

Peer Review File

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Review Comments

Comment 1: However, authors didn't explain how this topic is related with palliative care in stroke, and it isn't really easy to link. Authors should consider highlighting the association with stroke mortality in poor LMC cases and, at least, try to develop about this topic in the discussion.

Reply 1: This is a very good advice as MCAO is really a devastating disease in spite of recent advances in treatment, especially for patients with poor LMCs status. Palliative care is needed in these patients. Therefore, in the section of discussion, we have highlighted the adverse neurological outcomes in poor LMC cases and tried to develop the topic of palliative care in the discussion (see Page 14-15, Line 285-298).

Changes in the text: We added the paragraph in the discussion:

“Last but not the least, in spite of recent advances in revascularization therapy at emergency, MCAO still frequently led to adverse neurological outcomes, especially in patients with poor LMCs status. Consistent with prior studies, our study revealed that poor LMCs patients had higher baseline NIHSS score, higher mortality rates and poorer functional outcomes. Additionally, successful recanalization was achieved more often in patients with good collateral system, even though our findings were not statistically significant. A physical trajectory for those patients with poor LMCs status has been proposed of sudden decline in functional status at stroke onset, and ending in death or survival with long-term disability. For this reason, in order to increase the quality of life, there is need for a comprehensive palliative care to provide psychosocial support, determination of patient-focused care objectives, and symptom management for these patients after stroke (23). More research and more palliative care specialists are needed in the future to better understand palliative care need of stroke patients and their families (24).”

Comment 2: Line 64: “disabling neurologic deficit, how much brain is salvageable”...

Authors might consider using “and” instead of a comma.

Reply 2: we have modified our text as advised (see Page 4, Line 67).

Changes in the text: “disabling neurologic deficit and how much brain is salvageable”

Comment 3: Line 76: “Therefore, this is an imprecise process...”

Authors must consider defining LMC grading as slightly imprecise or not totally precise.

Reply 3: we have modified our text as advised (see Page 4, Line 80).

Changes in the text: “Therefore, this is not a totally precise process based on information extracted only from radiologic assessment.”

Comment 4: Line 202: “Then, two subgroups of patients...”

Although it is evident, authors should specify which these subgroups are.

Reply 4: We have modified our text as advised (see Page 10, Line 209).

Changes in the text: “Then, two subgroups of poor LMCs and good LMCs were compared...”

Comment 5: The discussion is difficult to read and subsequently to understand, due to the presence of a very long paragraph that might be summarized and/or divided into smaller ones.

Reply 5: We have carefully reviewed our discussion section and made a major revision by summarizing and simplifying some contents, as well as by dividing long paragraph into smaller ones to make the discussion more easily to read and understand (see Page 12-14).

Changes in the text:

(1) We simplified the long paragraph of explanation of arteriogenesis to make it less confusing and more easily to understand (Page 12, Line 241-249). See as follows:

“Although there is no dispute about the importance of baseline LMCs status in setting of MCAO, questions regarding the potential causes for inter-individual variability in human leptomeningeal collaterals are, however, unanswered. Animal studies have been informative, reporting that several genetic factors controlling arteriogenesis such as vascular endothelial growth factor a (VEGFa), chloride intracellular channel 4 (Clic4) , Delta-like 4 (Dll4) and midkine impacted the extent of LMCs in ischemic disease through regulating endothelial cell function (1, 15, 20-22). However, studies exploring the determinants of LMCs status in humans have been limited.”

(2) We re-organized the discussion on the association between RGMA and leptomeningeal collaterals. In the meantime, we divided the long paragraph about

'RGMa' into smaller ones to make the discussion section more logic and more easily to read (Page 13-14, Line 257-284). See as follows:

“RGMa, which was originally identified as an axon repellent in the chick retinotectal system, has now emerged as a molecule which could regulate various functions in the developing and pathological CNS depending on its cellular and environmental context (12-13, 22). Previous studies reported that after inhibition of RGMa, angiogenesis, coupled with functional recovery was enhanced in a rat middle cerebral artery occlusion/reperfusion (MCAO/R) model, indicating that RGMa may play a negative role in angiogenesis after stroke (12). Furthermore, in vitro, the addition of recombinant RGMa significantly decreased the proliferation, migration, and tube formation of ECs, indicating that RGMa may induce endothelial dysfunction in ischemia (13-14). While endothelial function also plays a main role in leptomeningeal collateral formation (4, 7, 15-16), a role for RGMa in leptomeningeal arteriogenesis following ischemic stroke can be hypothesized.

Furthermore, accumulating discoveries have elucidated that VEGF signaling pathway is a key element in growth and development of new arteries. Recent animal studies have reported that the level of VEGF expression strongly influenced the formation of LMCs (1, 17, 20). Interestingly, Fujita Y, et al recently reported that RGMa treatment of human umbilical artery endothelial cells (HUAECs) decreased VEGF-induced phosphorylation of focal adhesion kinase (FAK) (13). Additionally, in animal models, after inhibition of RGMa, a significantly increased VEGF expression levels was detected (12). Zhang G et al. implicated that RGMa was involved in VEGF-induced angiogenesis and may be an antagonist of both endogenous and exogenous VEGF (14).

Therefore, given the findings that RGMa contributes to endothelial dysfunction, together with evidence for the role of RGMa in VEGF-mediated vessel formation, we speculated that RGMa might be involved in arteriogenesis by inducing endothelial dysfunction via VEGF singling pathways, and thus determined LMCs status in ischemic stroke. Subsequent work should be performed both in vivo and in vitro to further explore the underlying role and mechanism of RGMa in leptomeningeal arteriogenesis in AIS.”

Comment 6: Line 232: “In the event of MCAO, angiogenesis results in an overall increment of vessel resistance and is insufficient to improve tissue perfusion, only LMCs from arteriogenesis are capable of providing crucial blood flow to the penumbra”. Authors sustain a crucial role in arteriogenesis as the only factor implicated in LMC status. This statement cannot be supported by the referenced articles in this study or any other. There are many factors that can strongly influence LMC status and function. Therefore, I recommend this paragraph to be reviewed.

Reply 6: We have carefully reviewed this paragraph and found our description was confusing. Therefore, we have modified the first paragraph in discussion as advised

(see Page 12, Line 236-249).

Changes in the text: “Penumbral ‘life expectancy’ in ischemic stroke is greatly dependent on the status of collateral circulation, of which the circle of Willis and leptomeningeal anastomoses are typical intracranial collateral arteries in humans (1-2). In the event of MCAO, the circle of Willis is no longer able to contribute collateral blood supply and LMCs provides crucial nutritional support to the penumbra by contributing to retrograde filling of pial arteries distal to an occlusion (3-4). Although there is no dispute about the importance of baseline LMCs status in setting of MCAO, questions regarding the potential causes for inter-individual variability in human leptomeningeal collaterals are, however, unanswered. Animal studies have been informative, reporting that several genetic factors controlling arteriogenesis such as vascular endothelial growth factor a (VEGFa), chloride intracellular channel 4 (Clic4) , Delta-like 4 (Dll4) and midkine impacted the extent of LMCs in ischemic disease through regulating endothelial cell function (1, 15, 20-22). However, studies exploring the determinants of LMCs status in humans have been limited.”

Comment 7: Authors might consider showing LMC in MCA territory at ganglionar level (upper).

Reply 7: This is a very good advice. Actually, we chose one of the CTA images based on rLMC score system. However, it is true that only one level image can not show the whole picture of LMC in MCA territory. Therefore, we added an image on upper level (ganglionar level) as advised (see Figure 1A, 1B).

Changes in the text:

