Author's response to reviews

Title: Serum VEGF levels are related to the presence of Pulmonary Arterial Hypertension in Systemic Sclerosis

Authors:

Andriana I Papaioannou (andriana78@vodafone.net.gr)
Epaminondas Zakynthinos (ezakynth@yahoo.gr)
Konstantinos Kostikas (ktk@otenet.gr)
Theodoro Kiropoulos (ktheod@med.uth.gr)
Angela Koutsokera (ilkoy@otenet.gr)
Athanasiou Ziogas (aziogas@yahoo.gr)
Athanasiou Koutroumpas (thanoskoutr@msn.com)
Lazaros Sakkas (lsakkas@med.uth.gr)
Konstantinos I Gourougoulianis (kgourg@med.uth.gr)
Zoe D Daniil (zdaniil@med.uth.gr)

Version: 3 Date: 1 January 2009

Author's response to reviews: see over
Dear Editor

Thank you for providing us the chance to revise our manuscript entitled “Serum VEGF levels are related to the presence of Pulmonary Arterial Hypertension in Systemic Sclerosis” for BMC pulmonary medicine. The revision has been done by the authors according to the reviewers’ comments, and we are submitting you the revised manuscript. Below we provide a list with the changes that have been done, preceded by the corresponding comments for your convenience.

REVIEWER 1

In the present study the authors detected significantly higher serum VEGF levels in patients affected by SSc with PAH compared to those without PAH and to control subjects. Furthermore they found a positive correlation of sVEGF levels both with PAH and MRC dyspnea score and a negative correlation between sVEGF levels and DLCO. The methods seem appropriate and well described and the data are sound.

Major Compulsory Revisions

1) The question posed by the authors is not well defined. The main aim of the study is to determine serum VEGF levels in patients with SSc and to evaluate whether those levels are different in patients with and without PAH not secondary to a lung interstitial involvement of the disease. Furthermore, the authors will evaluate the relationship between VEGF levels and several clinical and functional parameters.

The aim of the study has been rewritten according to the reviewers’ suggestions (page 6)

2) In the discussion the authors state that “further prospective studies are needed to investigate whether the worsening in sPAP is followed by increases in serum VEGF levels” and they conclude that “our findings suggest that serum VEGF levels may be used as a predictor of sPAP in
patients with SSc”. The authors should better clarify their hypothesis on the role of VEGF in the pathogenesis of PAH in patients with SSc.

As we have provided in the discussion it is not clear whether increased serum VEGF levels in patients with SSc are the cause of the development of pulmonary hypertension or if the increases of the pressure in the pulmonary artery result in the elevation of serum VEGF levels. However, as it seems that serum VEGF levels correlate to sPAP we hypothesized that serum VEGF levels and sPAP are closely related and though serum VEGF levels might be used as a predictor of sPAP in SSc patients. Further studies are needed to examine this hypothesis.

3) Patients with SSc and PAH have significantly lower DLCO compared to those without PAH. Is there a significant difference in arterial oxygen partial pressure between the two groups of patients? Measurements of PaO2 and PaCO2 should be presented in table 1. Considering the potential role played by chronic hypoxia in inducing VEGF expression the authors should discuss this aspect in patients affect by SSc with and without PAH.

The arterial oxygen partial pressure did not differ between the two groups of our patients. Measurements of PaO2 and PaCO2 have been presented in table 1 as requested by the reviewer. A plausible explanation for the above findings is presented in the first paragraph of page 18.

4) Is the effect of sVEGF level on the clinical and functional parameters mediated by the increase in PAH? Is there a correlation of sPAP both with MRC dyspnea score and DLCO?

The effect of sVEGF levels on the clinical and functional parameters is probably mediated by the increase of PAH. A significant correlation was observed between sPAP both with MRC dyspnea Score and DLCO. Those findings have been added in the results section and a comment has been added in page 14.
5) Some patients of the group with PAH showed an overlap in sVEGF levels with others of the group without PAH. Have these patients other characteristics that could explain the increase in PAH? In any case it could be very interesting to strictly monitor those patients who have not developed PAH.

There is indeed an overlap in the sVEGF levels between patients with and without PAH. As we have excluded patients with conditions that could affect sVEGF levels, the causes that could cause this overlap are not clear. However, we agree with the reviewer that it would be very interesting to strictly monitor the patients with high sVEGF who do not have PAH in order to determine if they will develop PAH in the future. A phrase has been added in page 16-17.

Minor Essential Revisions

1) On page 15 “It has also been reported” instead of “It has been also reported”

2) On page 17 the statement “Additionally, in severely hypoxic rats treated with an flk-1 inhibitor, the development of more marked PH accompanied by a marked increase in endothelial cell proliferation in the pulmonary artery[32]” is not clear.

Grammatical mistakes have been corrected according to the reviewers’ recommendations.
REVIEWER 2

The study is comparing VEGF levels to elevated right heart pressures via echo in SSc. Although interesting, there are changes that are needed. For instance it is not PAH that is being studied but elevated pressures on echo. The total N of SSc is not known and exclusion criteria are not provided. The sample is relatively small at n=40. How many were excluded via lack of consent, cardiac findings already on left heart and smoking, etc.

Exclusion criteria are provided in the “patients” section in the “materials and methods”. A flow chart of the patients which were screened is presented in Figure 1, in response to the reviewers’ request.

Was the project ethics approved and was a consent form signed?

The study protocol was approved by the local ethics committee and all patients gave written informed consent. The phrase is presented in page 8, paragraph 3.

The reference 18 needs to be given on page 8 after the first mention of the MRC dyspnea score.

The reference for the MRC dyspnea scale has been added in page 8 after the first mention of the MRC dyspnea score.

How were the controls selected? Who were they? They are not fully matched for gender.

The controls were healthy volunteers, non smokers with no history of lung disease and normal pulmonary function tests. Indeed, the two groups were not fully matched for gender, yet similar proportions (23% of patients with SSc
and 17.5% of controls) were male. The characteristics of the control groups are provided in page 7 and in table 1.

You need to give the data for those with true R heart cath proven PAH. This would make the results fare more meaningful.

We did not perform right heart catheterisation in any of the participants of this study. This was a study limitation that has been discussed in the last paragraph of page 18.

State in methods that logistic regression was done to adjust for skin score, limited vs. diffuse, disease duration etc.

In the present study, multiple linear regression analysis was used, since sPAP is a continuous (not a binary) variable. In this analysis, sPAP was used as dependent variable, whereas age, gender, disease duration, total skin score, MRC dyspnea score, $D_{LCO}$ and serum VEGF concentrations were used as independent variables. This is stated in page 11 of the Materials and Methods section. Total skin score and disease duration were not predictors of sPAP, and this is stated in the Results section and in Table 2.

On page 14 the $R^2$ squared of each should be provided in the text, not just the $p$ values (DLCO, dyspnea, etc).

Linear regression is a form of regression analysis in which the relationship between one or more independent variables and the dependent variable is modeled by a least squares function which is a linear combination of one or more model parameters called regression coefficients (Cohen J, Cohen P, West S.G. &Aiken LS (2003) Applied multiple regression/correlation analysis for the behavioural sciences (2$^{nd}$ edition) Hillsdate, NJ.: Lawrence Erlbaum
and Associates). On page 14, the $\beta$ (standardised coefficient) for each independent determinant of sPAP has been added in the text next to each $p$ value. The adjusted $R^2$ of the whole model is provided both in the text in page 14 and in Table 2.

**Page 16 - How do we know that VEGF is not abnormal due to local hypoxia?**

We agree with the reviewer that we do not know that VEGF is not abnormal due to local hypoxia. In our group of patients, no significant correlation was found between serum VEGF levels and either the severity of skin lesions, (expressed as the total skin score, or the presence of finger ulcers). Furthermore, tissue hypoxia described in SSc is not limited to the skin but also involves several internal organs which may contribute to the overall elevation of VEGF levels in the serum of these patients. However, in our study serum VEGF levels do not differ between patients with SSc without PAH and controls. Our findings may support the hypothesis that elevated serum VEGF levels might reflect an increase in VEGF production at sites of vascular injury due to tissue hypoxia. The above hypothesis is presented on page 16.

We remain at your disposal for any further clarifications.

Looking forward for your favorable decision.

Yours sincerely

Andriana I Papaioannou, MD