of niche-supporting cells to facilitate cell survival, angiogenic cytokine secretion, and can incorporate capacity and preserve the progenitor status of endothelial progenitor cells (EPCs).[17-20] Many studies also reported that a certain subpopulation of $CD34^-$ cells ($CD34^-/CD133^+$) can differentiate into $CD34^+/CD133^+$ EPCs and then acquire a mature endothelial phenotype, which is functionally more active than the supposedly more mature $CD34^+/CD133^+$ EPC subpopulation.[21,22] We inferred that the new lesion-proof effect from PBMNCs might result from this important $CD34^-$ subpopulation.

There were several limitations in the current study. First, this was a retrospective single-center study, and although a relatively large cohort of patients with AICLI was enrolled, the number of patients with recurrence was still small due to the relatively low rate of recurrence.[3,6] Second, recurrence is an event that must be associated with some postoperative factors. However, in the current study, only smoking cessation and persistent drug therapy were recorded; in addition, although many patients quitted smoking after transplantation, they were still exposed to second-hand smoke, so it was difficult to strictly defined smoking cessation. Finally, only patients whose transplanted limb developed the CLI condition again were enrolled as patients with recurrence while some patients with deteriorated claudication distance was excluded. This might lead to the underestimation of the real number of patients with recurrence.

Conclusion

For patients with AICLI who responded to cell transplantation, the durability of this therapy was satisfactory, with 5- and 10-year recurrence-free rates of 88.5% and 71.7%, respectively. Multiple limbs involved at admission and ischemia relief period ≥5 months were demonstrated to be independent risk factors for recurrence after transplantation, suggesting that strict follow-up is needed for such patients. Additionally, it seemed necessary to take active intervention for patients with new lesions for their poor prognosis when they have conservative treatment only.

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Conflict of Interest

The authors indicated no potential conflicts of interest.

Author Contributions

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

Data Availability

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

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