Author's response to reviews

Title: Patients with migraine with aura have increased flow mediated dilation

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Author's response to reviews: see over
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Dear Sir,

we would like you to consider the revised version of manuscript entitled: “Patients with migraine with aura have an increased flow mediated dilatation” MS: 1521809529278321 which my coauthors and I are going to submit for publication in BMC Neurology as Original Communication.

We revised our manuscript in light of the reviewers’ comments and made any required changes to the format of our paper. We gave a point-by-point response to the concerns that you’ll find below.

Hoping that the manuscript will be now suitable for publication, I send you my best regards.

Yours faithfully

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Referee 1:

Major Compulsory Revisions

Methods

1. Sensitivity and specificity of the measurement of forearm blood flow and changes induced by flow mediated dilation should be reported. Are there validation studies on the method which have been already published?

A: Although the assessment of brachial artery reactivity is technically challenging and it is not available a gold standard for the measurement of endothelial function, an expert in ultrasonography trained for several months is believed to ensure high quality data (19). Robust evidence of sensitivity and specificity of the measurement of forearm blood flow and changes induced by flow mediated dilation is still lacking and, as far as we know, each paper investigating FMD refers to the Guidelines of the International Brachial Artery Reactivity Task Force by Corretti et al., 2002. A new automated method of FMD quantification based on B-mode echographic images and edge detection algorithms has been recently proposed to reduce the within-reading and within-subject FMD% coefficients of variation in order to improve manual measurements (Craiem et al, 2008), but this new approach needs to be validated.

2. Differences in methodological approach from similar studies should be discussed in discussion section.

A: In the first version of the manuscript we discussed in the in Discussion section the main differences between our study and those previously published. In particular, the main distinction consists in the probe frequency. We used a 14 Hz probe which allows for a better definition of vessel walls and thus to a more accurate measurement of FMD. We modified the text to better outline this and other minor differences (page 9, line 3).

Statistical analysis

3. According to the method chosen, the distribution of FMD values is not normal. Why the authors did not chose the non-parametric median test considering that they reported in results section that FMD was over the median value (19%) in 23.1% of controls, 45.5% of MwoA patients and 90.0% of MwA patients?

A: We used the general linear model (GLM) approach (page 6, 1st paragraph) because it allowed to adjust the analysis for age and gender. Although the distribution of FMD was not normal, the departure from normality was not dramatic (skewness: 1.29), and the
least-square estimation of the significance levels are good approximation even in cases of violation of the normality assumption. The non-parametric test confirmed the difference shown by the GLM approach (median test P<0.0001). It should be noted that the dichotomization of the FMD using the median value was performed to obtain an estimate of the risk of having migraine in groups with higher/lower FMD, which is an information useful in clinical practice.

Discussion

4. How the authors do explain the quantitative difference in flow mediated dilation between MwA and MwoA patients? In changes in FMD is expressive of an hypersensitivity to EDNO, why this increased sensitivity is greater in MwA patients? These latter often have a painful phase less severe than that occurring during the ictal phase of patients with MwoA.

5. Could this greater increase be related to the aura phase? Is there experimental evidence for this also using other methodological approaches?

A: we agree with the reviewer that MwoA and MwA are two different clinical entities mainly, but not only, for the fact that the latter is preceded by aura phase. In the aura phenomenon cerebral blood flow undergoes a sequence of changes, with vasodilation preceding oligoaemia and the development of neurological symptoms, peculiar of MwA. “Spreading oligoaemia” is then followed by prolonged depression of cortical neuronal activity, defined as cortical spreading depression. So, cerebral arteries in MwA patients, differently from MwoA ones and controls, undergo to peculiar hemodynamic changes and this process is probably characterized by particular mechanisms of response. On this account, previous studies investigating cerebral blood flow by transcranial Doppler and also by near infrared spectroscopy, including one by our group (14), demonstrated that patients with MwA in the interictal period have an increased cerebral vasomotor reactivity to hypercapnia compared with MwoA patients and controls. Different studies suggested that this response could be related to an autonomic dysfunction leading to over-sensitivity of cerebral vessels to NO as well as to other chemical stimuli, such as CO2, i.e. in the case of increase of vasomotor reactivity. Our study results confirm that MwA patients’ arteries could be peculiarly characterized by a super-sensitivity to chemical and physical stimuli throughout the whole arterial system.

6. The authors asserted that excessive response to hyperemia could reflect similar changes in the cerebral circulation. Is there evidence for this using transcranial Doppler or echodoppler of carotid and vertebro-basilar vessels?

A: Flow mediated dilation investigation is typically assessed by measuring the change in brachial artery diameter after reactive hyperemia, compared with baseline measurements. There are a lot of studies by means of transcranial Doppler demonstrating that patients with MwA have an alteration of cerebral hemodynamics after hypercapnic stimulus, measuring from middle cerebral artery and/or basilar one. No study explored cerebral hemodynamic changes after hyperemia so far, thus there are not standardized methods to do that. This is mainly due for safety reason. In fact inducing hyperemia is potentially dangerous for cerebral circulation.
7. Methodological differences should be mentioned between this study and other(s) using similar methodology.

In our study, measurements of FMD were performed according to standardized guidelines. However, as we reported in methods section: ‘FMD depends on the shear stress on the blood vessels, that is directly related to the velocity and the viscosity of the blood but inversely related to the vessel diameter. Vessels with different diameters may have the same flow but substantially different levels of shear stress and thus a different degree of stimuli for FMD. In other words, FMD values derived from subjects with a comparable endothelial function but with different vessel diameters may result dissimilar. To avoid this bias, FMD raw values need to be corrected for flow velocity and diameter. A shear rate was then estimated as velocity divided by diameter. Peak shear rate, estimated as peak flow velocity divided by baseline diameter, was calculated to quantify the FMD stimulus in each subject. FMD responses were normalized by dividing the maximal percentage change in diameter by the peak shear rate’. However, our results did not change when we analyzed the FMD normalized for the peak shear rate with respect with the FMD raw ones. Another important difference in terms of methodology with respect to other studies is that we used an ultrasound probe with a frequency of 14MHz, higher than that used in other studies (7.5 -10 MHz). As we stated in Discussion section higher frequency probes have a better imaging resolution than lower ones, especially when evaluating superficial vessels, i.e. the brachial artery, and allow for a more precise detection of minimal calliper variation, as it is that resulting from a FMD evaluation (see above).

Results

8. They are clearly reported. Median test analysis results are lacking.

A: according to reviewer’s criticism, we added the sentence: ‘The median test confirmed the result (P<0.001)(page 6, Results section, 2nd paragraph).

Discretionary Revisions

Subject section

9. The time from the last and next attack should be specified.

A: Patients were headache-free for at least 15 days at the moment of the examination. No patient had a migraine attack in the five days subsequent the examination. We modified the text accordingly (page 4, last paragraph).
Referee 2:

Major Compulsory Revisions

Specific comments:
1. FMD is a non-invasive but not an easy technique. Its major limitation is the reproducibility, which is optimized by the use of automatic edge detection systems to measure changes in diameter. Even using this method the sample size would be inadequate. Indeed, coefficient of variation for repeated measures of FMD over time in the lab as well as a formal calculation of the power of the study should be reported.

A: the reviewer correctly points out the fact that FMD measurements have a wide variability, and this is of importance with respect to the power of the study. To make things worse from this point of view, our sample is relatively small. Since statistical power is a linear function of the probability of making a type II error (i.e. incorrectly fail to reject the “null” hypothesis), it is of concern in negative studies. When, as in the case of our study, the differences found are statistically significant, by definition the power is adequate. In fact, the power calculation performed on the basis of the data at hand yielded a value of 84%.

2. Results should be discussed also considering separately FMD as percentage in diameter, together with data on shear rate (baseline and peak) estimation (see table 1).

A: in the revised version of the paper according to the reviewer’s criticism we added a sentence in Discussion section (page 8, last paragraph). We have found no differences in our results after this adjustment, and therefore we had not previously discussed this issue.

The degree of vasodilation to ischemia is indeed quite high in all the groups (even in controls) as compared to the literature, raising some questions about accuracy in FMD measurement. Have the authors alternative explanation for this issue?

A: In our opinion, our finding of a higher vasodilation to ischemia than that reported in other studies is due to the use of a higher frequency (14 Mhz) probe than that utilized in literature; so we were able to detect a more precise detection of minimal calliper variation, resulting in higher values respect to other studies.

Which is the mechanism by which a lower shear rate (the stimulus) can result in a greater FMD? Accordingly, why should FMD be adjusted for peak shear rate?

The rationale for adjusting FMD for peak shear rate is reported in the Methods (page 5, 2nd paragraph). As there is no significant difference in diameter among the three groups investigated, the adjustment for peak shear stress is necessary not to underestimate FMD increase. In our study MwA patient presented a higher FMD not because of a lower shear rate but despite of a lower shear rate.
3. The authors cannot raise any conclusion concerning a possible increased sensitivity to endothelium-derived NO in absence of a control experiment with an endothelium-independent NO donor, namely GTN. Low dose GTN (e.g. 25 mcg) could be safely administrated to the study’s patients. This issue is only partially but not substantially reported in the discussion.

A: We highlighted this limitation of our study in the discussion section (page 9, last paragraph).

4. Controls should be better matched (age, gender) with patients. Details on confounders (smoking habits, blood pressure, plasma glucose and cholesterol) should be presented.

A: The demographic differences between groups could actually bias our results, but since this was a convenient sample, a better matching was not possible. This is why we used statistical methods to adjust for age and gender. We added the information on smoking, hypertension, and dyslipidemia in the results (Results section, first paragraph). Unfortunately, we have no information on blood glucose levels, but none of the participants had an established diagnosis of diabetes mellitus.

Minor Essential Revisions
Introduction: trinitrat should be trinitrate;

A: We changed the text accordingly (Introduction section, line 9).

reference 14 does not refers to migraine.

A: Reference 14 does refer to Migraine. The referee probably meant to outline that it did not refer to FMD but to cerebral VMR assessment in migraine patients. We corrected the mistake.