Author's response to reviews

Title: Increased plasma soluble endoglin levels as an indicator of cardiovascular alterations in hypertensive and diabetic patients

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Author's response to reviews: see over
Dear Dr. Aulakh:

Thank you very much for your mail regarding our above referenced manuscript. According to your instructions we are submitting a new version of the manuscript, which addresses all the specific comments from the reviewers. We consider that these comments have much improved our manuscript, and we hope that you consider that the present version is appropriate for publication in BMC Medicine.

We include a detailed response to all the reviewers’ comments.

Yours sincerely,

Carlos Martinez-Salgado.

Reviewer: S. Ananth S. Karumanchi

Reviewer’s report:

This paper evaluates the role of soluble endoglin as a biomaker for cardiovascular alterations. I have the following comments for the authors to consider.

1. Plasma endoglin should be changed to plasma soluble endoglin. Endoglin is a cell surface protein and is not present in plasma. Only the soluble form is present in the circulation.

We agree with the reviewer, and plasma endoglin has been changed to plasma soluble endoglin throughout the manuscript.

2. Strength of association for soluble endoglin levels (for example in retinopathy)
could be tested for example with an odds ratio so the findings presented are more meaningful to clinicians.

According to the reviewer suggestion, we have tested the strength of association between soluble endoglin and retinopathy with an odds ratio, and these data are now in the results section of the manuscript, considering that values above 5 ng/mL endoglin are pathological, as either diabetic patients (odds ratio: 4.72) or hypertensive-diabetic patients (odds ratio: 3.54) categorised as grade III and grade IV in the Keith-Wagener retinal changes classification showed endoglin levels above this value.

3. What about relationship between soluble endoglin and renal dysfunction?

As we indicated in the methods section, we analyzed the relationship between plasma levels of soluble endoglin and renal dysfunction, assessed by measuring plasma creatinine concentrations, glomerular filtration rate (estimated by Chronic Kidney Disease Epidemiology Collaboration - CKD-EPI - and the Modification of Diet in Renal Disease-Isotopic Dilution Mass Spectrometry - MDRD-IDMS – formulas, and proteinuria was assessed by the albumin/creatinine ratio (following the 2007 European Society of Hypertension / European Society of Cardiology Guidelines criteria). However, in our study we have not found any significant relationship or correlation between soluble endoglin and renal dysfunction. Thus, we have not included those data in the results section of the manuscript.

4. In the discussion, a discussion on cardiovascular disease amongst patients with HHT would be nice. In patients, with HHT where they have one allele of endoglin mutated, the soluble endoglin levels would theoretically be low - what is known about cardiovascular disease and retinopathy in this population.

We agree with the reviewer, and the next paragraph about cardiovascular disease in HHT patients has been included in the discussion section of the manuscript.

The relevance of endoglin in the cardiovascular system is reflected by the fact that mutations in the endoglin gene causes a vascular disease called the Rendu-Osler-Weber syndrome or hereditary hemorrhagic telangiectasia Type 1 (HHT1) [McAllister et al., Nat Genet 1994, 8:345-351], which is characterized by vascular dysplasia, frequent episodes of epistaxis, mucocutaneous telangiectases, and arteriovenous malformations of the lung, brain, liver, and gastrointestinal tract [Govani and Shovlin, Eur J Hum Genet 17: 860–871, 2009]. A reduction in Sol-endoglin levels has been recently described in HHT-1 patients [Ojeda-Fernandez et al., Clin Chim Acta. 2010 Apr 2;411(7-8):494-9]. About 15-35% of HHT patients show pulmonary arteriovenous malformations, 10% have cerebral arteriovenous malformations, 25-33% suffer significant gastrointestinal blood loss from gastrointestinal tract telangiectasia, and an unknown but high percentage have liver involvement. In summary, 10% of HHT patients die prematurely or suffer major disability, largely because of bleeding or paradoxical embolization through pulmonary or cerebral arteriovenous malformations [Brady et al., Ir J Med Sci. 2009 Jun;178(2):135-46]. Ocular
manifestations include conjunctival, retinal and choroidal telangiectasia [Geisthoff et al., Graefes Arch Clin Exp Ophthalmol. 2007 Aug;245(8):1141-4]. Although it has been recently shown that the loss of endoglin leads to retinal vascular abnormalities in mice [Mahmoud et al., Circ Res. 2010 Apr 30;106(8):1425-33], the frequency of ocular manifestation in HHT patients is estimated as of 1.9% for retinal involvement [Geisthoff et al., Graefes Arch Clin Exp Ophthalmol. 2007 Aug;245(8):1141-4]. The prevalence of cardiovascular disease other than the above mentioned in HHT-1 patients does not seem to be higher than in the general population, when adjusted by other endoglin-independent risks factors [Govani and Shovlin, Eur J Hum Genet 17: 860–871, 2009]. Nevertheless, the results obtained in our study seem to suggest that S-endoglin levels are associated with cardiovascular damage, which does not mean that they are themselves the cause of such damage.

Reviewer: Michael Shechter

Reviewer's report:

Major Compulsory Revisions

1. What was the exact definition of “healthy”? The inclusion/exclusion criteria should be more comprehensive. How were the patients enrolled in the study? What were the criteria for “diabetes”, “hypertension”? This should be clarified in the “Methods” section.

We have considered healthy patients those who were not diagnosed with hypertension or diabetes, who were not taking antihypertensive or antidiabetic drugs, and at the time of inclusion in the study did not meet criteria for either diagnosis.

Hypertension was diagnosed when the mean of three separate measurements over time of blood pressure was ≥ 140 mmHg for systolic blood pressure (SBP) and / or ≥ 90 mmHg for diastolic blood pressure (DBP). At each measurement, blood pressure was measured at least twice, separated by more than 1 minute, as it is recommended by The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension and of the European Society of Cardiology (Mancia et al., J Hypertens 2007, 25:1105-1187).

Diabetes was diagnosed when fasting glucose was ≥ 126 mg / dL or ≥ 200 mg / dL 2 hours after oral glucose overload (repeated on 2 occasions) or after detection of symptoms of diabetes and random blood glucose ≥ 200 mg / dL, as it was recommended by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (Genuith et al., Diabetes Care 2003, 26:3160-3167).

All this criteria have been also included in the Methods section of the manuscript.

2. No data is provided regarding baseline characteristics which could impact the endothelial function results, such as concomitant medications in all the groups.
(including the “healthy” controls, time of the day when the endothelial function were assessed, fasting blood glucose (also in the control group), white blood cell count, etc. In addition physical examination parameters such as blood pressure, heart rate, body temperature, body mass index, etc. which could potentially impact the endothelial function results.

Drug therapies in all groups (except healthy controls) are described in table 1; controls did not receive any antihypertensive or anti-diabetic medication, as well as any medication that could potentially modify the endothelial function. Endothelial function was tested in every patient in the morning (between 8:30 and 11:00 AM), as it is stated in the new version of the manuscript. Fasting blood glucose (basal glycaemia) in all groups is indicated in table 2. White blood cell count has been also included in table 2 in the new version of the manuscript. Blood pressure, heart rate and body mass index are also indicated in table 2. Body temperature is not indicated in table 2, because all analyzed patients showed similar normal values of body temperature.

3. The endothelial function assessment using in this study needs a better clarification. At what time after/before blood withdrawn? Who performed the endothelial function tests? Was the operator blinded to the patients’ clinical data?

Endothelial function and blood withdrawn were performed in different days, as in the Primary Care Health Centres involved in the study blood samples were obtained every Friday (between 8:30 and 9:00 PM), and the assessment of pressure wave velocity was performed one or two days before (every Wednesday or Thursday). Every endothelial function test was performed in a blind basis by the same trained researcher (José I. Recio-Rodriguez), who was completely unaware of the health status of the patients analyzed. This information is now included in the Methods section of the new version of the manuscript.

4. What was the power calculation for this project?

The power of our study is 0.95. Given that our sample size is high (288 patients), that our variability is low and the α value we have selected (0.05), we obtain a β value of 0.05, which makes our study powerful enough to detect most of the relationships between endoglin and the other variables. The high power of our study shows that our results are statistically consistent. This has been indicated in this new version of the manuscript in the “Statistical analysis” subsection of the “Methods” section.
POINT-BY-POINT DESCRIPTION OF THE CHANGES MADE IN THE MANUSCRIPT.

All changes are marked in blue in this new version of the manuscript.

Page 1, line 1: “Increased plasma endoglin” in the title has been changed to “Increased plasma soluble endoglin”

Page 2, line 5: “plasma levels of endoglin” has been changed to “plasma levels of soluble endoglin”

Page 2, line 10: “the relationship of endoglin plasma levels” has been changed to “the relationship of soluble endoglin plasma levels”

Page 2, line 24: “between plasma endoglin” has been changed to “between plasma soluble endoglin”

Page 7, line 5: There is a new paragraph with a new bibliographic reference, explaining the criteria for the diagnosis of hypertension and diabetes. Moreover, reference 33 is now reference 26. Next references have been renumbered.

Page 9, line 9: “measurements of systolic (SBP) and diastolic blood pressure (DBP)” has been changed to “measurements of SBP and DBP”

Page 12, line 1: “PWV was calculated using the SphygmoCor System” has been changed to “PWV was calculated in the morning (between 8:30 and 11:00 AM) 1-2 days before blood withdrawn, using the SphygmoCor System”

Page 12, line 3: There is a new sentence: “This analysis was performed in a blind basis by the same trained researcher, who was completely unaware of the health status of the patients analyzed”.

Page 12, line 24: The heading “Plasma endoglin determination” is now “Plasma soluble endoglin determination”

Page 12, line 25: “Endoglin” is now “Sol-endoglin”

Page 13, line 19: There is a new sentence: Due to our sample size (288 patients), our low variability and the selected α value (0.05), the power of our study is 0.95 (β value: 0.05).

Page 14, lines 6, 11, 14 and 23: “endoglin” is changed to “Sol-endoglin”

Page 15, lines 1, 6, 11, 19 and 25: “endoglin” is now “Sol-endoglin”

Page 15, line 12: “endoglin levels were found in...” has been changed to “endoglin levels (above 5 ng/mL) were found in...”

Page 15, lines 12 and 13: odds ratio data has been included
Moreover, endoglin levels were higher…” has been changed to “Although we have not found any significant relationship or correlation between Sol-endoglin and renal dysfunction, endoglin levels were higher…”

“(one-way ANOVA, p<0.05)” has been removed.

“endoglin” is changed to “Sol-endoglin”

A new paragraph, discussing cardiovascular disease in HHT patients, has been included.

“endoglin” is now “Sol-endoglin”

“endoglin” is changed to “Sol-endoglin”

“plasma endoglin determinations” is changed to “plasma soluble endoglin determinations”.

reference 26 (Mancia et al., 2007) is former reference 33; next references have been renumbered

There is a new reference (27): Genuth et al. (2003).

There are new references, 43-47.

“endoglin” is now “Sol-endoglin”

White blood cells count data have been included in table 2.