Author’s response to reviews

Title: Endothelial function in migraine: a cross-sectional study

Authors:

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Author’s response to reviews: see over
Detailed response to reviewers

Response to review by Franco Granella:

Major compulsory revisions:
None

Minor essential revisions:
1. The paper of reference 39, quoted as in press, has already been published.
   Response: This reference (nr 34 in the revised manuscript) has been corrected.

Discretionary revisions:
2. The Authors should quote and discuss the recent paper by Silva et al. (Headache 2007), in which no alterations of endothelial function were found by measuring forearm flow-mediated vasodilation.
   Response: This paper is discussed in the revised manuscript (reference nr 35).
Response to review by David Newby:

Major compulsory revisions:

1. The estimation of tissue plasminogen activator (t-PA) release is incorrect. There is no correction for the plasma fraction and the investigators need to know the subjects’ hematocrit (see ATVB 2005;25:2470-9 for review of the issues). Obviously t-PA is measured in plasma not whole blood. The release of t-PA should be the arteriovenous plasma concentration gradient multiplied by the plasma flow (not blood flow).

Response: The estimation of t-PA release has been corrected for the plasma fraction using the subjects’ hematocrit (methods, data analysis and statistics section, page 8-9 and results, page 10)

2. The arguments against measuring t-PA activity have limitations. The authors are correct in that they often track in parallel to antigen concentrations but at different gradients. There is no measurement of plasminogen activator inhibitor type 1 (PAI-1). This will inhibit t-PA activity. Thus if migraine sufferers have a persistently elevated PAI-1 concentration, then t-PA activity may be greatly reduced even if t-PA antigen release is normal.

Response: We addressed this issue as a limitation of our study in the discussion, page 14.

3. For the main variables, the mean differences between patients and controls should be provided along with 95% confidence intervals. This will permit some assessment of how big an effect could have been missed.
Response: 95% confidence intervals for the main variables (FBF responses) are provided in the revised manuscript (methods, data analysis and statistics section, page 9 and results, page 10).

4. L-NMMA takes more than 6 minutes to achieve its maximal inhibitory effect. It also takes a prolonged time to washout and the offset of action is longer than 12 minutes. How often was it infused before substance P or sodium nitroprusside?

Response: To our knowledge, the effect of L-NMMA on FBF in the doses used in our study reaches a plateau by 5 min (Vallance et al. Lancet 1989 336: 1589-9). In all experiments, L-NMMA was infused at the end of the experiment (Figure 1). Therefore the FBF responses to SNP or Substance P were not influenced by an ongoing effect of L-NMMA.

Minor essential revisions:

5. Why are the blood flow data (Figure 3) for L-NMMA given as percentage change in ratio rather than absolute blood flow (c.f. Figure 2; substance P and sodium nitroprusside)?

Response: The way vasodilator or vasoconstrictor FBF responses are expressed effects their variability. This issue is discussed in detail in the Discussion section of Vanmolkot et al. Br J Clin Pharmacol 2005 59: 387-97, to which we refer in the revised manuscript (methods, data analysis and statistics section, page 8, reference nr 20).

6. The full dose response to substance P should be provided in Figure 4.

Response: As an exploratory analysis, plasma t-PA was only assessed at baseline and at the end of infusion of the highest dose of Substance P (Figure 1). Therefore, we cannot provide a full dose response. To limit the number of figures,
Figure 4 was removed from the revised manuscript and results are given in text format (Results, page 10)
Response to review by Paola Sarchielli:

a) Answers to general questions (point 1 of your guideline)

6. Do the title and abstract accurately convey what has been found?

Reply: yes Why has the cross-sectional nature of the study been emphasized in the title, if this nature is not mentioned nor discussed in the text?

Response: This is advocated by the journal. The cross-sectional design should be clear to the reader after reading the Methods section.

b) Detailed concerns and suggestions

Abstract

Change the first sentence of the Background as follows: “Migraine has been associated with an increased risk of cardio and cerebrovascular diseases”.

Response: We believe that the term “cardiovascular” covers all types of vascular disorders, including cerebrovascular disorders, and is widely used as such in the medical literature.

Introduction

The sentence regarding the association between migraine and cardiovascular disease should be rewritten: the relation between migraine (with and without aura) and cardiovascular diseases should be
better explained and related references reported. Are only data available for the vasospastic disorder variant angina? For which migraine subtype?

**Response:** The sentence has been rewritten. The relation between migraine subtypes (with or without aura) and cardiovascular disorders is discussed in more detail in the Discussion, with related references (nr 4 and 38). Two examples of vasospastic disorders associated with migraine (variant angina and primary Raynaud disease) are referred to (refs nr 5 and 6). Unfortunately, no distinction was made between migraine with and without aura in these case-control studies.

I suggest to put “Moreover “before sentence “ Migraine with aura is a risk factor for ischemic stroke in the young” (eliminate in particular) because stroke is a cerebrovascular not a cardiovascular disorder.

**Response:** We believe that the term “cardiovascular” covers all types of vascular disorders, including cerebrovascular disorders, and is widely used as such in the medical literature.

A sentence for migraine without aura and cerebrovascular risk should be written briefly reporting epidemiological and clinical evidence as well as related references.

**Response:** The relation between migraine subtypes (with or without aura) and cardiovascular disorders is discussed in more detail in the Discussion, with related references (nr 4 and 38).

Based on the above, the sentence “ The underlying mechanisms ……”
should be modified as follows: “The underlying mechanisms for the
association between migraine and cardio and cerebrovascular disorders
are currently unknown.

Response: We believe that the term “cardiovascular” covers all types of vascular
disorders, including cerebrovascular disorders, and is widely used as such in the
medical literature.

Methods

Why did the authors choose a peripheral resistance vasculature for in
vivo measuring of stimulated endothelial NO release, basal endothelial
NO release and stimulated endothelial t-PA release? Is this a better model for
studying endothelial dysfunctioning than that used in the past by the same authors?
Using the previous model, the authors found a difference in the
flow-mediated dilatation of peripheral conduit arteries between migraine
with and without aura patients compared with controls, like in previous
studies of other groups.

Why have different vascular beds (resistance arteries vs conduit
arteries), different methodology (plethysmography vs ultrasound) and
different applied stimulus (pharmacologic agonists vs shear stress)
been chosen for this research? Is this an improved method to detect
endothelial dysfunctioning in migraineurs?

Response: The human forearm model is a classical model for evaluating endothelial
function. The model has some advantages over brachial artery flow-mediated
dilation: different aspects of endothelial function (endothelial NO release, fibrinolytic
capacity) can be evaluated and measurements are less variable compared to brachial flow-mediated dilation (FMD). An important difference is the difference in vascular bed studied: small resistance vessels (human forearm model) versus a large conduit artery (FMD). Therefore, use of the forearm model may expand our knowledge concerning the association between migraine and endothelial function.

How do the invasive nature of the forearm model and its complexity influence data, independently from the limited number of patients included into the study?

Response: We only intended to explain to the reader the reasons why a relatively limited number of patients was included, which may turn influence the power of the study. To avoid misunderstanding, the sentence has been rewritten (page 14).

Small variations in the variables assessed could be undetectable between patients and controls and between different migraine patients subtypes.

Response: This issue is discussed as a limitation of our study in the discussion (page 14).

Patients

The new ICHD-II classification should be reported not the old (1988) and the relative reference replaced.

Response: Reference was made to the updated ICHD classification (ref nr 14).

Patients were all assessed during the interictal period. Time lag from
the previous and next attack should be specified.

**Response:** Exact time lag from the previous and next attack was not recorded. However, as specified in the Methods, Experimental conditions section, page 5, patients were headache free for at least 72 hours before the start of measurements. If a migraine attack ensued within 24 hours after the visit (ascertained by telephonic interview), measurements were repeated in another headache-free period. The experiment was repeated in 1 patient because an attack started during the first experiment.

Plasma t-PA concentration

Was it determined using commercially available kits? If yes, company, city and country should be reported. Detection limits, linearity range, intra and intraassay CV and reproducibility should be provided.

**Response:** These important test characteristics are provided in the article by Holvoet et al., to which we refer in our manuscript (ref nr 19).

Results section

No mention has been made to differences between migraine with and without aura for all variables assessed. A sentence in this regard should be written.

**Response:** We added a sentence in this regard (Results section, page 10).

Discussion

Rewrite the sentence at the bottom of page 12 as follows:

“The response to L-NMMDA did not differ between migraine patients and
control subjects, indicating that the basal endothelial release of NO from peripheral resistance vessels is unaltered in migraineurs. This does not exclude that basal NO release from cranial resistance and perhaps conduit vessels is altered in the same patients.”

**Response:** This issue is addressed as a limitation of the study in the Discussion section, page 14.

Rewrite also the sentence at the top of page 13 as follow:

“Our findings suggest that, if basal NO production is indeed increased in migraine patients other NO synthases than endothelial NOS in peripheral circulation are implicated”.

**Response:** We have rewritten the sentence as suggested (Discussion section, page 11).

It cannot be excluded that variation in endothelial function can differ in relation to the time lag from the attack. Patients in this study were all assessed in the interictal period. A sentence in this regard should be written.

**Response:** We added this issue to the discussion, as suggested (Discussion section, page 11).

Better clarify the sentence: Previous work in migraine patients suggest abnormalities of haemostasis including abnormal platelet function (which abnormalities?) antiphospholipid antibodies and congenital thrombophilia (in particular migraine subgroups). Epidemiological and
clinical evidence should be reported.

**Response:** To limit the length of our manuscript, we would like to refer the reader for more details on this topic to the review article by Crassard et al. we refer to (ref nr 36).

The assertion “…leading to an increased thrombotic risk “is too marked.

**Response:** This sentence was rewritten and this assertion is now less marked (page 13).

The authors should explain if there are differences between patients with and without aura and if there are particular migraine patients subpopulations with haemostasis abnormalities.

**Response:** To limit the length of our manuscript, we would like to refer the reader for more details on this topic to the review article by Crassard et al. we refer to (ref nr 36).

How do they explain differences found in previous studies in flow-mediated dilation between migraineurs and controls?

How do these findings reconcile with the negative findings of this paper in the light of migraine pathophysiological mechanisms?

**Response:** A substantial part of the discussion section (page 11-13) has been rewritten to discuss in detail the differences between our findings and those found in previous studies.

The authors asserted that consistent findings between peripheral and
coronary circulation support the notion that the forearm model is a reasonable surrogate for coronary circulation.

Can this be asserted for the forearm model with regard to the cranial circulation? Only if this is true, the peripheral forearm model can be assumed as an informative surrogate for studying endothelial disfunctioning of cranial circulation in migraineurs.

**Response:** This cannot be asserted with regard to the cranial circulation. This issue is added as a limitation of our study to the Discussion section (page 14)