Safety and efficacy of internal carotid artery infusion of autologous bone marrow-derived stem cells in subacute middle cerebral artery infarct

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Summary

Objective: To evaluate the safety and the efficacy of internal carotid artery (ICA) infusion of autologous bone marrow-derived stem cells (BMSC) in subacute middle cerebral artery (MCA) infarct. *Subject and method:* A prospective, open-label, non-randomized was conducted in patients with MCA infarct, within 7-40 days from onset. Sixty-two patients satisfying the inclusion criteria were enrolled and allocated into either BMSC group (n = 31) or control group (n = 31). Follow-ups were performed at 6 months and 1 year after therapy. Adverse events were noted to conclude safety outcome. The primary efficacy outcomes were percentages of recovered patients with a score of 0 to 2 on the modified Rankin Scale (mRS). The secondary efficacy outcomes were evaluated by the National Institutes of Health Stroke Scale (NIHSS), Barthel Index (BI), Brunnstrom stages of hand (BRS-H), and infarct volume on head MRI. *Result:* There were no significant differences in the percentages of noted adverse events. The percentages of the mRS 0-2 in BMSC group were remarkably higher as compared to control group at both 6-month and 1-year follow-up, but not statistically significant (25.8% vs 6.9%, p=0.08 and 26.7 vs 9.7, p=0.1, respectively). BI at 6 months was significantly better in the BMSC group, however no significant differences on other secondary efficacy measures. *Conclusion:* ICA infusion of BMSC was safe and tolerated in patients with subacute MCA infarct. Although the difference in the primary efficacy outcomes was not statistically significant, a favorable trend was found in BMSC group representing by the BI at 6 months and the percentages of mRS 0-2 at both main follow-ups.

Keywords: Bone marrow stem cells, ischemic stroke.

1. Background

Ischemic stroke, of all strokes, is a major cause of serious long-term disability, in particular, MCA infarction poses an increased risk of mortality (by 17%) and severe disability (by 50%) compared to other infarcts [1]. For the treatment of cerebral infarction in the subacute stage, except for physical therapy and rehabilitation, other options of current medical treatment being used showed limited effectiveness, leading to the majority of long-term disabled patient. One of the most encouraging innovative treatment options is restorative therapy using stem cell replacement in the injured cerebral tissues. Although evidence of the beneficial effects of stem cells in animal stroke models is growing, there is a lack of clinical data [2-5]. Autologous BMSC have shown some promise for this group of patients, therefore, this study was conducted to evaluate the safety and the efficacy of internal carotid artery infusion of autologous BMSC in MCA infarct.

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2. Subject and method

2.1. Study design and setting

This was a prospective, non-randomized, open-label clinical study conducted at 108 Military Central Hospital, Hanoi, Vietnam from July 2018 to April 2022. Ethical approval was obtained from Independent Ethics Committee of 108 Military Central Hospital. Written informed consent was obtained from each patient or their next of kin (in case of being unable to perform due to aphasia or other stroke-related reasons) after being provided the information about the study.

2.2. Participants

The study recruited patients who met all the elements in the inclusion criteria and did not have any elements in the exclusion criteria. Inclusion criteria were as follows: (1) Patients 20-75 years old; (2) Ipsilateral MCA infarct confirmed by head MRI; (3) Onset of stroke between 7 and < 40 days; (4) NIHSS at day 7 from stroke onset ≥ 7; (5) Written informed consent.

Patients with the following conditions/diseases were excluded: (1) Hemorrhagic stroke or symptomatic hemorrhagic transformation; (2) Lacunar infarction; (3) Pre-existing stroke with mRS ≥ 2; (4) Hematological causes of stroke; (5) History of malignancy; (6) Renal impairment, with serum creatinine ≥ the normal upper limit; (7) Liver impairment, with serum aspartate transaminase and alanine transaminase 3 times greater than the normal upper limit; (8) Any other severe comorbidity; (9) Contraindication for MRI or for bone marrow harvest; (10) Pregnancy, childbearing potential (unless it is certain that pregnancy is not possible), or breast feeding; (11) Participation in any clinical trial in the last three-months.

All patients received standard medical care and were admitted to a stroke rehabilitation center according to current guideline but patients in the BMSC group, in addition, received BMSC infusion as protocol.

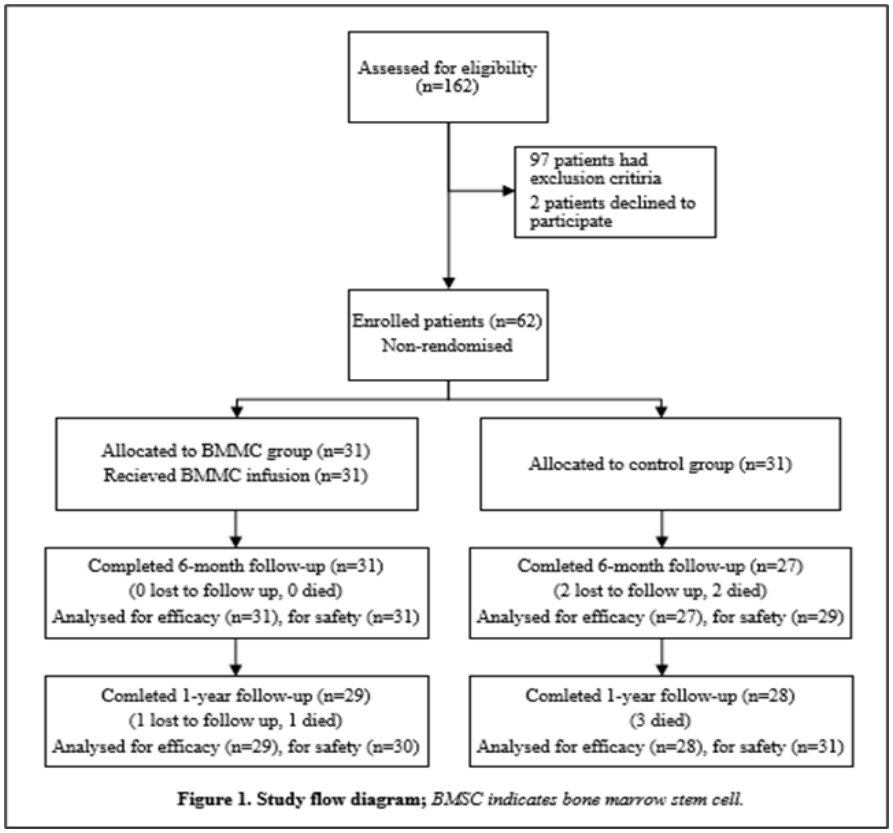

Figure 1. Study flow diagram; *BMSC indicates bone marrow stem cell.*

2.3. Bone marrow aspiration, cell separation, and BMSCs preparation

Bone marrow aspiration and subsequent cell preparation were accomplished on the same day as BMSCs infusion. The collection was performed under local anesthesia in operation room, through a puncture and repeated aspirations at the posterior iliac crest regions. A total of 300ml of bone marrow and anticoagulant solution was collected, and after removal of the bony and fatty residues, the mononuclear cells were isolated by density gradient (using a ficoll density gradient centrifugation procedure). Quality control of the final cell product was performed to determine the total number of cells, total CD34+ cells, CD34+ cell percentage, sterility, and viability. Total nucleated cells and the bone marrow mononuclear cells were counted using an automated hematology analyzer. CD34 cell enumeration was performed with an anti-CD34 antibody using the International Society of haemato-therapy and Graft Engineering guidelines. Viability was assessed by 7AAD dye on flowcytometry machine.

2.4. Internal carotid artery BMSC infusion

ICA infusion was carried out within 24 hours after separation of BMSC using a digital subtraction angiography. Patients were brought to the angiography suite and placed in the supine position. Ascending through the RT femoral artery to the aorta by diagnostic catheter and guiding hydrophilic J-shaped wire to reach the aortic arch, and from there, we select either right or left common carotid artery, then the internal carotid artery. With a 5F diagnostic catheter, ipsilateral internal carotid artery angiography was performed to ensure patency of the ICA and the segments of the middle cerebral artery. Through the microcatheter, a total of 20ml of the BMSC solution was slowly injected into the ICA for 10 minutes. After the infusion, a check diagnostic run was performed to ensure patency of all the vessels and rule out any thromboembolic complication.

Monitoring for infusion-related adverse events

Main vital signs including heart rate, blood pressure, body temperature, and oxygen saturation (SpO2) were monitored at pre-, mid-, and 24-hour post-procedure. The presences of rash, urticaria, chills, and rigors or any other complication were noted. Neurological assessment and main vital signs were monitored daily until hospital discharge. Adverse events (AEs) were still followed and noted up to 1 year after therapy. At 6-month follow-up, the complete blood count (CBC) was performed to assess the blood system disorders, and head MRI to rule out any abnormal lesions as expanding intracerebral processes (neoplasms benign, malignant).

2.5. Assessment and follow-up schedule

Clinical assessment: All patients assessed by neurological scales at baseline and at follow-ups, including NIHSS (0 to 42, with higher scores indicating greater stroke severity) [6], Barthel Index (0 to 100, with higher scores indicating greater ability to complete activities of daily life) [7], and mRS (0 to 6, 0 as no symptoms to 6 as death) [8, 9] to measures the degree of disability or dependence in the daily activities. Improved NIHSS or improved BI at each time point were calculated by changes in comparison to baseline. Item 5 in the NIHSS (arm motor-NIHSS, range 0-5) [6], and the Brunnstrom recovery stages of hand (BRS-H, range 1-7) [10], were used as motor outcome measures. The 6-month follow-up was performed in hospital but 1-year follow-up was perform by video call (due to COVID-19 pandemic).

MRI assessment: Head MRI was performed at baseline and 6-month follow-up including T1-weighted image (T1WI), T2-weighted image (T2WI), fluid attenuated inversion recovery (FLAIR), diffusion weighted imaging (DWI) and 3D TOF MRA. Infarct volume was measured on DWI sequence using the ABC/2 formula (ellipsoid) [11], expressed in milliliters. Changed volume of infarct lesion at 6 months was calculated in comparison to baseline.

2.6. Outcome measures

The primary outcome was percentages of survivals with no symptoms to slight disability, defined as a score of 0 to 2 on the mRS at 6-month follow-up and 1-year follow-up. This approach was

based on reference to several large clinical trials studying on patients with MCA infarction as REVASCAT trial or DEFUSE trial [12, 13]. The following secondary outcomes were also assessed: The global neurological scales including NIHSS (only at 6 months) and BI; the degree of motor recovery measured by motor-arm NIHSS and BRS-H; infarct volume on head DWI-MRI at 6 months. The reason for using the motor-arm NIHSS and BRS-H to measure motor function outcome is that the ability to recover motor function of the upper extremities and especially the hands is extremely difficult causing by the injury of the corticospinal tract in MCA infarct, that not able to solve by current conventional treatment.

2.7. Statistical analysis

Descriptive statistics included median (interquartile range [IQR]) for numeric data and percentages for categorical data. Comparisons between the BMMC group and control group for safety and efficacy endpoints were explored at 6-month follow-up and 1-year follow up, using Mann Whitney test for continuous data and either Chi-squared test or Fisher exact test for categorical data. All analyses were 2-sided, and p-values <0.05 were considered statistically significant. IBM SPSS version 24.0 (IBM Corp., Armonk, NY, United States) was used for data analysis.

3. Result

Patient recruitment

A total of 62 patients were recruited, of which 31 receiving BMSC were allocated to BMSC group (n = 31), and remaining patients were allocated to the control group (n = 31). In the BMSCs group, one patient lost to follow-up at 1-year end point due to the inability to contact the patient's family and one patient died before the 1-year assessment. In control group, 2 patients lost to 6-month follow-up, 2 patients died before this follow-up and one more patient died before the 1-year follow-up (Figure 1).

Table 1. Baseline characteristics and group comparisons

Measure	BMSC (n = 31)	Control (n = 31)	p value (2-sided)
Demographics			
Age (year)			
Median (IQR)	59 (35-74)	60 (54-67)	0.60
< 65 years old (n, %)	24 (77.4%)	20 (64.5%)	0.40*
Gender (male) (n, %)	23 (74.2%)	27 (87.1%)	0.33*
Stroke risk factors			
Hypertension history	22 (71.0%)	21 (67.7%)	1.00*
Atrial fibrillation	1 (3.2%)	4 (12.9%)	0.35**
Diabetes	9 (29.0%)	5 (16.1%)	0.36
Heart failure	0	3 (9.7%)	0.23**
Preexisting stroke (n, %)	2 (6.5%)	4 (12.9%)	0.67**
mRS 1 (n, %)	2 (6.5%)	4 (12.9%)	0.67**
mRS 2-6 (n, %)	0	0	
Dyslipidemia	9 (29.0%)	8 (25.8%)	1.00
Stroke clinical scale at baseline			
Total NIHSS (median (IQR))	13 (10-17)	12 (10-16)	0.48
Motor Arm NIHSS (median (IQR))	4 (3-4)	4 (3-4)	0.83

Measure	BMSC (n = 31)	Control (n = 31)	p value (2-sided)
BRS-H			
Stage 1 (n, %)	25 (80.6%)	27 (87.1%)	0.73
Stage 2-7 (n, %)	7 (22.6%)	4 (12.9%)	
Total BI (median (IQR))	25 (20-30)	25 (20-30)	0.24
Lesion on head MRI			
Left lesion side (n, %)	21 (67.7%)	15 (48.4%)	0.19*
Total volume (ml) (median (IQR))	58 (28.3-113.9)	114.8 (73.8-165.3)	0.09
Symptom onset to BMSC infusion (day)			
Median (IQR)	17 (12-20)	NA	NA

*BMSC, bone marrow stem cells; NIHSS, National Institutes of Health Stroke Scale; BI, Barthel Index; BRS-H, Brunnstrom Recovery Stages- Hand; IQR, interquartile range; NA, Non-assessment; *, Pearson Chi-Square; **, Fisher's Exact test.*

Baseline characteristics

The baseline characteristics of the BMSC and control groups were comparable. There were no significant differences in terms of the main clinical scores and the infarct volume. Although the infarct volume was larger in the control group, but not statistically significant (Table 1).

Intervention and doses

Bone marrow aspiration was successfully completed without any adverse events in 31 patients of the BMSC group. The mean number of infused bone marrow derived-mononuclear cells was 602.87 ± 285.20 x 10^6, containing 12.56 ± 6.72 x 10^6 CD34+ cells with viability of 94.2 ± 5.7%. Median time from onset to BMSC infusion was 17 days (IQR, 12–20 and range, 7–37).

Safety outcomes

Regarding short-term safety, there were no adverse events during bone marrow sampling, and no adverse event was attributable to BMSC infusion procedure.

Table 2. Adverse events observed during the time periods of follow-up

Adverse events (AEs)	6-month follow-up		1-year follow-up	
	BMSC (n = 31)	Control (n = 29)	BMSC (n = 30)	Control (n = 31)
Death	0	2 (6.9%)	1 (3.3%)	3 (9.7%)
Nervous system disorders				
Convulsion	2 (6.5%)	1 (3.4%)	3 (10.0%)	3 (9.7%)
Recurrent ischemic stroke	1 (3.2%)	1 (3.4%)	1 (3.0%)	1 (3.2%)
sICH	0	0	0	0
Cardiovascular disorders				
Deep vein thrombosis	0	0	0	0
Infusion-related allergic reaction	0	0	0	0

Adverse events (AEs)	6-month follow-up		1-year follow-up	
	BMSC (n = 31)	Control (n = 29)	BMSC (n = 30)	Control (n = 31)
Infections and infestations				
Cryptogenic fever	0	4 (13.8%)	0	4 (12.9%)
Blood and lymphatic system disorders	0	0	0	0
Surgical and medical procedures	0	0	0	0
All AEs	3 (9.7%)	8 (27.5%)	4 (16.3%)	11 (35.5%)
Noted: p>0.05 with all noted AEs between two groups, using Fisher's Exact test.				

Regarding long-term safety, in control group, two patients died before the 6-month follow-up with the causes identified as recurrent stroke and heart failure, respectively; one more patient died due to prolonged bedridden state after 11 months after baseline time. In BMSC group, the number of deaths was 1, with the cause identified as recurrent stroke at 7 months. Most of the adverse events occurred within 6 months from baseline, with no significantly higher rate in the control group (Tables 2). Head MRI at 6-month follow-up did not reveal any evidence of new abnormal lesions as expanding intracerebral processes (neoplasms benign, malignant).

Efficacy outcomes

Table 3. Outcomes at 6-month and 1-year follow-up and group comparisons

Outcome measure	6-month follow-up			1-year follow-up		
	BMSC (n = 31)	Control (n = 27)	p	BMSC (n = 29)	Control (n = 28)	p
mRS *	3 (2-3)	3 (3-3)	0.10	3 (2.75-3)	3 (1-6)	0.19
mRS ≤ 2 (n, %) *	8/31 (25.8%)	2/29 (6.9%)	0.08	8/30 (26.7%)	3/31(9.7%)	0.1
Total NIHSS	5.0 (3-9)	7.0 (5-9)	0.15	NA	NA	NA
Improved NIHSS	7.0 (5-9)	7.0 (4-8)	0.41	NA	NA	NA
Total BI	90 (80-95)	75 (55-90)	0.01	90 (85-100)	85 (60-90)	0.13
Improved BI	65 (35-70)	45 (30-60)	0.03	65 (32-70)	55 (31.2-63.7)	0.18
Motor Arm-NIHSS	2 (0-4)	2 (2-3)	0.77	2 (0-4)	2 (1-2.75)	0.90
BRS-H						
Stage 1-3 (n, %)	20 (64.5%)	23 (85.2%)	0.13	19 (65.5%)	23 (82.1%)	0.23
Stage 4-7 (n, %)	11 (35.5%)	4 (14.8%)		10 (34.5%)	5 (17.9%)	
Lesion on MRI				NA		
Total volume (ml)	43.8 (12.8-63.2)	60.4 (47.2-85.5)	0.07		NA	NA
Changed volume	6.4 (-9.1-38.8)	71.9 (27.3-86.7)	0.07			

Data are median (IQR) or n (%); BMSC, bone marrow stem cells; NIHSS, National Institutes of Health Stroke Scale; BI, Barthel Index; BRS-H, Brunnstrom Recovery Stages-Hand; NA, Non-assessment.

**, data included died patients as mRS6.*

Primary outcomes: The percentages of patients reaching mRS0-2 in BMSC group were higher compared to control group at both 6-month and 1-year follow-up, but not statistically significant (25.8% vs 6.9%, p=0.08 and 26.7 vs 9.7, p=0.1, respectively) (Table 3).

Secondary outcomes: At 6-month follow-up, the Barthel index was significantly better in the BMSC group compared to control group in terms of both total BI and improved BI, however, other scales including NIHSS, motor arm-NIHSS, BRS-H were similar between two groups. In terms of lesion on brain MRI, there were no significant differences in total infarct volume and in changed infarct volume compared to baseline. At 1-year follow-up, there were no statistically significant differences in variables mentioned above.

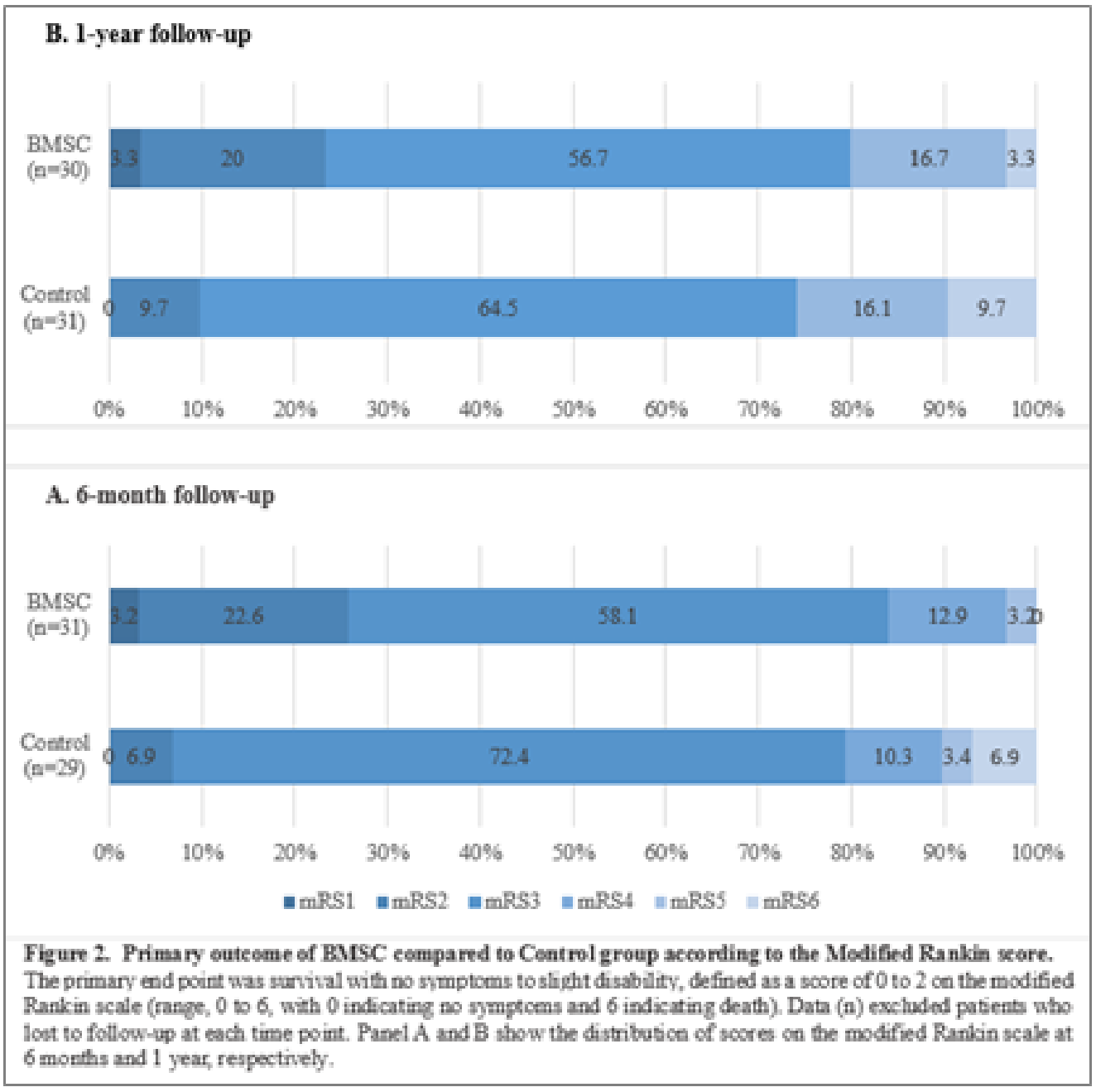

Figure 2. Primary outcome of BMSC compared to Control group according to the Modified Rankin score. The primary end point was survival with no symptoms to slight disability, defined as a score of 0 to 2 on the modified Rankin scale (range, 0 to 6, with 0 indicating no symptoms and 6 indicating death). Data (n) excluded patients who lost to follow-up at each time point. Panel A and B show the distribution of scores on the modified Rankin scale at 6 months and 1 year, respectively.

4. Discussion

Normally, the prognosis of patients with a large MCA infarction is extremely poor. Even with the current optimal medical treatment, MCA infarct may lead to death in 17% of cases, and survivors frequently suffer from long-term severe disability (50%), especially in the motor and speech function [14]. Unfortunately, the survivors have no effective treatment available other than neurorehabilitation to improve neurological functions, and restoring the motor function of upper limb, and especially of the hand is still an unsolved challenge. In recent years, regenerative medicine in general, and cell therapy, have shown the potential to find a way to overcome this difficulty both in terms of pathological mechanisms and experimental studies. Most clinical trials of cell therapies for the acute and subacute stages of stroke have used unsorted bone marrow mononuclear cells or purified adherent stem cells, such as multipotent adult progenitor cells, marrow stromal cells, adipose cells. Autologous BMSC was used this study due to its advantages including being easily accessible through the aspiration of the bone marrow, can be isolated from patients themselves thereby bypassing the ethical matter.

In this study, the subacute phase of cerebral infarction was selected as the timing of BMSC infusion for several reasons. Firstly, this phase in stroke was thought to provide an optimal window of native brain repair that may be enhanced by paracrine effects of exogenous stem cells [5]. Secondly, to eliminate two major confounding factors for the assessment of therapeutic efficacy, which often happen in acute phase of cerebral infarction including either the clinical deterioration or spontaneous recovery. In fist week from ischemic stroke onset, a certain percentage of patients presents clinically deterioration by cerebral edema or hemorrhagic transformation, while a small number of other patients have spontaneous recovery [15, 16].

The intra-carotid route was chosen to directly target the MCA territory and the leptomeningeal collateralization from the anterior cerebral artery and the infusion site was located at the proximal segment of ICA for the purpose of taking advantage of this collateral circulation system. In comparison with the intravenous route, intra-arterial infusion was shown to be more effective in delivering stem cells to the site of cerebral ischemic injury by avoiding the loss of stem cells attributable to pulmonary first passage [2, 17].

Regarding short-term safety, there were no related AEs during bone marrow sampling, and no adverse event was attributable to BMSCs infusion procedure, especially AEs such as allergic reactions or embolism were not noted. Regarding long-term safety, in BMSC group, the number of deaths was 1, with the cause identified as recurrent stroke at 7th month. Most of the adverse events occurred within 6 months after baseline, with no significantly higher rate in the control group (Tables 2). Head MRI at 6-month follow-up did not reveal any evidence of new abnormal lesions as expanding intracerebral processes (neoplasms benign, malignant). This safety result was consistent with most previous studies of intracarotid BMSC infusion in stroke [3-5].

The efficacy results were mixed. Although there were no differences in the preplanned analyses between the groups for primary outcome and some of the secondary outcomes, the trends moved in different directions. The Barthel Index at 6-month follow-up was significantly better in the BMSC group compared to control group, and the percentages of patients achieving mRS0-2 at both 6-month and 1-year follow-up were much higher in this group compared to control group, although not statistically significant.

In comparison with results of several intracarotid BMSC infusion trials performing on patients with subacute ischemic stroke, several authors reported positive results while a few other studies published negative results. Moniche F (2012) [18] and Bhatia V. (2018) [3] with prospective, randomized, open-label, blinded-end point trials, performing on ischemic stroke patients, had observed improved clinical outcomes significantly in

BMSC group. In these two trials, timing for BMSC infusion was from 3-15 days from stroke onset, earlier than in our study. In terms of BMSC dose, Moniche F. used dose of 1.59 ± 10^8 BMSCs containing 3.38 ± 10^6 CD34 cells and Bhatia V. used higher dose (6.1 ± 10^8 BMSCs with 1.2 ± 10^7 CD34 cells), the dose in our study was between these two doses. Thus, timing for BMSC infusion could be a difference.

In terms of limitation in this study, non-randomized design is a point we want to mention. However, the allocation into each group was based on patient choice, not subjectivity of researchers. In this project, we also conducted a group of patients receiving intravenous infusion of BMSC, whereby patients who met the inclusion criteria were offered 3 options for including intravenous infusion, intracarotid infusion, and joining in control group, therefore, it was essentially a random allocation.

5. Conclusion

Internal carotid artery infusion of autologous bone marrow-derived stem cells was safe and tolerated in patients with subacute middle cerebral artery infarct. Although the difference in the primary efficacy outcomes was not statistically significant, a favorable trend was observed in autologous bone marrow-derived stem cells group, presenting by the Barthel Index at 6 months and the percentages of mRS 0-2 at both main follow-ups. The study provides a framework for the design and conduct of further cell therapy trials in stroke.

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