Author's response to reviews

Title: Admission Hypoxia-inducible Factor 1alpha Levels and In-hospital Mortality in Patients with Acute Decompensated Heart Failure

Authors:

- Gang Li (marty007@163.com)
- Wei-hua Lu (huaweiulwh@163.com)
- Xiao-wei Wu (wuxiaowei119@gmail.com)
- Jian Cheng (chengjian1201@163.com)
- Rong Ai (arlotus@mail.hzau.edu.cn)
- Zi-hua Zhou (zzhua2001@163.com)
- Zhong-zhi Tang (zhongzt2007@163.com)

Version: 2
Date: 26 April 2015

Author's response to reviews: see over
Dear Dr. Renato De Vecchis:

Thank you very much for your letter and advice. We have revised the paper, and would like to re-submit it for your consideration. We have addressed the comments raised by the reviewers. This manuscript has been edited and proofread by International Science Editing, Compuscript Limited.

We hope that the revision is acceptable, and we look forward to hearing from you soon.

Sincerely,

Dr. Gang Li
Reviewer: Dr. Armando Pucciarelli

Major Compulsory Revisions

1. The paper is written in bad English and it cannot be accepted for publication in its current form; it needs a heavy editing for the removal of the very numerous spelling and grammar errors.

In the revised version, we have been corrected the grammatical and multiples spelling errors and undertaken the extensive stylistic corrections by the help of International Science Editing Compuscript Limited.

2. Abstract, Conclusions, row 22: The sense of this sentence is understandable, however probably it should be written in a more correct manner, as follows: "It may become a prognostic biomarker of heart failure, however this potential role needs to be convalidated by means of further prospective studies in the future"

Many thanks for the reviewer’s good advice. We have been revised the sentence as your recommended a more correct manner.

3. Introduction page 1, row 4. “Some researchers have been proved that the synthesis of BNP was directly caused by hypoxia mediated through HIF-1α independent mechanism without the stimulation of hemodynamic and neurohormone, which was based on ventricular myocyte model system in vitro”. The sentence is poorly written and needs to be reworked. Please correct "Some researchers have demonstrated..."

Thank you for your careful and patient review of our manuscript. The sentence has been revised.

4. Results page 7, row2: The levels of HIF-1 are very high in this series, due to the
existence of a condition of cardiac acute decompensation. However, the reader needs to be adequately informed about the normal range of the serum level of HIF-1 among healthy subjects as well as patients with systolic or diastolic dysfunction; in addition, exhaustive information should be provided about the serum levels of HIF-1 among patients with ADHF. This information could be provided within a table, or, alternatively, it could be given in the text of the Results, so as to enable the reader to understand the meaning of the values (expressed as pg/ml or ng/ml or ng/liter). Please report the requested information about the normal and pathological serum levels of HIF-1 under the various conditions (healthy individuals, left ventricular dysfunction [systolic or diastolic], left ventricular failure).

Thank you for your advice. According to your suggestion, we enrolled 52 healthy volunteers (mean age: 43.8±18.5; male: 67.3%) at the last twenty days. As a result, the mean level of HIF-1α among healthy subjects was 1.31±0.47ng/ml. In our ADHF patients, the mean level of HIF-1α was 2.95±0.85ng/ml. The Doppler echocardiographic diastolic indices were used to differentiate systolic or diastolic dysfunction. Serum HIF-1α level in patients with systolic dysfunction was 3.37±0.79 ng/ml. Meanwhile, the HIF-1α level of diastolic dysfunction patients was 2.70±0.78 ng/ml. These information had been added in the new manuscript.

5. Results, page7, row7:” HIF-1α levels positively correlated with NT-proBNP (r =0.337, P<0.001), TnT (r = 0.357, P<0.001), and negatively correlated with LVEF(r = -0.332, P<0.001) and SBP(r = -0.145, P=0.013). ” Please add the correlation plots in order to better describe the correlations that have been found between HIF-1α and NTproBNP, HIF-1α and TnT,HIF-1a and LVEF ,HIF-1a and SBP (four scatter plots on the whole)

The correlation plots have been added in the revised version as suggested by the reviewer (Figure 3).
Reviewer: Dr. Aldo Clerico

General considerations

1. It is not clear whether Authors accurately evaluated the presence of diastolic dysfunction by echocardiography (pages 5 and 6), which is the most important parameter for the inclusion of HF patients in the HFpEF group, as reported by the most recent international guidelines (McMurray JJ et al. Eur J Heart Fail 2012; 33: 1787-847; Yancy CW et al. J Am Coll Cardiol 2013; 62: e147-239).

The Doppler echocardiographic diastolic indices are the most important parameter to evaluate diastolic dysfunction. According to the ESC guideline, we measured the common echocardiographic indices including e’, E/e’ ratio, left atrial volume index and LV mass index. The presence of at least two abnormal measurements is primary to diagnose left ventricular diastolic dysfunction. The diagnosis of HFpEF in our patients requires 3 conditions to be satisfied: typical symptoms and signs of HF, normal LVEF and LV not dilated, left ventricular diastolic dysfunction.

2. The criteria for the evaluation of novel biomarkers for cardiovascular risk were previously reported in detail (Hlatky MA et al. Circulation 2009; 119:2408-16). Authors should follow these criteria in the evaluation of the novel biomarker HIF-1α.

We were firstly evaluated the circulating levels of HIF-1α in ADHF patients. However, due to the limitations of study design and sample scale, serum HIF-1α levels cannot predict the short-term prognosis of ADHF patients. In general, a novel risk marker should be evaluated in several phases. The present study may be an initial phases----proof of concept. HIF-1α as a novel risk marker requires a sound research design, a representative at-risk population, and an adequate number of outcome events to evaluate.
3. Further information is needed about the methods used for the measurement of cardiovascular biomarkers (page 5) and statistical analyses (page 6) used in this study.

The information of measurement and statistical analyses had been added in the new edition.

**Specific points**

1. Abstract, Background, page 2, lines 6 and 7. Author should clarify the sentence: “…and describe the relationship between NT-proBNP in vivo.”

This sentence has been revised and clarified in the new edition.

2. Abstract, Conclusions, page 2, lines 18-23. The conclusions reported in the Abstracts are not supported by the results found in the study. The first two sentences of the conclusions (lines 18-20) are not related to the results found in this study, but reported data from the literature. The last sentence of the conclusions is misleading (“It can become a prognostic biomarker of heart failure need more prospective study to demonstrate in the future.”). Authors should better clarify this sentence (for example: “Further studies are needed in order to demonstrate the diagnostic and/or prognostic role of HIF-1α as risk biomarker in patients with ADHF”).

We appreciate the reviewer to put forward these important comments. Conclusions have been revised and the last sentence has been clarified.

Thank you for your advice. The references have been updated.

4. Patients, page 4. Authors should better clarify the criteria adopted for the diagnosis of HF and the classification of patients. Authors should specify whether the criteria recommended by the most recent guidelines of the European Cardiology Society (ESC) and the ACCF/AHA were adopted for the classification of patients enrolled in this study (McMurray JJ et al. Eur J Heart Fail 2012; 33: 1787-847; Yancy CW et al. J Am Coll Cardiol 2013; 62: e147-239).

We adopted ESC and ACCF/AHA guidelines to diagnose and distinguish patients. The conditions for diagnosis HFrEF and HFpEF were satisfied the requirement of ESC and ACCF/AHA guidelines. The information had been added in the new edition.

5. Biochemical measurements, page 5, lines 4-21. More information is needed about the analytical performance of ELISA method used for the HIF-1α assay. In particular, Author should report the lower limit of detection (LOD), the possible interferences in this assay method with other proteins related to HIF-1α and the reference values intervals for the reference population. The reference for the ECLIA methods for NTproBNP assay is updated (ref. 24) (lines 12-13). Roche Diagnostics recently introduced a new assay, using monoclonal antibodies instead of polyclonal antibodies used in the old method: Authors should specify if the new monoclonal antibody method was used in this study (for a reference on differences between these two methods: Prontera C. et al. Clin Chim Acta 2009; 400: 70-3). The recommended measurement units for the NT-proBNP are ng/L (not pg/mL) (Apple FS et al. Clin Chem 2005; 51:486-93; Apple FS et al. Circulation 2007; 116: e95-8). The analytical characteristics and performance of ECLIA method for cTnT assay were previously reported (Giannitsis E. et al. Clin Chem 2010; 56:254-61). The quality specifications for troponin assay were reported in some authoritative reviews; in particular, the recommended units for the hs-cTnT assay are ng/L and the recommended cut-off is 14 ng/L (not 0.03 ng/mL or 30 ng/L, as reported in the manuscript, line 19 of page 5).

We used the ELISA method to measure serum HIF-1α levels. The lower limit of detection (LOD) was 0.041ng/ml and the reference values intervals was 0.078 to 5.0 ng/ml. These information had been added in the new edition. However, we asked the Abnova’s technical support department that whether exist other proteins may be interfere to measure HIF-1α are still not clear. We confirm that the new monoclonal antibody method was used in our study to assay NT-proBNP. The measurement units for the NT-proBNP were corrected with ng/L.


We adopted the latest Doppler echocardiographic indices of ESC and ACCF/AHA guidelines to evaluate the diastolic dysfunction.

7. Statistical analysis, page 6. It is well known that circulating levels of several biological variables, including NT-proBNP and cTnT, are not normally distributed. Authors should specify if this important point is taken into account when parametric tests or the univariate and multivariate regression analyses were performed (for example by using log-transformation of the original values of some variable).

In the univariate and multivariate regression models, the nonnormal distribution variables including NT-proBNP and TnT had performed log-transformation. We have been clarified it in the Statistical analysis section.
8. Results, page 8, lines 1-2. Authors should specify that the cut-off values reported in the manuscript are the best cut-off values evaluated by ROC analysis (that is the biomarker value which maximizes the sum of true positive and negative values).

Many thanks for the reviewer’s advice. We have been updated the best cut-off values for the original cut-off values.

9. Table 2. Authors should add the units of measurement for the variables: HIF-1α, cTnT, hs-CRP, Creatinine, BUN, Uric Acid.

The units of measurement had been added in the Table 2.

10. Figures 1 and 2. Authors should add the units of measurement (i.e., ng/mL) for the HIF-1α assay in the ordinate axis.

The units of measurement had been added in the Figures 1 and 2.

11. Conclusion(s), page 11, lines 7-8. The first sentence of conclusions is misleading. Authors evaluated the circulating levels (not the expression) of HIF-1α. The term “expression” may suggest that Authors also evaluated the gene expression and/or the production (for example by cardiomyocytes) of HIF-1α.

Thank you for careful review of our manuscript. The term “expression” has been revised with “circulating”.