Dear Editor,

February 7, 2014

Enclosed the edited manuscript in Word format (file name: 6993-review.doc).

Title: Prognostic value of increased carbohydrate antigen concentration (CA-125) in patients with stable chronic heart failure.

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Name of Journal: World Journal of Cardiology
ESPS Manuscript NO: 6993

The manuscript has been improved according to the suggestions of reviewers:

1. Format has been updated.
2. Revision has been made according to the suggestions of the reviewers:

1) Review 1: reviewed by 02446337.

I'm writing to thank you for reviewing our manuscript. Your recommendations have been very helpful in improving the quality of our paper. We revised the observations and changes suggested and modified the text following your recommendations

Despite interesting, there are few important points that need to be addressed: In the analysis of the cardiovascular risk factors, hypertension should be considered as a continuous variable (not discrete, as the Authors did), reporting the average of blood pressure values.

The average of blood pressure values are reported in the new table 1 and a new sentence has been added in the introduction pag. 4
….Despite of high number of clinical parameters described associated with poor outcome the assessment of prognosis in patients with stable heart failure is still a challenge[5,6].

And following your advice we have included the reference:

- Santulli G. Coronary heart diasease risk factors and mortality. JAMA 2012;307,1137

And a new one

- S Ather, W Chan, A Chillar, D Aguilar, AM Pritchett, K Ramasubbu, XH Wehrens, A Deswal, B Bozkurt. Association of systolic blood pressure with mortality in patients with heart failure with reduced ejection fraction: A complex relationshipAm Heart 2011;161:567-73

In the discussion we have added the following sentence according to the reviewer suggestion:
….Furthermore new biomarkers are under development to improve diagnosis and prognosis assessment in heart failure. Recently, experimental studies have suggested that changes in circulating microRNAs can be used as a biomarker of disease. However, these new molecular markers are still under investigation.

We also added the references suggested by the reviewer:
Iaccarino G. High Blood Press Cardiovasc Prev. 2013;20(1):5-12;
Van Rooij E. Eur J Heart Fail. 2013;15(6):650-0),
Iccarino G. Am J Cardiol. 2011; 107(8):1125-30,

Following your recommendations to update the epidemiology of heart failure we have added the reference suggested by the reviewer:
Santulli G. Epidemiology of Cardiovascular Disease in the 21 Century: update numbers and update facts. J Cardiovas Dis 2013;1:1-2.

2) **Review 2: reviewed by 01964825.**

I would like to express our gratitude to reviewer 2 for reviewing our manuscript. Your recommendations have been very helpful in improving the quality of our paper. We revised the observations and changes suggested and modified the text following your recommendations

- Retrospective data has been added to the abstract:
….The predictive value of CA-125 was retrospectively assessed in 156 …

- In the methods, following your suggestion, we have included a paragraph improving the inclusion and exclusion criteria and how HF was diagnosed. The HF treatment has been moved to this section

….Patients were included in the study if they had a previous documented episode of heart failure and were receiving heart failure treatment. The diagnosis of HF was made following the Clinical Practice Guidelines of the European Society of Cardiology[24]. Heart failure treatments included angiotensin-converting-enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARB) if ACEI were contraindicated, betablockers, diuretics, and aldosterone antagonists, with individualized assessment of treatment indication and optimized doses per recommendations of the Clinical Practice Guidelines of the European Society of Cardiology[24]

And added

We excluded patients in unstable HF (NYHA class IV), patients with hemodynamic instability, or those diagnosed with cancer or systemic diseases that could shorten life expectancy. Patients with valve heart disease wanting for surgery repair were also excluded from the study.

Was TDI utilized?
Unfortunately, TDI was not systematically used in these patients

How was valvular defined?
A Vahanian, O Alfieri, F Andreotti, MJ Antunes, G Baron-Esquivias, H Baumgartner, M AndrewBorger, TP. Carrel, M DeBonis, A Evangelista, V Falk, B Lung, P Lancellotti, L Pierard, S Price, HJ Schafers, G Schuler, J Stepinska, K Swedberg, J Takkenberg, UO Von Oppell, S Windecker , JL Zamorano, M Zembala. Valve heart disease was diagnosed following the European valvular heart guidelines: Guidelines on the management of valvular heart disease (version 2012). The Joint Task Force on the Management of Valvular Heart Disease of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS). European Heart Journal (2012) 33, 2451–2496.

Regurgitant lesions were considered significant if they were moderate or severe and they were the cause of HF. No patients had mitral stenosis. AS is considered in the following paragraph

How was dealt with concomitant disease, e.g. reduced systolic function and moderate aortic valve stenosis: valvular? systolic?
This is a difficult challenge, if the AS was only moderate and was not considered to be the cause of left ventricular dysfunction, in terms of etiology they were classified as dilated cardiomyopathy, if the AS was

severe was classified as valve disease. Moreover, they were also classified as HF with low EF: HFrEF. Furthermore, since the etiology of HF was not significantly different between groups, as is shown in table 1, it can be considered as not relevant for the main purpose of the study.

- How was dealt with patients with A-Fib?
The % of pts with permanent AF is reported in table 1. Presence of permanent AF, since it is risk factor for increased mortality was included in the multivariate analyses, as is mentioned in page 6:

… Variables included in the propensity score were left atrial diameter, age, atrial fibrillation, left ventricular ejection fraction, eGFR (estimated glomerular filtrate rate), hemoglobin and interventricular septum thickness…

- Mortality was mentioned but follow-up was inconsistent
We have added the range of follow-up on pag. 7
…mean follow-up was 17±8 months (2 to 32 months)

- So, what were the hazard ratios for each biomarker and its combinations?
- What is the additional value of CA125? Could a ROC analysis be provided?
- Who was CA positive and BNP negative? Could the baseline characteristics be dichotomized into CA125pos+BNPneg versus CA125neg+ BNPneg? Minors: NT-proBNP is not normally distributed, therefore median and interquartile range should be displayed and not mean and standard deviation

The observation is well received. Therefore, we add a table of baseline characteristics according to the combined category of CA 125 and NT-proBNP. Moreover, the hazard ratios for each biomarker and its combinations are shown in the new table 1.
Discrimination is the model's ability to assign the right outcome to a randomly selected pair of subjects; in other words, it allows the model to classify subjects in a binary prediction outcome setting. The area under the ROC curve (AUC) is the most common performance measure used in the evaluation of the discriminative ability for normal-error models with a binary outcome. The equivalent for censored data is the C-statistic. In our case, as we used Cox regression model to analyze censored data, then Harrell's C-statistic was performed.
Finally, the additional value of CA 125 combined with NT-proBNP vs NT-proBNP alone was assessed by the integrated discrimination improvement (IDI) index. When two models are compared, IDI quantifies the increment in the predicted probabilities for the subset of patients experiencing the event and the decrement for those not experiencing the event. (It does reflect an improvement in the average of the true positive rate without sacrificing its average true negative rate). In our case, the average of IDI was 4%. In practice it is quite difficult to obtain significant changes in the ROC curve by adding a new predictor to a complex model. In fact this new predictor should have a significantly high odds ratio to capture the difference by simply comparing the ROC curves (in our case: model 1=0.744 AUC and model 2= 0.766 AUC). Age is a clear example of strong discriminative predictor. Therefore, as other studies have reported, we used the integrated discrimination improvement (IDI) index that is more sensitive to the changes made by a new predictor in a model’s ability for discrimination.

- It should state ARB not ARA
Following your suggestion we have changed ARA for ABR throughout the manuscript

- Multiple annoying typos and inconsistency with abbreviations (ST2 its not defined; sometimes CA-125 changes to CA 125; sometimes NTproBNP changes to NT-proBNP
We have reviewed our manuscript and corrected all the typos left.

- Several subtitles are not in english (Antigeno carboanhidrato!)
Sorry about that we have corrected the error

- Discussion: It would need multivariate analysis to state that CA125 is an independent predictor. Please delete.
Multivariate analyses were done. They were reported in table 4. In order to avoid overfit multivariate models, two propensity score were built to adjust in a multivariate Cox model. This technique is proposed in: *Núñez E, Steyerberg EW, Núñez J. [Regression modeling strategies]. Rev Esp Cardiol. 201;64:501-7*.
We would like you to considerer the possibility of not delete the sentence you mentioned

- Consistency needed: either 0,014 or 0.014 (points or commata)
Again sorry about the error we have reviewed all the decimals numbers to be sure there is a dot separating decimals

3) **Review 3: reviewed by 00742171.**

I would like to express our gratitude for so thoroughly reviewing our manuscript. Your observations have been extremely helpful in improving the quality of the manuscript.
Following your observations we have done the following changes:

- 1. Following your recommendations we have updated the epidemiologic reference changing the previous to a new one:

Santulli G. Epidemiology of Cardiovascular Disease in the 21 Century: update numbers and update facts. J Cardiovas Dis 2013;1:1-2.

- 2. Some clarifications needed: CA 125 was drawn only once…..
Only one determination of CA 125 was measured at entering in the study, no further determinations during follow-up were performed.
We agree that in the case of BNP, that a change from baseline might be better predictor of outcome, especially in hospitalized patients with worsening HF. The change in BNP from admission to discharge was describe as a better predictor of prognosis. However, we included outpatients followed in a heart failure unit and we don't do routine sequential determinations of BNP, the repeated determinations are reserved for individualized cases.

- 3. you mentioned "cost" as an advantage over BNP three times in study, but do not actually address it. Need to do so.
The low cost of CA 125 determination has been reported previously. In page 4 we have added a reference in relation to the low cost of CA 125:
Vizzardi E, Nodari S, D'Aloia A, Chiari E, Faggiano P, Metra M, Dei Cas L. CA 125 tumoral marker plasma levels relate to systolic and diastolic ventricular function and to the clinical status of patients with chronic heart failure. Echocardiography. 2008;25:955-60

In page 9 we have suppressed the sentence:
…The advantages of CA 125 determination would be its greater stability as a biomarker and low cost….
and added
Therefore the addition of Ca 125 may help to improve prognosis assessment in patients with stable heart failure

In page 10 .

….Its easy determination and low cost may encourage its expanded use.

We considerer as a possibility that the use of CA 125 determination added to NT-proBNP will not significantly increased the global cost of HF assessment

- 4. Clarification for myself - page 6, CA 125 was negatively associated with sodium concentrations

We agree with you that the text was misleading we have clarified it by changing the sentence to:

…) and negatively with left ventricular ejection fraction (ß=-0.23, p=0.003) and sodium concentration (ß=-0.24, p=0.003).

- 5. page 8, two of the conclusions as pointed out in the attached document – are speculations rather than conclusions that can be drawn from the study data. Looking at the models-…..

The observation is well received. Therefore, we add a new table of baseline characteristics according to the combined category of CA 125 and NT-proBNP.
The additional value of CA 125 combined with NT-proBNP vs NT-proBNP alone was assessed by the integrated discrimination improvement (IDI) index, as we pointed out in methods and reported in results (6-7 pages).
The value of CA-125 alone is reported in hazard ratio terms (table 1) in a multivariate cox model (model 2, Harrell's C-statistic 0.730).

Finally, we agree with the reviewer comment **"Patient with elevated CA125, but negative BNP value was not significantly different than patient with negative Ca125 and negative BNP"**
However, we will like to point out that it is in this group, the smallest group and the closest to the referent group, where the lack of statistical power is more evident, but as it is written in the limitation sections
"Although the cohort was relatively small, mortality and hospitalization rates were similar to previous studies analyzing stable HF patients. When the study population was divided according to CA 125 and NT-proBNP values, some subgroups were quite small. Nonetheless, increased CA 125 concentration was very effective in identifying stable HF patients at high risk of death and new admissions for worsening HF, and despite having a relatively small sample size the results are consistent with those obtained in previous studies in acute HF.**"**
Finally, we could not achieve a design study with balanced big 4 groups with CA125 and NT-proBNP combination, which could present better statistical power.

4) **Review 4: reviewed by 00060499.**

I am writing to thank you for revising our manuscript. As a result of your suggestions we have review the following items:

- We have modified the methods section:

The population was a prospective cohort of 156 patients diagnosed with HF and remitted to the outpatient heart failure unit for monitoring, from 2009 to 2011. Patients were included in the study if they had a previous documented episode of heart failure and received heart failure treatment. The diagnosis of HF was made following the Clinical Practice Guidelines of the European Society of Cardiology[20]. Heart failure treatments included angiotensin-converting-enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARB) if ACEI were contraindicated, betablockers, diuretics, and aldosterone antagonists, with individualized assessment of treatment indication and optimized doses per recommendations of the Clinical Practice Guidelines of the European Society of Cardiology[20]…

- We have used the terms HFpEF and HFrEF for referring to patients with preserved EF (≥50%) or reduced EF (<50%) throughout the manuscript.

- A reference has been added referring to the EF value that differentiate preserved or reduced EF

Nguyen PK, Schnittger I, Heidenreich PA. A comparison of echocardiographic measures of diastolic function for predicting all-cause mortality in a predominantly male population. Am Heart J. 2011;161:530-7.

- Global % of pts under BB treatment was 58%. However, the % of pts taking BB in the group of HFrEF, that is the group with BB indication was 76%. The same for aldosterone, in the group of HFrEF the % of pts under aldosterone therapy was 56%.

The different treatment in both groups: HFrFE anf HFpEF has been added to the text

…The percentage of patients with HFrEF treated with ACEI/ARB was 85%, with beta-blockers 76% and with mineralocorticoid receptor antagonists 56%, while in the HFpEF group the percentage of patients treated with ACEI/ARB was 69%, with beta-blockers 42%,and with mineralocorticoid receptor antagonists 39%. The percentage of patients treated with furosemide was similar in both groups 84%.

- References and typesetting were corrected.