Reviewer's report

Title: Circulating miR-30a, miR-126 and let-7b as biomarker for ischemic stroke in humans

Version: 1 Date: 9 September 2013

Reviewer: Olof Gidlof

Reviewer's report:

Major Compulsory Revisions

1. The rationale behind the selection of miRNAs analyzed in this manuscript is a little unclear. As the authors themselves state, miR-126 has already been investigated as a biomarker in ischemic stroke (Tan et al 2009), limiting the novelty of this study. If the reason to chose miR-126 was to confirm the results of Tan et al, this needs to be more clearly stated. The reason for choosing let-7b is also unclear. One study shows that it is involved in neurodegeneration, but that has little bearing on the pathophysiology of ischemic stroke.

2. Inclusion/exclusion criteria when enrolling patients/controls must be included in the methods section.

3. It is unclear whether blood samples were drawn from the same patients at the different time points or if each time point is represented by a different set of patients. If the latter is the case, then that constitutes a major flaw in the experimental design. Could the authors please clarify this in the methods section.

4. U6 RNA was used for normalization of qPCR data. To my knowledge, it is unclear whether U6 RNA is suitable as a house keeping gene in the context of ischemic stroke. Could the authors please provide evidence for the stability of U6 RNA levels over the time course and between patients and controls?

5. The authors show that the three miRNAs under investigation can accurately distinguish between healthy controls and ischemic stroke patients. However, the clinical problem is not whether an ischemic stroke can be distinguished from a healthy individual but whether an ischemic stroke can be distinguished from conditions that can be mistaken for stroke, such as hemorrhagic stroke, seizure or migraine. In order to claim that these miRNAs might be clinically useful biomarkers, the authors should compare ischemic stroke patients with these kind of patient groups as well.

Minor Essential Revisions

1. It would be interesting if the authors could speculate on why the miRNA levels decrease after ischemic stroke. If the miRNA is removed from the circulation, where does it end up?

2. The authors should mention the paper by Gan et al 2012, which shows that miR-145 is increased in circulation after ischemic stroke patients.
3. In the discussion, the authors state "we identified that circulating miRNAs were associated with human AMI". It would be advisable to additionally mention at least some of the many other studies which have showed this.

Discretionary Revisions

1. It would be interesting to see if these miRNAs also have any prognostic value or reflect the extent of brain damage following the ischemic stroke.

**Level of interest:** An article of limited interest

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests