Response to Reviewer "Revision Ms. No. 02641240

Title: Combined assessment of myocardial damage and electrical disturbance in chronic heart failure.

Reviewer 1:

The authors are grateful for the helpful comments and constructive suggestions of reviewer. The following comments are in response to the reviewer's points:

Q1. Abstract In the abstract and paper the Primary endpoint of the study which appeared to be composite of Cardiovascular death and hospitalisation for heart failure and as such the primary endpoint needs to be more specifically stated. Similarly in the in the results it would clearer if they stated that there were 117 primary endpoints which included '27 cardiac deaths and 90 re-hospitalizations for worsening CHF.'

Response to Q1:

Thank you for your helpful suggestion. According to your suggestion, we added the following sentence in abstract. "The primary endpoint was cardiac deaths and rehospitalization for worsening CHF." We rewrote the following sentence in abstract. "There were 117 primary events, including 27 cardiac deaths and 90 rehospitalizations."

In result section, we rewrote the following sentence. "There were 117 primary events, including 27 cardiac deaths and 90 re-admissions for worsening CHF, during the follow-up period." (Page 10, Paragraph 2)

Q2. The definition of a prolonged QRS used in their study of >120ms should be stated in the abstract. I would then state that 'Multivariate analysis demonstrated that high H-FABP levels (hazard ratio 1.745, p = 0.021) and QRS prolongation (hazard ratio 1.612, p = 0.0258) were independent predictors of the primary endpoint'.

Response to Q2:

Thank you for your helpful suggestion. According to your suggestion, we added the following sentence in abstract. "A prolonged QRS duration was defined as 120 ms or longer."

Q3. Introduction The authors make a big statement without any reference to this: 'The role of biomarkers in the evaluation and risk stratification of patients with CHF continues to increase in importance.' This sentence should be referenced eg with some BNP evidence

Response to Q3:

According to your suggestion, we added the following reference.

Ahmad T, Fiuzat M, Felker GM, O'Connor C. Novel biomarkers in chronic heart failure. Nat Rev Cardiol 2012;9:347-359.

(門脇先生へ依頼し、番号変更を。)

Q4. Methods I am confused by the inclusion criteria. Are the authors stating that patients with new LBBB were excluded or any LBBB? I don't fully understand why patients with LBBB would be excluded and if so you could be excluding >25% of potential patients limiting the utility of H-FABP. Who were the patients with QRS >120 were they RBBB? Were patients with CRTs included?

Response to Q4:

Thank you for your comments. In the present study, we did not include patients with CRT and candidates for CRT, since there is a great difference in prognosis between responders and non-responders for CRT. Therefore, we did not include patients with LBBB as candidates for CRT. Please check the exclusion criteria for this study in Study population in Methods section.

Q5. Again under the methods instead of 'The end points were cardiac death, defined as death due to progressive heart failure, myocardial infarction or sudden cardiac death, and progressive heart failure requiring prehospitalization I would be more specific and state that the primary endpoint was cardiovascular death (defined as heart failure, myocardial infarction or sudden cardiac death) and heart failure hospitalization.

Response to Q5:

Please see response to Q1.

Q6. Results I find it strange that patients with hospitalized HF were included yet >50% were

NYHA II. I am not sure why NHYA Class II and II should increase the hazard ration for the primary endpoint. Do the authors mean NYHA IV vs II and III rather than as quoted as II/III vs IV?

Response to Q6:

We enrolled 322 patients with CHF, who were admitted to our hospital for the diagnosis or treatment of CHF. There were many stable CHF patients with NYHA class II when they were admitted to our hospital for diagnosing etiologies of heart failure. Our university hospital has many patients who are referral from affiliated hospitals.

Q7. Where did the H-FABP cut off of ≤4.5 ng/ml come from as it was 4.3 in one other authors previous studies?

Response to Q7:

Thank you for your comments. We had several reports investigating H-FABP in patients with CHF as follows. We used several cutoff of H-FABP from 4.1 ng/ml to 5.4 ng/ml. In the present study, we used 4.3 ng/ml of H-FABP as a cutoff. If we use 4.3 ng/ml of H-FABP as a cutoff, results did not change.

1. Otaki Y, Arimoto T, Takahashi H, Kadowaki S, Ishigaki D, Narumi T, Honda Y, Iwayama T, Nishiyama S, Shishido T, Miyashita T, Miyamoto T, Watanabe T, Kubota I. Prognostic value of myocardial damage markers in patients with chronic heart failure with atrial fibrillation. Intern Med. 2014;53(7):661-8. PubMed PMID:24694473. (H-FABP, 5.4 vs. 4.6 ng/mL)
2. Kutsuzawa D, Arimoto T, Watanabe T, Shishido T, Miyamoto T, Miyashita T, Takahashi H, Niizeki T, Takeishi Y, Kubota I. Ongoing myocardial damage in patients with heart failure and preserved ejection fraction. J Cardiol. 2012 Dec;60(6):454-61. doi: 10.1016/j.jjcc.2012.06.006. PubMed PMID: 22819040. (H-FABP >4.3 ng/ml)
3. Ishino M, Takeishi Y, Niizeki T, Watanabe T, Nitobe J, Miyamoto T, Miyashita T, Kitahara T, Suzuki S, Sasaki T, Bilim O, Kubota I. Risk stratification of chronic heart failure patients by multiple biomarkers: implications of BNP, H-FABP, and PTX3. Circ J. 2008 Nov;72(11):1800-5. PubMed PMID: 18832778. (H-FABP >4.1 ng/ml)
4. Niizeki T, Takeishi Y, Arimoto T, Nozaki N, Hirono O, Watanabe T, Nitobe J, Miyashita T,

Miyamoto T, Koyama Y, Kitahara T, Suzuki S, Sasaki T, Kubota I. Persistently increased serum concentration of heart-type fatty acid-binding protein predicts adverse clinical outcomes in patients with chronic heart failure. Circ J. 2008 Jan;72(1):109-14. PubMed PMID: 18159110. (H-FABP >4.3 ng/ml)

5. Arimoto T, Takeishi Y, Niizeki T, Nozaki N, Hirono O, Watanabe T, Nitobe J, Tsunoda Y, Suzuki S, Koyama Y, Kitahara T, Okada A, Takahashi K, Kubota I. Cardiac sympathetic denervation and ongoing myocardial damage for prognosis in early stages of heart failure. J Card Fail. 2007 Feb;13(1):34-41. PubMed PMID:17339001. (H-FABP >5.2 ng/ml)
6. Niizeki T, Takeishi Y, Arimoto T, Takabatake N, Nozaki N, Hirono O, Watanabe T, Nitobe J, Harada M, Suzuki S, Koyama Y, Kitahara T, Sasaki T, Kubota I. Heart-type fatty acid-binding protein is more sensitive than troponin T to detect the ongoing myocardial damage in chronic heart failure patients. J Card Fail. 2007 Mar;13(2):120-7. PubMed PMID: 17395052. (H-FABP >4.3 ng/ml)
7. Arimoto T, Takeishi Y, Niizeki T, Koyama Y, Okuyama H, Nozaki N, Hirono O, Tsunoda Y, Miyashita T, Shishido T, Okada A, Takahashi K, Kubota I. Ongoing myocardial damage relates to cardiac sympathetic nervous disintegrity in patients with heart failure. Ann Nucl Med. 2005 Oct;19(7):535-40. PubMed PMID: 16363617.
8. Niizeki T, Takeishi Y, Arimoto T, Takahashi T, Okuyama H, Takabatake N, Nozaki N, Hirono O, Tsunoda Y, Shishido T, Takahashi H, Koyama Y, Fukao A, Kubota I. Combination of heart-type fatty acid binding protein and brain natriuretic peptide can reliably risk stratify patients hospitalized for chronic heart failure. Circ J. 2005 Aug;69(8):922-7. PubMed PMID: 16041160. (H-FABP >4.3 ng/ml)
9. Niizeki T, Takeishi Y, Arimoto T, Okuyama H, Takabatake N, Tachibana H, Nozaki N, Hirono O, Tsunoda Y, Miyashita T, Fukui A, Takahashi H, Koyama Y, Shishido T, Kubota I. Serum heart-type fatty acid binding protein predicts cardiac events in elderly patients with chronic heart failure. J Cardiol. 2005 Jul;46(1):9-15. PubMed PMID: 16095226.
10. Arimoto T, Takeishi Y, Shiga R, Fukui A, Tachibana H, Nozaki N, Hirono O, Nitobe J, Miyamoto T, Hoit BD, Kubota I. Prognostic value of elevated circulating heart-type fatty acid binding protein in patients with congestive heart failure. J Card Fail. 2005 Feb;11(1):56-60. PubMed PMID: 15704065. (H-FABP >4.3 ng/ml)

Q8. Discussion In the discussion the authors really need to explain why BNP failed to predict outcomes in this study despite the known excellent data to support its prognostic utility form other studies. H-FABP measurement is time consuming and at present more rapid measures of assessment and data from a larger clinical study needs to be available for it to be considered for mainstream clinical practice. Also the AUC was modest at best and this is not mentioned.

Response to Q8:

We think that H-FABP levels provide different information from BNP levels. BNP level easily alters dependently on fluid balance. However, H-FABP level is a marker of ongoing myocardial damage and is not altered by fluid balance. As we stated in discussion section, "We recently determined that the AUC for prediction of cardiac events in heart failure was greater for H-FABP level than for BNP level [9]"

Q9. I found the discussion quite short and the translational advantages were not discussed nor was the specificity of the marker which is new to me. Is this an epimarker for something else or specific for Heart failure over Myocardial infarction of other types of ACS.

Response to Q9:

Thank you for your comments. As described in Introduction, H-FABP is a low molecular weight protein (14-15 kDa) and abundant in the cytosolic fraction of cardiomyocytes. Therefore, H-FABP is rapidly released into the circulation from myocardium when myocardium is damaged. We and others repeatedly reported that serum H-FABP level is a feasible marker for prognosis in heart failure as well as ischemic heart disease. We also reported that elevated H-FABP can predict cardiovascular mortality in general population (PLoS One. 2014 May 21;9(5):e94834.). To eliminate redundancy, we did not describe usefulness of H-FABP in discussion.

Q10. References Reference 2 (Funk M, Krumholz HM. Epidemiologic and economic impact of advanced heart failure. J Cardiovasc Nurs. 1996;10:1-10. [PMID: 8656234]) is outdated for the comments that The authors are trying to make re reduction in clinical endpoints with HF: Please change this reference to a more contemporary reference:

Response to Q10:

Thank you for your helpful suggestion. According to your suggestion, we changed the reference 2.

[2] Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, et al. Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study Investigators.. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. N Engl J Med. 2003;348:1309-1321. [PMID: 12668699. DOI: 10.1056/NEJMoa030207]

Q11. Figures I am not sure as to the point of showing an r of <0.1 in figures 1 for BNP and H-FABP with QRS.

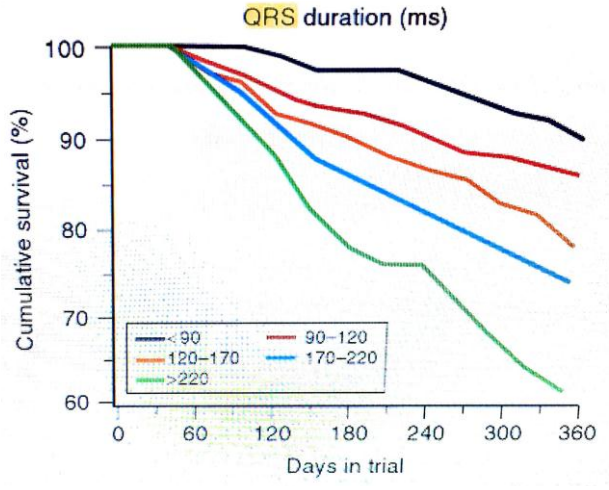
Response to Q11:

Thank you for your comments. As described in discussion, since there was no correlation between QRS duration and H-FABP or BNP levels, each parameter reflects different pathophysiological backgrounds. Therefore, we performed combined assessment of H-FABP and QRS duration for clinical outcomes in CHF.

Q12. Other comments I was wondering whether the authors have considered looking simply at Heart rate and H-FABP given that Heart rate is so simple to measure and is already a very well established markers of HF outcomes. That way they could include patients with all types of bun

Response to Q12:

Thank you for your comments. We agree that heart rate is an important factor for prognosis in CHF patients. However, heart rate easily alters dependently on their condition and medications such as beta blockers. Both H-FABP levels and QRS duration represent severity of myocardial damages such as left ventricular fibrosis and cardiac myocyte loss and are not altered by medications. Therefore, we chose these 2 markers. Previous study revealed that prolonged QRS duration stratified patients with CHF (JACC 1999;33(2):145A (abstract 847-4)).



Cumulative survival as a function of QRS duration on 12-lead electrocardiogram at trial entry in the VEST study. JACC 1999;33(2):145A (abstract 847-4)

Reviewer 2:

The authors are grateful for the helpful comments and constructive suggestions of reviewer.

Reviewer 3:

The authors are grateful for the helpful comments and constructive suggestions of reviewer. The following comments are in response to the reviewer's points:

Q1. Kadowaki S et al. performed a prospective single center study included 322 chronic heart failure (CHF) patients to evaluate whether a combined measurement of biochemical [(heart-type fatty acid binding protein (H-FABP)] and electrophysiological (QRS prolongation) markers can be used to risk-stratify patients with CHF, the results showed either high H-FABP levels or QRS prolongation was independent predictor of cardiac events that included cardiac death, myocardial infarction or sudden cardiac death, and progressive heart failure requiring rehospitalization, whereas high H-FABP + QRS prolongation confers the highest risk for cardiac events in patients with CHF. The subject is clinically of interest, and the methodology is sound and well described.

Response to Q1:

Thank you very much for the concise statements on our work.

Q2. The authors should give a more detailed description of follow up, eg. visit cycle, related examination, etc.

Response to Q2:

Thank you for your helpful suggestion. According to your suggestion, we added the following sentence in methods (End points and follow-up). "Patients were followed in our hospital outpatient clinic every month. The other patients were followed by telephone twice a year until 2555 days after discharge."

Q3. The mean HF duration of the patients need to be provided.

Response to Q3:

Thank you for your helpful suggestion. We agree that is an important point. Almost patients were referral from affiliated hospitals for first-ever heart failure within 1 month. However, there were patients who suffered from acute exacerbation of chronic heart failure. We did not know

exact duration of heart failure.