Significant improvement of Barthel index scores in patients with subacute middle cerebral artery infarct after intravenous autologous bone marrow-derived stem cells infusion

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Summary

Objective: To assess the safety and the efficacy of intravenous 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 performed in patients with MCA infarct, within 7-40 days from onset. Sixty-three patients, satisfying the inclusion criteria, were enrolled, and allocated into the IV-BMSC group (n = 32) or into control group (n = 31). Main follow-ups were at 6 months and 1 year after therapy. Adverse events were noted to conclude safety outcome. The primary efficacy outcomes were proportions of patients achieving 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 statistically significant differences in the rates of noted adverse events. There were no significant differences between the two groups on the proportions of the mRS 0-2 at both 6-month and 1-year follow-up (3.2% vs 6.9% with p=0.6, and 6.9 vs 9.7 with p=1.0, respectively). The improvement of BI at 6 months was significantly better in the IV-BMSC group compared to control group, however no significant differences on other secondary efficacy measures. *Conclusion:* Intravenous infusion of BMSC was safe in patients with subacute MCA infarct. Although the difference in the primary efficacy outcomes was not statistically significant, a favorable secondary outcome was observed in IV-BMSC group, presenting by the statistically significant improvement of the Barthel index at 6-month follow-up.

Keywords: Bone marrow stem cells, cerebral infarct, intravenous infusion.

1. Background

Both the short-term and long-term outcome of patients with a large MCA infarction are 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 [1]. Unfortunately, current medical therapies using for treatment for subacute stage in cerebral infarct have limited efficacy, and with patients suffering MCA infarct, restoring the motor function of upper limb, especially of the hand is still an unsolved challenge. In recent years, stem cells have shown a promising role in ischemic stroke, in preclinical studies as well as initial pilot studies and it could be a potential direction to discover a way to solve the above-mentioned difficulties.

***Received**: 13 November 2022, **Accepted**: 18 December 2022*

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Coming from the context that research data on this therapy is still limited and published results in those previous trials are inconsistent about the efficacy [2], our study was performed to assess the safety and the efficacy of intravenous infusion of autologous BMSC in subacute MCA infarct.

2. Subject and method

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 (IEC) of 108 Military Central hospital. Written informed consent was obtained from each patient or their next of kin (if they lacked capacity due to aphasia or other stroke-related reasons) after being provided the information about the study.

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 IV-BMSC group, in addition, received BMSCs infusion as protocol.

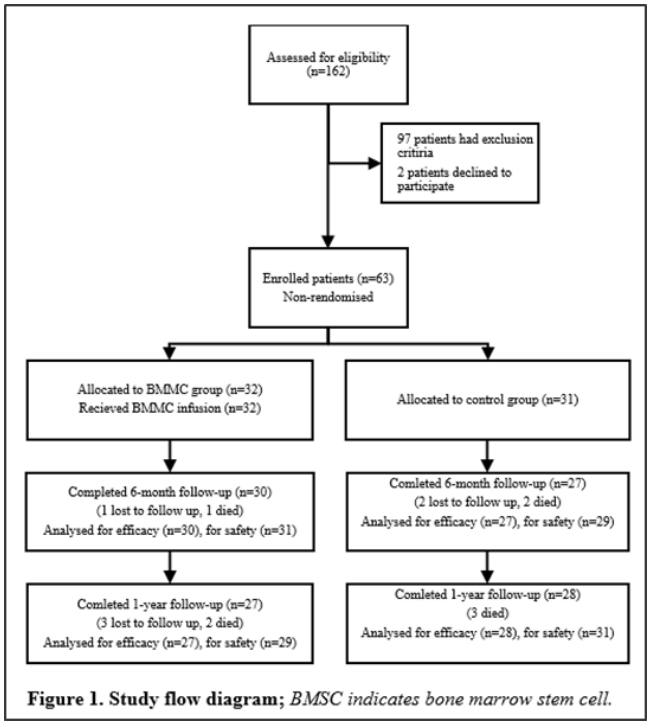

Figure 1. Study flow diagram; *BMSC indicates bone marrow stem cell.*

Bone marrow aspiration, cell separation, preparation and intravenous BMSCs infusion

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.

The cells were suspended in 250mL of NaCl 0.9% solution just before being infused into antecubital vein of the patients in IV-BMSC group, intravenously at 6mL/min, on the same day as the harvest of bone marrow cells.

Monitoring for BMSCs infusion-related adverse events

Main vital signs including heart rate, blood pressure, body temperature, and oxygen saturation (SpO_2) were monitored at pre-, mid-, and 24 hours 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, and laboratory test including complete blood count (CBC) was performed at 6-month follow-up to assess the blood system disorders, head MRI at 6 months to rule out any abnormal lesions as expanding intracerebral processes (neoplasms benign, malignant).

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) [3], Barthel index (0 to 100, with higher scores indicating greater ability to complete activities of daily life) [4], and mRS (0 to 6, 0 as no symptoms to 6 as death) [5, 6] 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) [3], and the Brunnstrom recovery stages of hand (BRS-H, range 1–7) [7], were used as motor outcome measures. The 6-month follow-up was performed in hospital, but the 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. Cerebral infarct volume was measured on DWI sequence, using the ABC/2 formula (ellipsoid) [8], and being expressed by milliliters. Changed volume was calculated as the difference between the infarct volume at 6 months compared with baseline.

Outcome measures

The primary outcome was proportions 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 [9, 10]. 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 and changed 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.

Statistical analysis

Descriptive statistics included median (interquartile range [IQR]) for numeric data and proportions 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

Table 1. Baseline characteristics and group comparisons

Measure	IV-BMSC (n = 32)	Control (n = 31)	p-value (2-sided)
Demographics			
Age (year)			
Median (IQR)	60.5 (55-66)	60 (54-67)	0.97
< 65 years old (n, %)	22 (68.8%)	20 (64.5%)	0.79*
Gender (male) (n, %)	22 (68.8%)	27 (87.1%)	0.12*
Stroke risk factors			
Hypertension history	20 (62.5%)	21 (67.7%)	0.79*
Atrial fibrillation	4 (12.5%)	4 (12.9%)	1.00**
Diabetes	8 (25%)	5 (16.1%)	0.53*
Heart failure	1 (3.1%)	3 (9.7%)	0.35**
Preexisting stroke (n, %)	3 (9.4%)	4 (12.9%)	0.70**
mRS 1 (n, %)	3 (9.4%)	4 (12.9%)	0.70**
mRS 2-6 (n, %)	0	0	
Dyslipidemia	10 (31.3%)	8 (25.8%)	0.78*
Stroke clinical scale at baseline			
Total NIHSS (median (IQR))	14.5 (11.25–18)	12 (10–16)	0.09
Motor Arm NIHSS (median (IQR))	4 (3–4)	4 (3–4)	0.61
BRS-H			
Stage 1 (n, %)	29 (90.6%)	27 (87.1%)	0.70**
Stage 2–7 (n, %)	3 (9.4%)	4 (12.9%)	
Total BI (median (IQR))	22.5 (20–25)	25 (20–30)	0.05
Lesion on head MRI			
Left lesion side (n, %)	22 (68.8%)	15 (48.4%)	0.12*
Total volume (ml) (median (IQR))	93.5 (54.3–149.8)	114.8 (73.8–165.3)	0.29
Symptom onset to BMSC infusion (day)			
Median (IQR)	16 (13.25–19)	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.

A total of 63 patients were recruited, of which 32 were received the BMSCs treatment and 31 were allocated to the control group. In the IV-BMSC group, the number of patients who lost to follow up at 6-month and 1-year follow-up due to the inability to contact the patient's family were 1 and 3, respectively, while 2 patients in the control group lost follow-up at 6-month follow-up. On the number

of patients who died before the 6-month and 1-year follow-up, the IV-BMSC group had 1 and 2 patients for each time point, respectively, and the control group had 2 and 3 patients, respectively (Figure 1).

Baseline characteristics

The baseline characteristics of the treatment and control groups were comparable. There were no significant differences between two groups in terms of the clinical scores, baseline infarct volume (Table 1).

Intervention and doses

All patients received the conventional treatment. Bone marrow aspiration was successfully completed without any adverse event in 32 patients in the BMSCs group. The mean number of infused bone marrow derived-mononuclear cells was 523.27 ± 242.31 x 10^6, containing 13.21 ± 9.66 x 10^6 CD34+ cells with viability of 96.4 ± 2.9%. Median time from onset to BMSC infusion was 16 days (IQR, 13.25-19 and range, 10-38).

Safety outcomes

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

SAE	6-month follow-up		1-year follow-up	
	IV-BMSC (n = 31)	Control (n = 29)	IV-BMSC (n = 29)	Control (n = 31)
Death (with any cause)	1 (3.2%)	2 (6.9%)	2 (6.9%)	3 (9.7%)
Nervous system disorders				
Convulsion	2 (6.5%)	1 (3.4%)	5 (17.2%)	3 (9.7%)
Recurrent ischemic stroke	1 (3.2%)	1 (3.4%)	2 (6.9%)	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
Infections and infestations				
Cryptogenic fever	2 (6.5%)	4 (13.8%)	2 (6.9%)	4 (12.9%)
Blood and lymphatic system disorders	0	0	0	0
Surgical and medical procedures	0	0	0	0
All AEs	6 (19.4%)	8 (27.5%)	11 (37.9%)	11 (35.5 %)
Noted: p>0.05 with all valuables between two groups, using Fisher's Exact test.				

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

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

Measures	6-month follow-up			1-year follow-up		
	IV-BMSC (n = 30)	Control (n = 27)	p	IV-BMSC (n = 27)	Control (n = 28)	p
mRS *	3 (3-3)	3 (3-3)	0.96	3 (3-4)	3 (1-6)	0.82
mRS ≤ 2 (n, %) *	1/31 (3.2%)	2/29 (6.9%)	0.60	2/29 (6.9%)	3/31 (9.7%)	1.00
Total NIHSS	6.0 (4-9.25)	7 (5-9)	0.64	NA	NA	NA

Measures	6-month follow-up			1-year follow-up		
	IV-BMSC (n = 30)	Control (n = 27)	p	IV-BMSC (n = 27)	Control (n = 28)	p
Improved NIHSS	8.0 (4.75-10.25)	7.0 (4-8)	0.06	NA	NA	NA
Total BI	90 (78.75-90)	75 (55-90)	0.05	90 (75-95)	85 (60-90)	0.39
Improved BI	62.5 (53.7-70)	45 (30-60)	0.007	60 (35-70)	55 (31.2-63.7)	0.11
Motor arm NIHSS	2 (1-3)	2 (2-3)	0.65	2 (1-3)	2 (1-2.75)	0.48
BRS-H						
Stage 1-3 (n, %)	26 (86.7%)	24 (88.9%)	1.00	27/27 (100%)	24/25 (96%)	0.70
Stage 4-7 (n, %)	4 (13.3%)	3 (11.1%)		3 (11.5%)	5 (17.9%)	
Lesion on head MRI				NA	NA	NA
Total volume (ml)	49.1 (32.9-97.5)	60.4 (47.2-85.5)	0.61			
Changed volume	20.3 (-3.3-73.9)	71.9 (27.3-86.7)	0.24			

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; IQR, interquartile range; NA, Non-assessment.
* The data (n) included patients who died as mRS6.

Regarding long-term safety, in control group, 2 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 following stroke onset. In IV-BMSC group, the number of deaths was 2, one patient died at 3rd month due to infection and the other died at 7th month due to heart disease. 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).

Efficacy outcomes

Primary outcomes: There were no significant differences between the IV-BMSC and control group on the proportions of the mRS 0-2 at both 6-month and 1-year follow-up (3.2% vs 6.9% with p=0.6, and 6.9 vs 9.7 with p=1.0, respectively) (Table 3, Figure 2).

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

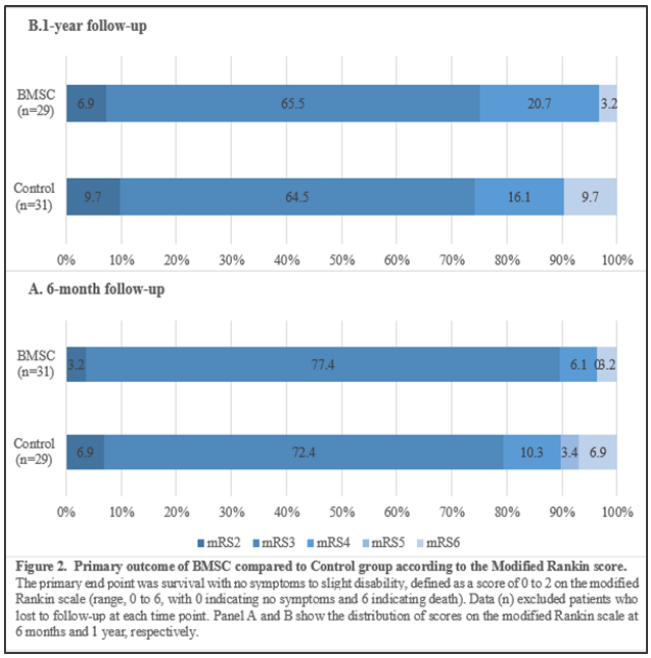

Figure 2. Primary outcome of BMSC compared to Control group according to the Modified Rankin score. The primary end point was survival with no symptoms to slight disability, defined as a score of 0 to 2 on the modified Rankin scale (range, 0 to 6, with 0 indicating no symptoms and 6 indicating death). 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

The main finding in this study was that intravenous infusion of BMSCs was safe and tolerated. Regarding short-term safety, there were no noted AEs attributing to bone marrow sampling and BMSCs infusion procedure, especially AEs such as allergic reactions or embolism that people are often concerned about when infusing the blood and cell-related products were not noted. In terms of long-term safety, in BMSC group In BMSC group, the number of deaths was 2, one patient died at 3rd month due to severe infection developing from focal infection at a pressure ulcer on upper back, and the other died at 7th month due to heart disease. Most of the adverse events occurred within 6 months after baseline time, with not statistically significantly higher rate than those 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 intravenous BMSC infusion in stroke [11-13]. 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 improved score of BI at 6-month follow-up was significantly better in the BMSC group than that in control group. In comparison with results of the intravenous BMSC infusion trials performing on patients with subacute ischemic stroke, several authors reported positive results while a few other studies published negative results. ZK Law et al. (2021) [14] reported the apparent improvement in the BI score at 6 weeks in the BMSC group, that is consistent with our result.

Additionally, Jaillard A, et al (2020) [12] and Lee J et al (2022) [15] with RCT trials, using the bone marrow - derived mesenchymal stem cells (MSCs) for infusion, had observed significantly favorable outcomes in terms of motor recovery, however we did not find out the significant differences when analyzing the motor function improvement in the arm and the hand on the paretic side based on the arm motor-NIHSS and BRS-H. In these two trials, timing for BMSC infusion were 32 (IQR, 28–40) and 24.6 (SD, 21.0) days, respectively, from stroke onset, like that in our study. Thus, the kind of cells for infusion could be a difference.

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 [16]. 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 [17, 18].

In this study, intravenous rout was used to infuse the BMSC due to its advantages. In comparison with other routs such as arterial, intracerebral, or intraventricular infusion, it is the least invasive and safest method to deliver the cells. In addition to the advantages of autologous BMSC mentioned above, this therapy has potential to easily and widely deployed if it is proven to have real benefits. However, this method may be suboptimal in achieving successful cell migration to the infarcted area due to the loss of stem cells attributable to pulmonary first passage [2, 19].

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 researcher's subjectivity. In this project, we also conducted a group of patients receiving internal carotid artery infusion of BMSC, whereby patients who met the inclusion criteria were offered 3 options including intravenous infusion, intracarotid infusion, and control group, therefore, it was essentially a random allocation.

5. Conclusion

Intravenous infusion of autologous bone marrow-derived stem cells was safe in patients with subacute middle cerebral artery infarct. Although the difference in the primary efficacy outcomes was not statistically significant, a favorable secondary outcome was observed in intravenous infusion of autologous bone marrow-derived stem cells group, presenting by the statistically significant improvement of the Barthel index at 6 months follow-up. The study provides a framework for the design and conduct of further cell therapy trials in stroke.

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