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Analysis of possible factors relating to prognosis in autologous peripheral blood mononuclear cell transplantation for critical limb ischemia

LINGJIE SUN[1], LIHUA WU[1], ZHUOQING QIAO[2], JINGYI YU[1], LIANLIAN LI[1], SHANGZHU LI[1], QINGGUO LIU[1], YIMIN HU[1], NING XU[1] & PINGPING HUANG[1]

[1]*General Medical Center of the Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China, and* [2]*Department of Hematopoietic Stem Cell Transplantation, Hospital Affiliated with the Academy of Military Medical Sciences, Beijing, China*

Abstract

Background aims. Autologous transplantation of granulocyte colony-stimulating factor–mobilized peripheral blood mononuclear cells (M-PBMNCs) has been shown to be effective in treating critical limb ischemia (CLI); however, the studies of the possible prognosis predictors after autologous M-PBMNC transplantation are inadequate. The objective of the study was to assess the possible factors affecting the results of M-PBMNC transplantation for CLI. *Methods.* We reviewed the clinical profiles of 87 patients with CLI who were treated with M-PBMNC implantation in the Blood Diseases Hospital, Chinese Academy of Medical Sciences, between December 2002 and December 2011, and we followed these patients. The patients were divided into a good prognosis group and a poor prognosis group on the basis of whether amputation was performed. The significant differences of clinical variables between two groups were analyzed by means of the Mann-Whitney test and χ^2 test, and logistic regression analysis was used to study the variables representing the possible prognostic factors for amputation. *Results.* Of the 87 patients, three patients died and one patient was lost during the follow-up period. We analyzed 83 patients. The diseases included CLI complicated by diabetes mellitus gangrene (35 cases, 42.2%), arteriosclerosis obliterans (31 cases, 37.3%) and thromboangiitis (17 cases, 20.5%). The mean age was 62 years (range, 30–87). The median follow-up time for the surviving patients was 5 years. The 5-year amputation-free rate was 72.2%, and no adverse effects related to M-PBMNC transplantation were observed. *Conclusions.* The significant prognostic factors associated with poor angiogenesis were fibrinogen >4 g/L and fasting blood glucose >6 mmol/L.

Key Words: *critical limb ischemia, peripheral arterial disease, peripheral blood mononuclear cell transplantation, prognostic factor*

Introduction

Critical limb ischemia (CLI), which is the most severe form of peripheral arterial disease, has a dismal result for limb salvage and survival. CLI can arise from various types of vasculitis, including diabetes mellitus (DM) gangrene, arteriosclerosis obliterans (ASO) and thromboangiitis (TAO). The goals of treatment for patients with CLI are to relieve exertional symptoms, improve walking capacity, relieve ischemic pain at rest, heal ischemic ulceration, prevent limb loss and improve quality of life (1). A large number of patients still cannot avoid amputation despite recent progress has happened in drug, vascular surgery and interventional radiology for peripheral arterial disease. Since Folkman (2) reported the conception of "therapeutic angiogenesis" in 1998, more and more animal and clinical studies about cell transplantation for lower-limb ischemia, including bone marrow mononuclear cells (BMMNC)-mobilized and granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood mononuclear cells (M-PBMNCs) have arisen (3–13). In 2002, Tateishi *et al.* (4) first proved that autologous implantation of BMMNCs could be safe and effective for achievement of therapeutic angiogenesis. A phase II trial of autologous transplantation of BMMNCs for CLI showed an amputation-free survival rate of 75.2% at 12 months after the treatment (12). Dubsky *et al.* (13) proved that both autologous BMMNC and M-PBMNC transplantation improved ischemia in patients with diabetic foot, and there were no significant differences

Correspondence: **Pingping Huang**, MD, General Medical Center of the Institute of Hematology & Hospital of Blood Diseases, Chinese Academy of Medical Sciences & Peking Union of Medical College, 288 Nanjing Road, Tianjin 300020, China. E-mail: huangpp@ihcams.ac.cn

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between the two groups. Recently, a meta-analysis of all randomized, controlled trials on cell therapy in patients with CLI showed reduced amputation rates in the therapeutic arms, with a relative risk of major amputation of 0.58 (14). Gupta *et al.* (15) also proved the safety of the use of allogeneic bone marrow–derived mesenchymal stromal cells (BM-MSCs) in patients with CLI and showed positive trends toward improvement in ankle-brachial index through a randomized, double-blinded, placebo-controlled multicenter phase I/II trial (15). In 2004, we evaluated the clinical efficacy of M-PBMNC in five patients with severe ASO of the lower extremities. The results suggested for the first time that the autologous transplantation of M-PBMNCs is a practical, safe and effective treatment for lower-limb ischemia in China (6). Our team also conducted a randomized trial and demonstrated that the autologous transplantation of M-PBMNCs or BMMNCs significantly improves limb ischemia in patients with lower-limb arteriosclerosis obliterans 12 weeks after cell implantation; the improvement in the ankle-brachial index, skin temperature and resting pain was more significant in the patients in the M-PBMNC transplantation group than in the BMMNC group, and there was no significant difference between the two groups in pain-free walking distance, transcutaneous oxygen pressure, ulceration or rate of lower-limb amputation (7). The possible mechanism of therapeutic angiogenesis is that endothelial progenitor cells from bone marrow and peripheral blood could incorporate into the existing vasculature to increase capillary density and that a fraction of these cells support angiogenesis and vasculogenesis through the paracrine effects (8).

However, studies about long-term clinical outcome of autologous M-PBMNC transplantation for lower-limb ischemia were limited. In addition, the studies about the prognostic factors show contradictory results for M-PBMNC implantation. Our study aims to assess the possible prognostic factors regarding the long-term outcome for angiogenesis after autologous M-PBMNC transplantation in patients with chronic lower-limb ischemia in China.

Methods

Patients and variables

This clinical study was approved by the ethical committee board of the Institute of Hematology & Hospital of Blood Diseases, Chinese Academy of Medical Sciences & Peking Union of Medical College. Clinical profiles of 87 patients treated with autologous M-PBMNC transplantation for lower-limb ischemia in the Institute of Hematology & Hospital of Blood Diseases, Chinese Academy of Medical Sciences & Peking Union of Medical College from December 2002 through December 2011 were retrospectively studied. One patient with polycythemia vera was excluded. The 87 patients were selected to undergo M-PBMNC implantation because they were unresponsive to medication or surgical or endovascular procedures were deemed inappropriate. All patients received antiplatelet or anticoagulant drugs before transplantation. Angiography of all patients was analyzed, and no substantial heterogeneity was detected. The patients were followed up through telephone interviews, outpatient examinations or home visits. The variables studied included the following: the patient's sex, patient's age at first transplantation, Rutherford classification, vascular complications (including high blood pressure, ischemic cardiomyopathy and cerebral vascular disease), history of smoking, leukocyte counts after mobilization, blood concentrations of hemoglobin, fibrinogen, liver enzymes (including alanine aminotransferase and aspartate aminotransferase), creatinine, blood urea nitrogen, fasting blood glucose on first admission and times of transplantation. We evaluated the curative effect according to amputation (3). In this study, "lower-limb amputation" refers to any part of lower-limb amputation. Patients who avoided amputation were classified in the good prognosis group, and other patients were classified into the poor prognosis group. We retrieved all of the clinical records of the patients who received autologous M-PBMNC transplantation for lower-limb ischemia. The contents of the follow-up included whether death and amputation occurred and the cause of death.

M-PBMNC isolation and implantation

The method of transplantation was as previously described (5). The patients received 600 μg/d of recombinant human G-CSF (Kirin Pharmaceuticals, Tokyo, Japan) by subcutaneous injection for 5 days to mobilize the stem/progenitor cells. Meanwhile, a perfusion of 10,000 units/d heparin for 5 days by intravenous drip was used to avoid the possible risks of embolism. Approximately 300 mL of a suspension of blood circulating M-PBMNCs was collected from the patients treated with G-CSF. The superfluous cells were stored in liquid nitrogen for further use. Three hours later, each diseased lower limb was intramuscularly injected (40 sites, 3 × 3 cm distance, 1–1.5 cm deep, 7.5×10^8 M-PBMNC per site) into the thigh and leg, with a total of 3 × 109 mobilized PBMNCs. Every 40 days after transplantation, the severely diseased lower limb was given an additional transplantation of the same number of the cells frozen in liquid nitrogen.