Abstract. – OBJECTIVE: To investigate the efficiency of autologous peripheral blood stem cell transplantation (APBSCT) to treat severe the lower limbs ischemia caused by thromboangiitis obliterans (TAO).

PATIENTS AND METHODS: From April 2007 to December 2014, a total of 64 patients with TAO (80 affected limbs) received APBSCT at our hospital. The treatment effect was evaluated by subjective indicators including pains and cold sensation of the affected limbs, combined with objective indicators including claudication distance, ankle brachial index (ABI), transcutaneous oxygen pressure (TcPO2) and skin temperature.

RESULTS: Five patients (with 5 affected limbs) suffered from necrosis below the middle of the leg 4 weeks after transplantation and received amputation. For the remaining 59 patients (75 affected limbs), pain and cold sensation of the affected limbs were improved with varying extent 3 months after transplantation; there were statistically significant differences in pain score and cold sensation score of the affected limbs before and after APBSCT ($p<0.05$). Claudication distance, ABI, TcPO2 and skin temperature were also improved. Claudication distance increased from 85.69 m±43.48 m to 36.5±9.88 mmHg, and the skin temperature of the lower limbs increased from 27.70°C±0.53°C to 33.49°C±0.60°C. All four indicators were considerably improved after APBSCT ($p<0.05$). Arteriography was performed for 75 affected limbs in 59 patients 6 months after transplantation and found that new collateral vessels were formed in the affected limbs. No patients were complicated by retinal hyperplasia, malignant tumors, myocardial infarction and cerebral infarction during the follow-up examinations; no patients underwent symptom aggravation during 9-48 month follow-up (average, 28.5 months).

CONCLUSIONS: APBSCT is an easy, safe and reliable treatment for ischemia of lower limbs, especially for those with poor distal arterial outflow tract in the lower limbs that do not permit bridging.

Key Words: Autologous transplantation, peripheral blood stem cells, thromboangiitis obliterans (TAO), stem cell therapy.

Abbreviations

APBSCT = autologous peripheral blood stem cell transplantation; TAO = thromboangiitis obliterans; ABI = ankle brachial index; TcPO2 = transcutaneous oxygen pressure; CTA = CT angiography; MNC = mononuclear cells.

Introduction

Thromboangiitis obliterans (TAO) is a refractory disease that involves the artery of upper and lower limbs, leading to severe ischemia of the extremity. For patients with severe TAO, amputation has to be performed as last choice, which otherwise, may end up with life-threatening condition. The current treatments have limited indication and limited with poor long-term treatment effect. Autologous peripheral blood stem cell transplantation (APBSCT) is one of the major achievements in regeneration medicine as well as a new technique for revascularization that can salvage the limbs. In the current study, 64 patients with severe ischemia of lower limbs due to TAO and admitted to our hospital from April 2007 to December 2014 were recruited and treated by APBSCT, and the satisfactory results were achieved and presented below.

Patients and Methods

General Information

Of 64 included patients, 54 patients were male and 10 female, aged 26-44 years (average, 36.1 years). All patients had a smoking history of 5-21 years.
years (average, 13 years). They all presented the symptoms of coldness and pain of feet and (or) lower limbs and intermittent claudication, which was developed into rest pain in 36 cases, abnormal skin color of feet in 47 cases and foot ulcers in 12 cases; 21 cases were affected in the right lower limbs, 27 in the left lower limbs, and 16 in both limbs, making a total of 80 limbs being affected. Arteriography was performed in lower limbs in 22 cases, and CT angiography (CTA) of the lower limbs was performed in 42 cases. All patients were found to have femoral artery stenosis, popliteal artery occlusion and absent outflow tract below the knee. All subjects responded poorly to medication.

Case Selection
Inclusion criteria for the study included: confirmed TAO with popliteal artery occlusion and absent outflow tract below the knee detected with arteriography or MRI; poor response to other therapies. Patient was excluded if he/she fits any of the exclusion criteria, which included: focal infections in the lower limbs; history of malignancies in the past 5 years or an clinically significant elevation of blood tumor markers (AFP, CEA, PSA, CA19-9, CA125); still smoking without wish of quit.

Mobilization and Collection of PBSC
Recombinant human granulocyte colony-stimulating factor (rhG-CSF, filgrastim, Kirin Kunpeng Bio-Pharmaceutical, Shanghai, China) was injected subcutaneously at the dose of 150 μg/d for 4-5 days. During mobilization, low-molecular-weight heparin injection was administrated. Mononuclear cells (MNC) were serially collected using a blood cell separator. The total volume of circulation sample was 10,000 ml-13,000 ml, and volume of final collection 120-260 ml with total MNC of 2.3-3.6×10^10.

APBSCT
Deep intramuscular injection was performed at different points (with depth of 1.0-1.5 cm) along the orientation of blood vessels in the affected limbs following epidural or spinal anesthesia. For each point 0.4 ml-0.6 ml of MNC suspension was injected, and the spacing between two adjacent points was 2-3 cm.

Perioperative Management
Perioperative management included (1) Vasodilator and anti-coagulation therapy for promoting blood circulation and removing blood stasis, (2) ulcer clearing and dressing change, and (3) long-term oral administration of antiplatelet drugs (clopidogrel).

Observation Indicators
Subjective indicators and objective indicators were evaluated for all subjects 3 months after transplantation, and arteriography was performed 6 months after transplantation to detect the formation of new collateral vessels in the affected limbs. The complications such as retinal hyperplasia, malignancies, myocardial infarction and cerebral infarction were observed and reported, if any. Subjective indicators included (1) Pain scores (4): 4, no remission; 3, need for analgesics; 2, constant but endurable pain; 1, occasional pain; 0, no pain; (2) Cold sensation pain (4): 4, feeling icy cold; 3, obvious cold sensation; 2, constant cold sensation; 1, occasional cold sensation; 0, no cold sensation. Objective indicators included claudication distance, the distance from starting to walk to experience of severe pain that prevents walking; ABI, systolic pressure at the ankle, divided by the brachial systolic pressure; TcPO2, detected using TCM400 multi-channel tcpO2 monitor (Radiometer Ltd, Copenhagen, Denmark); skin temperature, measured at the back of feet using skin temperature sensor (Beaverton, Or, USA).

Statistical Analysis
Statistical analysis was performed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). The scores of pain and cold sensation in the affected limbs before and after transplantation were compared by χ^2 test. The indicator data were expressed as mean±standard deviation. One-way ANOVA was performed at α=0.05 significance level. P<0.05 was considered as statistically significant difference.

Results
General Outcomes
Five patients (5 affected limbs) suffered from necrosis below the middle of the leg 4 weeks after transplantation and received amputation. The limbs were successfully preserved in the remaining 59 patients (75 affected limbs).

Evaluation with Subjective Indicators
The pain and cold sensation in 75 affected limbs (59 patients) were improved to varying extent 3 months after transplantation. The scores of
pain and cold sensation before and after transplantation are shown in Table I. Thirty-five patients reported disappearance of pain and 24 with alleviation. Thirty-five patients reported disappearance of cold sensation and 14 patients reported improvement of cold sensation, and 10 with alleviated cold sensation. The scores of pain and cold sensation were both improved after transplantation \((p<0.05)\).

**Evaluation with Objective Indicators**

Foot ulcers were completely healed in 12 patients (12 affected limbs) 3 months after transplantation; claudication distance was improved in 59 patients \((p=0.02)\), and both ABI \((p=0.02)\) and TcPO2 \((p=0.03)\) were increased compared with values tested before transplantation; the skin temperature of feet was increased after transplantation \((p=0.03)\) (Table II). Arteriography was performed for 75 affected limbs in 59 patients 6 months after transplantation, and the result showed formation of new collateral vessels to varying extent.

**Follow-up Analysis Results**

Follow-up examination was conducted during 9-48 months (average, 28.5 months) and it was found there was no aggravation of symptoms after alleviation. No report of complications such as retinal hyperplasia, malignancies, myocardial infarction or cerebral infarction.

### Table I.

Scores of pain and cold sensation before and after transplantation (cases).

<table>
<thead>
<tr>
<th>Scores</th>
<th>Pain</th>
<th>Cold sensation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>0</td>
</tr>
</tbody>
</table>

**Discussion**

TAO is a segmental inflammatory occlusive disorder that affects the arm and leg arteries, including shank, calf, radial and ulnar arteries, palms of hand and soles of feet and distal arteries of toes. The first report about TAO dates back to 1869, Burger provided the first detailed account of its pathological manifestations in 1908 and named it as Burger’s disease. Typical TAO is most frequently seen in male smokers, and there has been a trend of rising incidence among females currently. Early manifestations include coldness of extremities and intermittent claudication. The disease will progress into rest pain, skin ulcers or even gangrene, and the patients may need amputation to relieve pain.

TAO mainly involves peripheral vessels and presents a diffusive segmental pattern with unknown pathogenesis, which makes the treatment of TAO very difficult. The common mediation therapies include stasis removing, thrombolysis and vasodilator administration (targeting the collateral circulation). These therapies are applied to patients with early or mild TAO or those with poor revascularization, however, without unsatisfactory outcome. Ohta et al. reported that the 5-year patency rate for TAO patients after vessel bypass grafting was...
47% and the 10-year patency rate was only 39%. Sympathectomy or chemical lumbar sympathectomy can alleviate rest pain and prevent amputation, but only to a limited extent. The priorities of treatment for TAO are to reconstruct collateral circulation of lower limbs, increase blood supply of the affected limbs, reduce amputation rate and improve the life quality of the patients. Isner et al proposed the concept of therapeutic angiogenesis in 1990, which relied on promoting the stem cells and the substrate participating in vascular endothelial growth factors (VEGF). This opened up a new prospect for treating ischemia of the lower limbs. Tateishi-Yuyama et al first reported successful treatment of ischemia of the lower limbs by autologous bone marrow stem cell transplantation in 2002.

Since then, considerable progress has been made in clinical application of stem cell transplantation. Ishida et al confirmed the safety, reliability and ease of manipulation in treating ischemia of the limbs by PBSC and mesenchymal stem cell transplantation. The principle of APBSCT for treating ischemia of the limbs is to increase the secretion of VEGF in ischemic and anoxic microenvironment. PBSC contains a variety of cellular components. CD34+ stem cells contain endothelial progenitor cells that can differentiate into vascular endothelial cells in ischemic tissues, thereby promoting angiogenesis. In APBSCT, the stem cells differentiate into endothelial cells in ischemic muscles and then evolve into capillaries, which are remodelled into small collateral vessels.

Four weeks after APBSCT in this study, 5 patients (5 affected limbs) suffered from necrosis below the middle of the leg and received amputation. The remaining 59 patients (75 affected limbs) had their limbs successfully preserved with improvement of pain and cold sensation. Claudication distance was increased in these 59 patients ($p=0.02$) 3 months after transplantation, with a rise of ABI ($p=0.02$), TePO2 ($p=0.03$) and skin temperature of feet ($p=0.03$). Arteriography at 6 months after transplantation revealed formation of new collateral vessels to varying extent. This indicated that APBSCT can alleviate symptoms of TAO and promote the healing of ulcers. No complications such as retinal hyperplasia, malignancies, myocardial infarction and cerebral infarction were observed during follow-up. Thus APBSCT is a safe and effective method for treating TAO.

**Conclusions**

To conclude, APBSCT can alleviate the symptoms and promote ulcer healing in TAO safely and effectively, especially for those with poor distal arterial outflow tract in the lower limbs that do not permit bridging. However, the long-term efficacy is not confirmed yet.

**Fund**
National Natural Science Foundation of China (31360227)

**Conflict of Interest**
The Authors declare that they have no conflict of interests.

**References**


