Title: Association between Extracellular Volume Excess and Renal Outcomes in Patients with Chronic Kidney Disease: Retrospective single-center cohort study

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Author's response to reviews: see over
May 20, 2014  
Re: MS: 1712093473122169  

Dear Dr. Liffert Vogt:

Enclosed please find a revised version of the manuscript (MS: 1712093473122169) entitled “Association between Extracellular Volume Excess and Renal Outcomes in Patients with Chronic Kidney Disease: Retrospective single-center cohort study” by Tai et al. We thank the reviewers and editor for their careful analysis of our work. Their comments have led to alterations that have significantly enhanced the quality of our manuscript. A point by point response to each comment is outlined below.

**Responses to Reviewer 1 comments:** We thank the Reviewer 1 for the positive and constructive suggestions.

1. **Regarding the rationale for using the ECW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio as a parameter of volume overload:** We agreed with the Reviewer 1 that the reference had used the ratio of measured TBW\textsubscript{(BIA)} to estimated TBW\textsubscript{(Watson)} but not the ECW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio as an adequate marker of volume overload (reference 8). We had compared the TBW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio with the ECW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio and had shown renal survival curves by the tertiles ratio of TBW\textsubscript{(BIA)} to TBW\textsubscript{(Watson)} in Supplementary Figure S1 and then regarded the ECW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio as a better marker of volume overload than the TBW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio. The measured TBW\textsubscript{(BIA)} includes the both of ECW content and ICW content, which is a serious drawback because ICW content decreases with aging (Supplement Figure 1). Besides ECW excess, decreased ICW content increases the ECW\textsubscript{(BIA)}/TBW\textsubscript{(BIA)} ratio. Therefore, the TBW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio may include the decreased ICW content. We addressed to have had modified the TBW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio to the ECW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio in the manuscript.
The ECW\textsubscript{(BIA)}/TBW\textsubscript{(BIA)} ratio is very interest marker for assessing fluid volume imbalance between intra- and extracellular water. The ECW\textsubscript{(BIA)}/TBW\textsubscript{(BIA)} ratio is 1:3 and then is gradually changing to 2:5 with aging in healthy subjects (Supplement Figure 2). In fact, the marker is associated with adverse outcomes in the references 2–7 and even in our study population. However, the increased ECW\textsubscript{(BIA)}/TBW\textsubscript{(BIA)} ratio does not always mean volume overload. Importantly, the ratio between ICW and ECW is not 2:1 in lean elderly subjects. The adverse outcomes with the increased ECW\textsubscript{(BIA)}/TBW\textsubscript{(BIA)} ratio is associated with the decreased ICW content with aging.
2. Regarding the underlying renal disease: We have modified the table 1 as suggested.

3. Regarding the relationship between measured TBW\textsubscript{(BIA)} and estimated TBW\textsubscript{(Watson)}: This is a very good point raised by the Reviewer 1. The estimated TBW by Watson formula expects the expected value and the measured TBW by the BIA device includes the unexpected value. We think that the unexpected values are mediated by fluid volume excess in patients with CKD. As reviewer 1 indicated, the larger TBW\textsubscript{(BIA)} had the larger difference in TBW\textsubscript{(BIA)} and TBW\textsubscript{(Watson)} in manuscript Figure 1. This is the main concept in this present study. We have mentioned this in the discussion session.

4. Regarding the correlation of body weight with fluid volume status: First, fluid volume increases when body weight gains regardless of whether volume overload exists or not. Second, both intra- and extracellular water volumes increase with body weight, although the trend was predominantly observed in ICW content and vice versa (supplement figure 3A). This result showed that the ECW\textsubscript{(BIA)}/TBW\textsubscript{(BIA)} ratios had a weak negative correlation to body weight ($r = -0.237$, $P = 0.004$) (Supplement Figure 3B). Thus, both markers of the ECW\textsubscript{(BIA)}/TBW\textsubscript{(BIA)} ratio and the ECW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio have the weak correlation of body weight.
The estimated TBW by Watson formula is adjusted for age, height, and body weight can minimalize the above issues. Consequently, we have showed the correlation between BMI and the ECW\textsubscript{(BIA)/TBW\textsubscript{(Watson)}} ratio in Figure 2. After adjusting for covariate, BMI was independently associated with the ECW\textsubscript{(BIA)/TBW\textsubscript{(Watson)}} ratio (Table 2). As a reference, we present the correlation of BMI with the ECW\textsubscript{(BIA)/TBW\textsubscript{(BIA)}} ratio by sex (Supplement Figure 4). There was no significant correlation between these two parameters.
5. **Regarding change in the main parameter**: Thank you for the reviewer 1’s suggestion. We agree with the Reviewer 1 if we have not sought a parameter for volume overload. However, we’d like to stick to the ECW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio because we believe that the ECW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio can express the degree of deviation from expected body composition better than the ECW\textsubscript{(BIA)}/TBW\textsubscript{(BIA)} ratio does. In addition, we do not have the Body Composition Monitor device that can compare measured ECW with expected ECW. This device may be able to assess the association of volume overload with adverse outcome in future study.

6. **Regarding the association of serum albumin with the ECW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio**: We agree with the Reviewer 1 that this association adds clarity to the study. We now provided the correlation of serum albumin to the ECW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio as Supplementary Figure S2. It may strongly support for our hypothesis that lower serum albumin has a significant correlation to fluid volume.

7. **Regarding the association between proteinuria and the ECW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio**: This is an excellent point. Proteinuria had a positive correlation to the ECW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio. After adjustment for covariates including proteinuria, the ECW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} was found to be significantly associated with adverse renal outcomes (Manuscript Table 3).

8. **Regarding the concern in the second paragraph**: Please refer to an above-mentioned point #1 regarding the rationale for using the ECW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio as a parameter of volume overload. We now caution the reader about this in the second paragraph of the Discussion section.

9. **Regarding the comparison between ICW, ECW, and TBW**: We agree that we did not directly study the comparison between ICW, ECW, and TBW. We have therefore showed changes in an imbalance between ICW and ECW with aging in Supplementary Figure S1 online.

10. **Regarding the description of expected ECW by new device based on data from healthy volunteers**: We described that the new device quantified fluid overload using reference values derived from the pooled data of healthy subjects. Therefore, it is unclear whether determination of adequate fluid balance is the same in healthy individuals and in those with disease. - Reviewer 1 pointed out that the TBW\textsubscript{(Watson)} values also are derived from healthy volunteers. In this regard, we fully agree with the Reviewer 1 that the ECW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio is also quantifying fluid overload using reference values. We
have therefore amended the discussion section.

**Responses to Reviewer 2 comments:** We thank the reviewer for the positive comments about our work.

1. **Regarding the rationale for using the ECW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} ratio as a parameter of volume overload:** Please refer to answers provided to the Reviewer’s point #1. We have modified our manuscript in the Introduction section to explain this point.

2. **Regarding mortality in this study periods:** This is a very good point raised by the Reviewer 2. There were no significant differences in cumulative survival by the ECW\textsubscript{(BIA)}/TBW\textsubscript{(Watson)} tertile and also by the ECW\textsubscript{(BIA)} tertile in body weight (Supplement Figure 5). We reviewed clinical charts until August 2013 (median, 1789 days [10\textsuperscript{th}–90\textsuperscript{th} percentile, 422–2689 days]) unless loss to follow-up or all-cause death occurred. We have now created the Kaplan-Meier survival curves for all-cause mortality as Supplementary Figure S4 online.

3. **Regarding the exclusion criteria and the representation of the sample in relation to fluid body composition:** We had 21 patients with incomplete clinical data in whom we could not assess anthropometric measurements, blood pressure, proteinuria, and kidney function at the same time. We have modified our manuscript in the Methods section to explain this point. In addition, we show that there are no significant differences in fluid body composition between two groups.
<table>
<thead>
<tr>
<th>Body composition</th>
<th>Patients with lack of data $n=21$</th>
<th>Patients with complete data $n=149$</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBW$_{\text{BIA}}$, L</td>
<td>33.9 ± 8.0</td>
<td>31.2 ± 6.8</td>
<td>0.105</td>
</tr>
<tr>
<td>ICW$_{\text{BIA}}$, L</td>
<td>20.7 ± 4.9</td>
<td>18.9 ± 4.3</td>
<td>0.145</td>
</tr>
<tr>
<td>ECW$_{\text{BIA}}$, L</td>
<td>13.3 ± 3.3</td>
<td>12.3 ± 2.6</td>
<td>0.200</td>
</tr>
<tr>
<td>Percentage of ECW$<em>{\text{BIA}}$ to TBW$</em>{\text{Watson}}$</td>
<td>39.9 ± 5.4</td>
<td>39.2 ± 3.5</td>
<td>0.553</td>
</tr>
</tbody>
</table>

4. **Regarding the definition of adverse renal outcomes**: This is an excellent point. If GFR decrease $\geq$ 50% from baseline, CKD stage advance to the next stage. This definition is used in some recent studies (PMIDs 24206458 and 22846257). We have now quoted these studies in our manuscript.

**Responses to Reviewer 3 comments**: We thank the Reviewer 3 for the positive and constructive suggestions.

1. **Regarding the other parameters in relation to fluid volume overload**: We agree with the Reviewer 3 that this present study is limited by the lack of data of NT-proBNP. We have showed that the ECW$_{\text{BIA}}$/TBW$_{\text{Watson}}$ ratio was associated with many factors in relation to fluid volume overload including resistant hypertension, lower eGFR and serum albumin, higher levels of proteinuria, and higher frequency of furosemide use. This suggests that fluid excess caused hemodynamic instability. We added a comment to the limitation paragraph to acknowledge this.

2. **Regarding the association between uric acid and fluid volume status**: According to the Reviewer 3’s comment, we analyzed about the correlation between uric acid and the ECW$_{\text{BIA}}$/TBW$_{\text{Watson}}$ Ratio. However, these had no significant correlation in the linear regression analysis ($r = 0.044$, $P = 0.60$).

3. **Regarding the association between congestive heart failure and the ECW$_{\text{BIA}}$/TBW$_{\text{Watson}}$ ratio**: We found that 5 patients had congestive heart failure during mean 4.9 year periods. These patients were categorized in Tertile 2 and 3 of the ECW$_{\text{BIA}}$/TBW$_{\text{Watson}}$ ratio; however, there was no significant association between congestive heart failure and the ECW$_{\text{BIA}}$/TBW$_{\text{Watson}}$ ratio in this present study ($P = 0.07$).

4. **Regarding the data on usage of NSAIDs**: Thank you for pointing this out. However, we have no data to explain this point.

5. **Regarding the associations of urinary creatinine excretion and body surface area with**
**fluid volume status:** According to the Reviewer 3’s comment, we added the data to Table 1 to show these additional parameters. However, these had no significant correlation in the linear regression analysis.

6. **Regarding the obesity paradox in patients with CKD (Why was obesity paradox seen in only patients with moderate CKD?):** This is an excellent point. As seen in Supplement Table 2, fluid volume can be distributed on the basis of age, sex, and body size in everyone including healthy subjects. If they have fluid volume excess, probably the excessive fluid volume is redistributed to ECW like the second column in Supplement Table 2. Thus, we assume that there are differences in the baseline fluid volume distribution and in the fluid volume redistribution when they are exposed to fluid accumulation. This tentative theory becomes obvious when patients have fluid volume imbalance status, including CKD and heart failure.

**Supplement Table 2**

<table>
<thead>
<tr>
<th>Cases</th>
<th>Baseline TBW</th>
<th>+3.0L volume excess from baseline TBW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICW</td>
<td>ECW</td>
</tr>
<tr>
<td>40 years old, Male sex, BW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>108kg, TBW 50% in BW, ICW:ECW = 64:36</td>
<td>54L</td>
<td></td>
</tr>
<tr>
<td>40 years old, Male sex, BW</td>
<td>34.56L</td>
<td>19.44L</td>
</tr>
<tr>
<td>70kg, TBW 60% in BW, ICW:ECW = 62:38</td>
<td>42L</td>
<td></td>
</tr>
<tr>
<td>40 years old, Female sex, BW</td>
<td>26.04L</td>
<td>15.96L</td>
</tr>
<tr>
<td>BW 60kg, TBW 50% in BW, ICW:ECW = 62:38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70 years old, Male sex, BW</td>
<td>18.6L</td>
<td>11.4L</td>
</tr>
<tr>
<td>48kg, TBW 50% in BW, ICW:ECW = 60:40</td>
<td>24L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16L</td>
<td>8L</td>
</tr>
</tbody>
</table>

7. **Regarding the P for Trend in Table 1:** In accordance with the suggestion of Reviewer 3, statistical significance has been reassessed using a linear regression model to compare the mean values of possible risk factors between the tertile groups (PMID: 24529154) and were
checked by one-way analysis of variance for continuous variables and the Pearson chi-squared test for categorical variables.

8. **Regarding the differences in TBW\textsubscript{(Watson)} among individuals of different races:** We agree with the Reviewer 3. Some studies pointed out the differences in TBW\textsubscript{(Watson)} among individuals of different races (PMIDs: 24145829 and 24145829). In the other hand, the estimated TBW for Asians has not been established; whereas the estimated TBW\textsubscript{(Watson)} is a parameter that is widely used in this area. Our proposal marker was associated with many factors in relation to fluid volume status, including age, diabetes mellitus, resistant hypertension, lower eGFR and serum albumin, higher levels of proteinuria, and higher frequency of furosemide use. We believe that the ECW\textsubscript{(BIA)}/TBW\textsubscript{(BIA)} ratio is an acceptable marker as ECW volume excess.

9. **Regarding the association of the ECW\textsubscript{(BIA)}/TBW\textsubscript{(BIA)} ratio with adverse renal outcomes:** It was not surprising that the ECW\textsubscript{(BIA)}/TBW\textsubscript{(BIA)} ratio was associated with adverse renal outcomes (Supplement Figure 5). However, the measured TBW\textsubscript{(BIA)} includes the both of ECW content and ICW content, which is a serious drawback because ICW content decreases with aging (Supplement Figure 1). Besides ECW excess, decreased ICW content increases the ECW\textsubscript{(BIA)}/TBW\textsubscript{(BIA)} ratio. The ECW\textsubscript{(BIA)}/TBW\textsubscript{(BIA)} ratio is very interest marker for assessing fluid volume imbalance between intra- and extracellular water. However, this maker does not express ECW volume excess. We have mentioned this in the discussion session.
10. **Regarding the lowest mean BMI in the second highest tertile of the ECW\(_{(BIA)}\)/TBW\(_{(Watson)}\) ratio:** This is a very good point raised by the Reviewer 3. The ECW\(_{(BIA)}\)/TBW\(_{(BIA)}\) ratio had a weak positive correlation to BMI. Nevertheless, the lowest mean BMI was observed in the second highest tertile of the ECW\(_{(BIA)}\)/TBW\(_{(Watson)}\) ratio. In this regard, we thought that the obvious fluid volume overload led to increase body weight in the highest tertile of %ECW\(_{(BIA)}\)/TBW\(_{(Watson)}\). Probably, leaner or elderly patients with CKD are more susceptible to volume overload. However, BMI become less and less associated with fat and macule with the increasing fluid volume. We added a comment to the discussion paragraph to acknowledge this.

11: **Regarding the estimated GFR by CKD-EPI equation:** In accordance with the suggestion of Reviewer 3, we reanalyzed all data sample by using CKD-EPI equation. This result was similar in both equations. We amended our Methods section and Table 2 to reflect this.

12: **Regarding the sort title:** Thank you for pointing this out. We have amended short title.

13: **Regarding the following word; ‘tend to be male’:** In accordance with the suggestion of Reviewer 3, we reanalyzed statistical significance using a linear regression model. As a result, the increased tendency of male sex was observed in the higher tertile of %ECW\(_{(BIA)}\)/TBW\(_{(Watson)}\).

14: **Regarding the following word; ‘followed up until august 2013’:** Thank you for pointing this out. We have amended abstract.