Author's response to reviews

Title: Brain Natriuretic Peptide Levels are Associated with Peripheral Arterial Disease in Type 2 Diabetic Patients

Authors:

JIN Qi-hui (jinqihui1228@sina.com)

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Author's response to reviews: see over
Dear editor:

Thanks for the work you and the reviewers have done for my paper. I really appreciate your help. We have revised the paper according the reviews. We hope we can do better, and we are sure we can get more advice and help from you if the paper still needs revision. Thank you for your efforts and time. The answers to the review were as following:

**Reviewer #1:**

**Question 1.** Diabetes duration was not very long, and yet almost 30% of patients had PAD, suggesting that this is a very selected sample. Please comment and emphasise this selection more.

**Answer:**

Patients enrolled in this study were outpatients. All patients had acceptable glycemic control within 2-3 months and took oral hypoglycemic agents to control blood sugar. None of them used insulin in the past. None had clinical cardiovascular disease, infectious diseases, or diabetic foot ulcer. Diabetes duration, starting from the initial diagnosis of type 2 diabetes, was obtained through interrogation. In China, many patients may not be diagnosed in time. This may impact the accuracy of the measurement of diabetes duration.

**Question 2.** Was the potential effect of neuropathy examined? Please add a brief comment.

**Answer:**

Diabetic neuropathy was examined by testing vibration (using a 128-Hz tuning fork), pin-prick sensation (using Neurotip), temperature sensation (using warm and cool rods), and achilles tendon reflex (using a tendon hammer). All patients were asked whether they experienced pain or discomfort in their legs. Patients with a description of burning, numbness, or tingling and symptomatic diabetic neuropathy affecting the lower extremities with at least one positive
sensory symptom such as pain, burning, paraesthesia or prickling were excluded because diabetic neuropathy may lead to abnormalities in heart rate and vascular dynamics.

**Question 3.** When summarising that BNP was index of PAD severity, the authors should bear in mind that this holds true to the inverse association with ABI but not with Fontaine’s classes, inasmuch as there was no difference between II and III categories.

**Answer:**

This comment is very reasonable. Measurement of the ankle brachial index (ABI) is an objective, non-invasive screening technique that effectively identifies the presence of PAD and assesses its severity. Fontaine’s classes categorized according to symptoms. ABI and Fontaine’s classes are two different grading systems, our conclusions should be considered with some caution, so this result needs to be modified based on reviewers suggestions. We have already revised.

**Question 4.** Why were patients with Fontaine’s IV category excluded?

**Answer:**

Patients with Fontainie’s IV category have culcer and gangrene. These patients were in relatively severe condition and were likely to have basic diseases and inflammation. Therefore, most of them were hospitalized. Since our study only enrolled outpatients, these patients were excluded.

**Question 5.** Please state the clinical implications in more detail.

**Answer:**

Revision was made according to the review.

**Reviewer #2:**
**Question 1.** The pages are not numbered; therefore, an accurate review of this manuscript is very difficult to do.

**Answer:**

The pages were numbered according to the review.

**Question 2.** Abstract. Authors should report a clear and specific aim of the study in the Abstract.

**Answer:**

Revision was made according to the review.

**Question 3.** Authors should provide an index of the (non-standard) abbreviations used in the manuscript.

**Answer:**

Revision was made according to the review.

**Question 4.** Background. Authors reported that: “Brain natriuretic peptide (BNP) ... is secreted predominantly from the ventricular myocardium in response to increased ventricular wall stretch [3]”. This reference [3] is very old (i.e., 1993). Authors should update this sentence, according to a more modern vision of the cardiac endocrine function. Some authoritative revisions of the more recent acquisitions on the physiological role and clinical utility of the BNP system has been recently published (for example: Clerico A. et al. Am J Physiol Heart Circ Physiol 2011; 301: H12-20). Furthermore, Authors should revise the Background and Discussion sections of the manuscript according to these more recent acquisitions concerning the pathophysiological role of the cardiac endocrine system, which better explain the results obtained in this study.

**Answer:**

The reference as mentioned in the review was updated accordingly to better explain our results.

**Question 5.** Patients, inclusion/exclusion criteria. Authors should better describe how the presence of cardiac systolic and diastolic dysfunction as well as of asymptomatic coronary artery disease was excluded in the patients enrolled in the study. Indeed, Authors reported that patients with cardiac systolic and diastolic dysfunction were excluded by means of echocardiography evaluation; however, Authors reported in Table 1 that mean left ventricular ejection fraction values were 58.4±16.2 and 54.7±14.9 in the two groups of patients, respectively. From these data, I would assume that some patients had less than 40% of left ventricular ejection fraction, and so
these patients should have heart failure with reduced ejection fraction according to the more recent international guidelines (Yancy CW et al. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2013; 62:e147-239). Authors should clearly report whether they have assessed the presence/absence of cardiac diastolic and systolic dysfunction according to the international guidelines.

Answer:

I appreciate the reviewer’s attention on the mean left ventricular ejection fraction values. I feel sorry that I made a mistake when copying statistic data from the statistical software. I have rechecked all the statistic data in my paper and corrected the errors.

Question 6. Patients. Authors reported that “subjects using any vasoactive drug” were excluded. The term “vasoactive drug” is not clear (almost for me). Authors should specify this type of drugs. Authors should report whether the diagnosis of arterial hypertension were made according to the more recent international guidelines (Mancia G. et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension. Eur Heart J 2013; 34:2159-219).

Answer:

Vasoactive drug in this article mainly refers to nitrates and diuretics. The 2010 Chinese guidelines for the management of hypertension was used as the diagnostic criteria of hypertension, which is consistent with the 2013 ESH/ESC Guidelines, both ≥ 140 and/or 90 mmHg.

Question 7. BNP peptide assay. The recommended international units for BNP assay are ng/L, not pg/mL (Apple et al. Clin Chem 2005; 51:486-93). Authors reported that the normal reference range for the ADVIA Centaur method is < 100 pg/mL (i.e., 100 ng/l), but some recent results suggest that the decisional level for this method should be lower (Clerico A. et al. Clin Chim Acta 2012; 414: 112-9).

Answer:

Most articles published recently used pg/ml, including the 2008 and 2012 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. ESC ‘rules out ’ heart failure when BNP level is < 100 pg/ml and ‘rules in ’ heart failure (typically implying referral for additional investigations, notably

Question 8. Statistical analysis. Authors reported the significance of statistical tests both with < 0.05 (or p < 0.01) and the exact p value (for example, p=0.021). This may generate confusion in the reader. I suggest that Authors should report only the exact value of p, when this is less than 0.05, while Authors should report that test results are not statistically significant when p > 0.05.

Answer:
The exact p values were added instead of < 0.05 (or p < 0.01) as suggested.

Question 9. Discussion. The data reported in Table 1 suggest that patients with PAD had more frequently higher HbA1c levels, hypertension, hyperlipidemia, and were more frequently aged, smokers and treated with RAAS blockade and calcium channel blockers (even if the statistical analyses reported are not all significant). These data, taking as a whole, indicate that patients with PAD have higher BNP levels because the cardiac endocrine function is more activated in these patients rather than in patients without PAD (see: Clerico A. et al. Am J Physiol Heart Circ Physiol 2011; 301: H12-20). As also reported in the most recent international guidelines, BNP levels are the most powerful predictor of cardiovascular events in general population as well as in patients in all stages of heart failure (from stage A to stage D)(Yancy CW et al. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2013; 62: e147-239). Natriuretic peptides (such as BNP) are hormones with a protective action on endothelial function, but BNP, if used as biomarker of cardiovascular risk, is a powerful index of mortality and adverse cardiovascular events. Accordingly to these considerations, the results of this study indicate that patients with PAD had higher BNP values (than patients without PAD) and so they are at increased risk of major and adverse cardiovascular events even in the short term period.

Answer:
The discussion part was revised and related references were added as suggested.

Reviewer #3:
Question 1. It has been already reported that PAD patients have higher
circulating BNP levels than controls (reference #6–#8). The only original point of this study is that subjects are diabetic. Then, the reviewer advises the authors to do the same analysis in subjects without diabetes, and compared the observed findings between diabetic and non-diabetic subjects.

Answer:

It has been reported that PAD patients have higher circulating BNP levels than controls. In addition, the incidence of PAD is high in diabetic patients and BNP level is higher in diabetic patients than in healthy controls. So this study focused on diabetic patients and investigated the possible role of BNP in the mechanism of lower extremity vascular diseases in diabetic patients. Corresponding data of non-diabetic patients over the same period were not collected, so a comparison with non-diabetic patients is not available in this study. We would like to improve this part in our future study.

Question 2. BNP is secreted predominantly from the ventricular myocardium. The authors described that patients with cardiac systolic and diastolic dysfunction were excluded from the study. What are the criteria of systolic and diastolic dysfunction? Recently, Yamasaki et al. reported that high BNP levels were associated with E/e’, but not E/A, in PAD patients (Angiology 64;540-543, 2013).

Answer:

Systolic function is conveniently (although not always accurately) measured as the ejection fraction (EF). The LVEF is easily interpreted. The lower limit of normal is 50%. The lower the EF is, the greater the reduction in systolic function. The diagnostic criteria of systolic dysfunction is LVEF <50% according to ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012. (European Heart Journal 2012; 33, 1787–1847)

In (Angiology 64;540-543, 2013) Yamasaki reported that high BNP levels were associated with E/e’, but not E/A, in PAD patients. In our view, this may be due to the following reasons. Firstly, diastolic function is more difficult to evaluate.
No single echocardiographic parameter is sufficiently accurate and reproducible to be used in isolation to make a diagnosis of LV diastolic dysfunction. E/A is the ratio of early to late diastolic mitral inflow waves: E/A<1 (Impaired relaxation); =1~2 (Normal); >2 (Restrictive). E/e’ is the ratio of the mitral inflow E wave to the tissue Doppler e’ wave: E/e’>15 (high LV filling pressure); =8~15 (intermediate); <8 (low). E/A and E/e’ are used to assess left ventricular diastolic dysfunction in patients with heart failure. The E/e’ ratio has been proposed as a reliable estimate of LV filling pressure, e’ is presumed to correlate closely with LV relaxation indexes, but e’ appears to be age dependent( J Am Soc Echocardiogr. 2004;17:132-138; Am J Cardiol. 2005;95:1020-1023; Diab Vasc Dis Res. 2005;2:24-30.), this age dependence also could detract from its prognostic value(J Am Coll Cardiol. 2003;41:820 – 826; J Am Coll Cardiol. 2005;45:272–277; J Hypertens. 2005;23:183–191.) The European Society of Cardiology consensus statement on how to diagnose diastolic heart failure suggests that an E/e’ ratio >15 alone or an E/e’<8 in combination with an elevated B-type natriuretic peptide >200 pg/mL can be used as the simplest noninvasive objective indication of diastolic dysfunction to confirm the presence of diastolic heart failure (Circulation. 2002;106:1333–1341). Value of E/e’>15 is usually proposed as evidence for elevated LV filling pressure and a value of E/e’<8 as evidence for normal LV filling pressure. As a consequence, there is a wide range of E/e’ values (8 <E/e’>15) for which additional investigations are required to obtain a LV filling pressure estimate. Further technical limitations include angle dependency, signal noise, signal drifting, spatial resolution, sample volume, and tethering artifacts. E/e’ might not reflect diastolic performance accurately in cases with regional wall motion abnormality. (J Echocardiogr 2013;11:138–146). So performance data obtained in other environments or under different conditions may vary significantly. Second, in this paper echocardiographic parameters could not be obtained in some cases. Besides, the number of patients in this study was small, and further studies are needed.
**Question 3.** Both ABI <0.9 and ABI = 0.9 are used as the cut-off value for PAD diagnosis in the text.

**Answer:**


**Question 4.** The reviewer found several typographical errors in the text.

**Answer:**

We checked through the whole article and corrected the typographical errors.