

## Dynamics of Human Mesenchymal Stem Cells, M1 Microglia/Macrophage, and Fractalkine in Ischemic Stroke Patients

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**Background:** About 85% of strokes are ischemic strokes, caused by occlusion of cerebral artery that induced brain inflammation. A deep understanding of ischemic stroke mechanism will lead to better neurorestorative treatment. **Objective:** This study investigates the dynamics of human mesenchymal stem cells, fractalkine, and M1 microglia/macrophage in ischemic stroke patients. **Results:** We found the same fractalkine levels and M1 microglia/macrophage cells on patients with stroke onset 0 to 14 days, then decrease until 30 days of stroke onset. MSCs was increase 7 days after stroke onset, peaked by 14 days, then decreased until 30 days after stroke ischemic onset. **Conclusions:** This study found an interaction between microglia/macrophage, fractalkine, and MSCs on ischemic stroke patients, so therapeutic strategy could be developed.

**Keywords:** ischemic stroke, microglia, fractalkine, mesenchymal stem cells

**DOI:** 10.15562/bmj.v5i1.201

**Cite This Article:** Herminawati, L., Wijaya, A., Arief, M., As'ad, S. 2016. Dynamics of Human Mesenchymal Stem Cells, M1 Microglia/Macrophage, and Fractalkine in Ischemic Stroke Patients. *Bali Medical Journal* 5(1). DOI:10.15562/bmj.v5i1.201

### INTRODUCTION

Stroke is one of the Non-Communicable Diseases (NCDs), that remains the third leading cause of death and the leading cause of severe disability in the United States, Europe and Asia, both in young and elderly people.<sup>1</sup> Ischemic stroke is 85 % of all stroke incidence. Tissue death caused by ischemia due to occlusion of cerebral artery lead to brain inflammation, followed by breakdown of blood-brain barrier and infiltration of leucocytes. Microglia, the resident macrophages of the CNS parenchyma, is sensitive to brain injury and disease, altering their morphology and phenotype into M1 and M2 phenotypes, which have been linked to functional properties including production of inflammation association molecules, phagocytic activity, and neuro-protective effect.<sup>2,3</sup>

Fractalkine is expressed at high levels by neurons, mostly in forebrain structure, and suppresses microglial activation through its microglial receptor CX3CR1 which expressed by microglia.<sup>4</sup>

Disrupted neuron-microglia communication system caused by neuron injury will released fractalkine and attract inflammation molecules to injury site.<sup>5</sup>

Mesenchymal stem cells (MSCs), as guardians of inflammation,<sup>6</sup> significantly increase microglial expression and release of molecules associated with a neuroprotective phenotype such as CX3CR1. Through the release of CX3CL1, MSCs are modulating constitutive “calming” receptors, typically expressed by “steady-state microglia”, thus switching microglia from a detrimental phenotype to a neuroprotective one.<sup>7</sup> This study investigates the dynamics of human mesenchymal stem cells, fractalkine, and M1 microglia /macrophage in ischemic stroke patients.

### MATERIALS AND METHODS

#### Research Subjects

The subjects were part of a cohort study about ischemic stroke in The Central Hospital of Army (RSPAD) Gatot Soebroto Jakarta. Subjects were patients that was at least 30 years old. Cerebral infarction was diagnosed based on medical history, neurological examination, and brain MRI. All subtypes of ischemic stroke were included. Individuals with ongoing therapy for malignancy, known history of brain tumor, and those taking immunomodulatory drugs were excluded. According to the onset of stroke, patients were

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subdivided into 3 groups: A group with onset stroke < 7 days, B group with onset stroke 7 to 14 days, and C group with onset stroke 15 to 30 days. Blood sample were taken as soon as possible when patients admitted to hospital. The study was approved by ethics committee of Faculty of Medicine, Hasanuddin University, Makassar, Indonesia (approval number: UH14110581).

### Clinical Data and Laboratory Studies

For every patient, demographic and clinical data such as risk factors (hypertension, diabetes mellitus, and lipid dysregulation) were obtained. Fractalkine (FKN/CX3CL1) levels were determined in EDTA plasma using ELISA kits (R&D Systems, Inc., Minneapolis, MN, USA). The sensitivity of the assay was 0.018 ng/mL. Mesenchymal Stem Cells (CD105<sup>+</sup>/CD73<sup>+</sup>/CD90<sup>+</sup>/CD45<sup>-</sup>/CD34<sup>-</sup>) and M1 microglia/macrophage (CD45<sup>+</sup>/CD11b<sup>+</sup>) was quantified using flow cytometry (BD Biosciences, FacsCanto).

### RESULT

The mean age of the study population (n = 57) was 58 years (range, 38 to 77 years). For further general characteristic of markers level see Table 1. Between February 2015 and September 2015, 57 patients were included in this study, 12 patients (21%) are female and 45 patients (79%) are male. We found 63% of subjects had hypertension, 61% of patients had dyslipoproteinemia, and 33% of patients had diabetes as risk factor of ischemic stroke.

**Table 1.** General characteristic of subjects (n = 57)

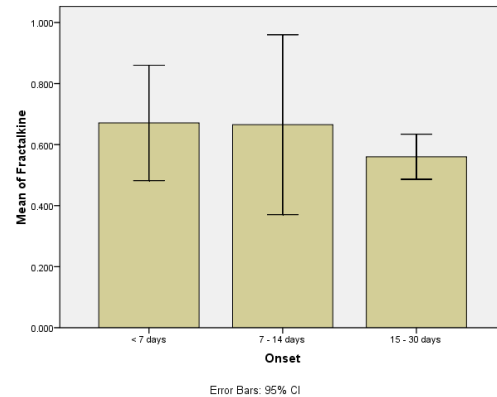
Variable	Min	Max	Mean ± SD
Age (year)	38	77	58 ± 10
CX3CL1 (ng/mL)	0.313	2.723	0.635 ± 0.403
M1 Microglia (10 <sup>3</sup> cells/mL)	10.0	66.2	37.6 ± 14.9
MSCs (10 <sup>3</sup> cells/mL)	13.0	65.6	22.8 ± 8.31

MSCs: Mesenchymal Stem Cells, CX3CL1: fractalkine

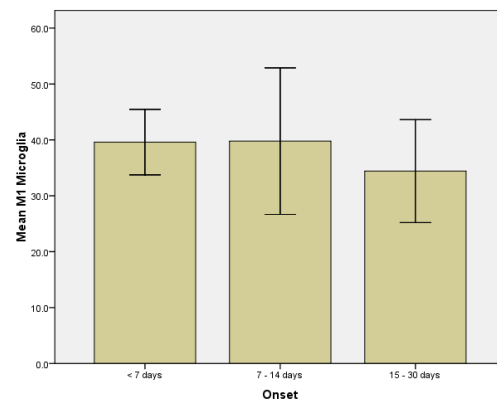
**Table 2.** Comparison of biomarkers among groups

Variables	Stroke Onset (days)		
	< 7 (n = 20)	7 - 14 (n = 17)	15 - 30 (n = 20)
CX3CL1 (ng/mL)	0.671 ± 0.367	0.665 ± 0.573	0.560 ± 0.133
M1 Microglia (10 <sup>3</sup> cells/mL)	39.6 ± 11.4	39.7 ± 17.1	34.4 ± 17.3
MSCs (10 <sup>3</sup> cells/mL)	22.9 ± 11.2	24.8 ± 4.34	21.6 ± 6.23

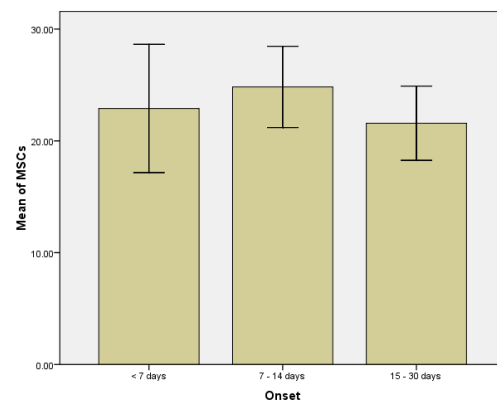
MSCs: Mesenchymal Stem Cells, CX3CL1: fractalkine



**Figure 1.** Graph of fractalkine levels in ischemic stroke patients.



**Figure 2.** Graph of M1 microglia/macrophage cells in ischemic stroke patients.



**Figure 3.** Graph of mesenchymal stem cells in ischemic stroke patients.

After neuronal death, loss of cell-to-cell interaction may promote microglial activation to M1 phenotype. We found the same profile of fractalkine and M1 microglia/macrophage cells on patients with stroke onset < 7 days and 7 – 14 days. They have almost the same fractalkine levels and M1 microglia/macrophage cells. Patients with onset

stroke 15 – 30 days have lower fractalkine levels and M1 microglia/macrophage cells (Figure 1 & 2).

Different model of MSCs, compared to fractalkine and M1 microglia/macrophage cells were found in this study. MSCs were increase in patients with stroke onset 7 – 14 days, then decrease in patients with stroke onset 15 – 30 days (Figure 3). A significant correlation was found between M1 microglia/macrophage cells and MCs ( $p = 0.002$ ) on patients with stroke onset 15 – 30 days.

## DISCUSSIONS

This study is a preliminary case report which also a part of cohort study to provide medical intervention and monitor the patients until 30 days after intervention. Ischemic stroke includes a series of events that began at the onset of ischemia and called as ischemia cascade. The length of time of each event and the overall time varies greatly, depending on many variables such as the size of infarction, onset and duration of ischemia and reperfusion effectiveness.<sup>8</sup> Brain ischemia causes failure of ion pumps, over accumulation of intracellular sodium and calcium, loss of membrane integrity, and necrotic cell death.

Fractalkine (CX3CL1), a cell surface-bound chemokine constitutively expressed by neurons, suppresses microglial activation through its microglial receptor CX3CR1. After neuronal death, loss of this cell-to-cell interaction will lead to microglial activation.<sup>9</sup>

In vitro experiment showed that M1 phenotype of microglia increased from day 3 and last up to 14 days after ischemia.<sup>10</sup> Correspond to this study, our results showed a constant fractalkine levels and M1 microglia cells until 14 days after stroke onset. M1 microglia/macrophages, which are characterized by reduced phagocytosis and increased secretion of pro-inflammatory mediators, begin to dominate the injured site and exacerbate neuronal demise. The role of MSCs as ‘guardian of inflammation’ against excessive inflammatory responses.<sup>6</sup>

Giunti et al.<sup>7</sup> reported that CX3CL1 led to the upregulation of neuroprotective gen such as CX3CR1 when LPS-activated microglia were cultured in the presence of MSCs. Correspond to these in vitro experiments, we found that increase of MSCs begin on 7 days after stroke ischemic and remained elevated for at least 14 days after ischemia. As fractalkine levels and M1 microglia/macrophage decrease, MSCs also decrease at 14 days after stroke onset. This finding will promote the research of MSCs as cell therapy at delayed time window.<sup>11</sup>

Fractalkine levels, M1 microglia/macrophage, and MSCs peaked by 14 days on ischemic stroke patients, then decreased until 30 days after stroke ischemic onset.

## CONCLUSIONS

This study found an interaction between microglia/macrophage, fractalkine, and MSCs on ischemic stroke patients, so therapeutic strategy could be developed. A deeper understanding of ischemic stroke pathophysiology should enable the development of medical intervention that are more effective and have a longer therapeutic time window.

## ACKNOWLEDGMENTS

We wish to thank Prodia StemCell Indonesia (ProSTEM), Prodia Research Laboratory, and Prodia Education and Research Institute (PERI) for their support of this study.

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