**Author's response to reviews**

**Title:** The Top Tertile of Hematocrit Change During Hospitalization is Associated with Lower Risk of Mortality in Acute Heart Failure Patients

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**Author’s response to reviews:**

Response to Reviewer 1:

Susana Ravassa (Reviewer 1): The authors have investigated the prognostic value of the hematocrit (HCT) change during hospitalization in patients with acute heart failure (AHF). The authors have categorized 510 patients hospitalized for AHF by tertiles of the HCT change measuring this parameter at baseline and discharge or close to day 7. The authors conclude that an increase in HCT during hospitalization is associated with lower risk of all-cause mortality.

Major concerns

1. There are several spelling and grammatical errors. Please have the manuscript reviewed by an expert in scientific English.

Response: Thank you for pointing this out. The manuscript has been thoroughly reviewed for spelling and grammatical errors.

2. It is already known that hemoconcentration is associated with better prognosis. The authors justify the novelty of the current study on the fact that the analysis of the prognostic potential of the HCT change by tertiles has not been previously approached. I do not believe this is enough justification. I think that the authors should reinforce this particular aspect.
Response:

Introduction:

A 2-classification system based on the presence or absence of hemoconcentration was standard in previous studies (reference 8-13). These studies established that hemoconcentration is associated with favourable prognostic outcomes. These criteria failed to differentiate between patients with no change in HCT and those with hemodilution. However, whether patients with a largely unchanged or decreased HCT values (no change in volume status or hemodilution) experience the same mortality risks or benefits as those with hemoconcentration respectively remained unclear.

Clinically, delta-HCT presents as a spectrum varying from hemoconcentration, through a state of no change, to hemodilution. We used a 3 classification system to better represent this spectrum and characterize the no-change group. In support of previous studies our study demonstrated that hemoconcentration is associated with lower risk of all-cause mortality. In addition, we also found that patients in the NC and hemodilution strata of the spectrum showed no difference in the risk for all-cause mortality, and were both at a higher risk. This is of clinical significance because it suggests that patients with small/no changes in delta-HCT should also be treated with intensive diuretic therapy, equivalent to those with hemodilution.

Additionally, our study had a longer follow up than previous studies (18.9 months vs. 90-day mortality(Breidthardt), 180-day all-cause mortality(Van der Meer), 180 days(Testani), 9.9 months(Greene), 347days(J.Oh), 461 days(Davila).

The value of this study lies in-

1). The additional characterization of patients with unchanged and decreased HCT values.

2). The length of the follow-up

We have added this to the background and discussion section: 1). “However, whether patients with a largely unchanged or decreased HCT values (no change in volume status or hemodilution) experience the same mortality risks or benefits as those with hemoconcentration respectively remained unclear.”. 2). “Previous research excluded NC or hemodilution, therefore the mortality rate in these subgroups were unknown.”

3. It is somehow confusing that the authors provide hazard ratios for the risk of mortality in patients with hemodilution or no change in HCT, considering those with hemoconcentration as the reference group, whereas in the conclusion the authors single out the lower risk of all-cause mortality for hemoconcentration (no specific data on the HR for the risk of mortality for hemoconcentration is provided). Either the authors change the conclusion by focusing the message in those patients with a higher risk of mortality (hemodilution or NC) as compared with hemoconcentration, or repeat the Cox regression analysis providing a HR for patients with hemoconcentration, taking hemodilution (or hemodilution plus NC) as the reference group.
Response: We have revised the manuscript according to reviewer’s suggestions. As is presented in revised table 2 (the original table was deleted), each of the 3 groups were taken as reference groups in the Cox regression analysis. The abstract(result) and Results section have revised accordingly.

4. The univariate analyses should consider medications at discharge as potential confounding factors to be included in the multivariate model.

Response:

1) We have revised according to reviewer’s suggestion. Originally, variables with >5% missing values, which included medications at discharge, were not included in the univariate analysis.

2) Missing values for medications at discharge were > 5% but <10%. In order to accommodate medications at discharge, we changed the criteria in the Statistical analysis section to >10% to accommodate this variable.

3) The revised results are reported in revised table 2.

5. The analyses proposed by the authors are based on tertiles of the HCT change between two time points: baseline and discharge (or close to day 7). In this regard, a table should be provided including changes in relevant clinical or echocardiographic parameters (e.g. ΔNYHA class, ΔLVEF, ΔNT-proBNP etc.), to obtain more information on the clinical and echocardiographic progression of patients between baseline and discharge by tertiles of the HCT change.

Response:

1) This is a very pertinent question. Owing to the retrospective nature of this design it was difficult to ensure that HCT, NYHA class, LVEF and NT-proBNP levels were available at both time points. Other papers published on this topic also failed to accommodate this data (e.g. JACC 2013;61:1973-81, International Jounal of cardiology 2013;168:4739-4743, J Cardiac Fail 2011;17:1018-1022).

2) This information was added to the limitations section of the paper: “Finally, longitudinal data on the change of relevant clinical or echocardiographic parameters (NT-proBNP, NYHA class, LVEF) over time was not available in our study.”.

6. In line with the previous comment, it is important to determine whether changes in the clinical and echocardiographic parameters of interest between baseline and discharged predict risk of death and therefore should be considered in the multivariate Cox regression models. For instance, have the patients that exhibit hemoconcentration at discharge experienced a higher improvement in LVEF? Does this factor explain the lower risk of death?

Response: Please refer to the comment no. 5.
7. In addition, if the HCT is proposed as a biomarker independently associated with mortality, it is necessary to demonstrate that this determination improves the classical predicting factors included in the basal models. For instance, does the change in HCT predict mortality with a higher efficacy that, for instance, changes in the NYHA class or LVEF or eGFR? In fact, these analyses could reinforce the novelty aspects mentioned in comment #2.

Response:

1). In previous research addition of HCT to the model has already been found to improve the prediction of mortality over classical predicting factors included in the basal models (NYHA class, LVEF, eGFR etc…) Greene et al. also used HCT to define hemoconcentration and demonstrated the feasibility of HCT in such a context.

2). Moreover, in our study after multivariable adjustment with NYHA class, LVEF, eGFR etc…, change in HCT remained a predicting factor.

Minor comments,

1. It is necessary to state whether the Cox models fulfil the proportional-hazards assumption.

Response:

1) we tested the proportional hazards assumption by using R, and found that all P >0.05. Thus, the analysis of time dependent Cox model is not needed.

2) This information was added to the Statistical analysis section of the revised paper: "Cox models fulfilled the proportional-hazards assumption (using R).”.

2. The sentence in the Abstract conclusion: "… independent risk factor of survival" has no sense.

Response: we have deleted this sentence.

3. CRP needs definition at the bottom of Table 1.

Response: Thank you for pointing out this flaw. We have added the full form of CRP.

Response to Reviewer 2:

Johann Auer (Reviewer 2): Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of
This study investigated whether changes in volume status among patients hospitalized with acute heart failure (AHF) and is associated with a favorable outcome.

The authors enrolled 510 consecutive patients hospitalized for AHF and hematocrit (HCT) levels were measured at admission and discharge or close to day 7.

Based on the results the authors conclude that in patients hospitalized with AHF, changes of HCT concentration during hospitalization is a significant independent predictor of survival. An increase in HCT during hospitalization is associated with lower risk of all-cause mortality relative to no change or a decrease in HCT.

No change or a decrease in HCT showed no difference in risk for all-cause mortality.

The paper is well written. However, there are some major points that have to be addressed:

1. Previous studies including the paper by Testani and colleagues (J Am Coll Cardiol. 2013 Aug 6; 62(6): 516-524.) report similar findings. Additionally, this paper found that only hemoconcentration occurring late in the hospitalization was associated with improved survival. Did the authors find similar results?

The data should analysed for this remarkable finding.

Response:

1) We agree with their results. However, considering that two studies (J Am Coll Cardiol. 2013 Aug 6; 62(6): 516-524. vs. EUR J HEART FAIL 2017, 19(2):226-236. ) have explored the role of timing of hemoconcentration on mortality in HF patients, we did not perform this analysis.

2) We have cited one of these studies in the background section in this revision of the manuscript: “Subsequently, Testani et al demonstrated that the subset of patients experiencing late hemoconcentration during hospitalization achieved better survival [14].”.

2. Which are the novel findings of this study? Is this study confirmatory only?

This should be thoroughly discussed.

Response:
Introduction:

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Clinically, delta-HCT presents as a spectrum varying from hemoconcentration, through a state of no change, to hemodilution. We used a 3 classification system to better represents this spectrum and characterize the no-change group. In support of previous studies our study demonstrated that hemoconcentration is associated with lower risk of all-cause mortality. In addition, we also found that patients in the NC and hemodilution strata of the spectrum showed no difference in the risk for all-cause mortality, and were both at a higher risk. This is of clinical significance because it suggests that patients with small/no changes in delta-HCT should also be treated with intensive diuretic therapy, equivalent to those with hemodilution.

Additionally, our study had a longer follow up than previous studies (18.9 months vs. 90-day mortality(Breidthardt), 180-day all-cause mortality(Van der Meer), 180 days(Testani), 9.9 months(Greene), 347days(J.Oh), 461 days(Davila) ).

The value of this study lies in-

1). The additional characterization of patients with unchanged and decreased HCT values.
2). The length of the follow-up

We have added this to the background and discussion section: 1). “However, whether patients with a largely unchanged or decreased HCT values (no change in volume status or hemodilution) experience the same mortality risks or benefits as those with hemoconcentration respectively remained unclear.”. 2). ” Previous research excluded NC or hemodilution, therefore the mortality rate in these subgroups were unknown.”

3. The design of this study cannot confirm causality. This, any findings are hypothesis generating. This should be clearly stated, thoroughly discussed and added to the limitations section.

Response: Thank you for your suggestion, we added this to the limitations section: “Due to the observational nature of the study, it is impossible to confirm causality.”

4. The authors should add information on diuretic doses used in individual patients. Did diuretic dose (any possibly renal function) significantly correlate with HCT (and outcomes)?
Response:

1) This is a very pertinent question. We agree that diuretic doses are important. However, data on diuretic doses were not available in the current study. Van der Meer et al. found that total dose diuretics was lower in patients with hemoconcentration (J AM COLL CARDIOL 2013, 61(19):1973-1981.) Several similar studies also failed to report these findings (e.g. (EUR J HEART FAIL 2017, 19(2):226-236.), (EUR J HEART FAIL 2013, 15(12):1401-1411.)…).

2) We have added this to the limitations section.

5. Did the authors perform sample size calculation?

Response:

For cox regression, sample size should $\geq 15$ times the number of variables. We were able to satisfy these standards.