Reviewer’s report

Title: Cardio-renal syndrome revealed increased neurohormonal activity, tubular and myocardial damage respect to heart failure patients with preserved renal function

Version: 2
Date: 10 January 2014

Reviewer: Carmelina Ariano

Reviewer’s report:

Comment by Carmelina Ariano

The paper is very interesting, but it shows some shortcomings which are listed below.

Major Compulsory Revisions

General considerations

-An aspect which should be clarified consists in the fact that for some of the measured markers (for example, osteoprotegerin), reliable and well encoded reference intervals are not available, so the choice of creating specific reference ranges for your laboratory through the enrollment of a control sample consisting of healthy subjects would have been desirable. I presume that this has not been done due to logistical and financial problems (need of avoiding further financial burden in addition to the already high costs of the kits?). Nevertheless, reporting this shortcoming in the text would perhaps be appropriate. In other words, the decision of emphasizing among the study limitations the lack of any comparable sample of apparently healthy subjects able to provide the reference range of biochemical parameters, for which encoded "ranges" of normality are unknown or unavailable, would perhaps be appropriate. Please make this adjunct in the text (for instance, in the Section “Study limitations).

-Conversely, the reference values for serum BNP are known; so, reporting them in the section “Methods” would be appropriate, due to the fact that the serum BNP at admission was included as part of the criteria for making the diagnosis of ADHF. By contrast, in the text of Methods, it is laconically stated that patients should have received a diagnosis of ADHF, so as to be considered for the inclusion in the study, if serum B-type natriuretic peptide was "greater than the upper limit of normal for age". Please report the adopted cut-off for serum BNP, which was used as diagnostic criterion for heart failure in the present study.

-Results “In all patients we found a significant inverse correlation between GFR and BNP (r = - 0.14), GFR and IL6 (r = - 0.16), GFR and NGAL (r = - 0.35), GFR and TnT (r = - 0.71), GFR and BUN (r = - 0.80) and GFR and BUN creatinine ratio (r = - 0.61)".

It is strange that the comparison has been established between all patients (n.246 patients), namely the sum of ADHF patients presenting with renal dysfunction plus those renal dysfunction-free, and the group with cardiorenal
 syndrome (n.126 patients). On the contrary, a comparison should be made
between the patient group with heart failure and normal eGFR (n.120 patients)
and the one with patients suffering from cardio-renal syndrome (n.126 patients).
Therefore, please redo the calculations or alternatively please rectify the text.

- Results “In all patients we found a significant inverse correlation between GFR
and BNP (r= - 0.14), GFR and IL6 (r= - 0.16), GFR and NGAL (r= - 0.35), GFR
and TnT (r= - 0.71), GFR and BUN (r= - 0.80) and GFR and BUN/ creatinine ratio
(r= - 0.61). In the CRS group we found a significant inverse correlation between
GFR and BNP (r= - 0.18), IL-6 (r= - 0.21), BUN (r= - 0.70) (Figure 1), TnT (r= -
0.41) and BUN / creatinine ratio (r= - 0.46); in this group we didn’t find a
significant correlation between GFR and NGAL. (Table 3)”

In my opinion, there is a paramount misunderstanding which deserves to be
evidenced. In truth, the fact that in many cases the correlations found are in
reality weak, although they attain the threshold of statistic significance, set at a
p-value of <0.05, should be highlighted. Actually, the r-value is higher than - 0.6 (clinically relevant correlation) only in some cases, namely in the cases of
correlations that involve GFR and TnT (r= - 0.71), GFR and BUN (r= - 0.80) and
GFR and BUN/ creatinine ratio (r= - 0.61), when the values of the entire
population are taken into account. As regards the CRS group, the r – value
exceed - 0.6 only in the case of the correlation involving GFR and BUN (r =
-0.70). In truth, several authors have offered guidelines for the interpretation of a
correlation coefficient. Cohen (1988) has observed, however, that all such criteria
are in some ways arbitrary and should not be observed too strictly. The
interpretation of a correlation coefficient depends on the context and purposes. A
correlation of 0.8 may be very low if one is verifying a physical law using
high-quality instruments, but may be regarded as very high in the social sciences
where there may be a greater contribution from complicating factors. A table
summarizing these concepts[ source: Wikipedia (“Pearson product-moment
correlation coefficient”) is represented below:

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>None:</td>
<td>from - 0.09 to 0.0 and from 0.0 to 0.09</td>
<td></td>
</tr>
<tr>
<td>Small:</td>
<td>from - 0.3 to -0.1 and from 0.1 to 0.3</td>
<td></td>
</tr>
<tr>
<td>Medium:</td>
<td>from -0.5 to -0.3 and from 0.3 to 0.5</td>
<td></td>
</tr>
<tr>
<td>Large:</td>
<td>from -1.0 to -0.5 and from 0.5 to 1.0</td>
<td></td>
</tr>
</tbody>
</table>

On this basis, the entire study design should be re-thought. In fact it seems to me
that an useful informative content cannot be derived from the presentation of a
number of correlation values which in the majority of cases are rather feeble,
although corroborated by the fact that the significance threshold (p <0.05) is
reached.

-Cox proportional hazards’ regression analysis was not performed. However, it
would have been easy to set up, by using as outcome variable the diagnosis of
cardiorenal syndrome, made on the basis of the criterion of a reduced eGFR (<50
ml / min / 1.73 m2) in the heart failure clinical scenario, and by assuming as
exposure variables the clinical and laboratory parameters of interest, the latter
expressed either as dichotomous (e.g., NGAL levels > 113.5 ng/ml) or as continuous variables. Please use the tabulated data that you possess, to build a regression model. Subsequently, please compare the results with the already drawn ROC curves.

-Discussion …“The findings of the current study can be summarized in three main points: 1- Our data demonstrated that, beyond the traditional laboratory parameters consisting in creatinine and GFR measurement, some others laboratory markers could help in early identification of patients with CRS and RI. ...

In this regard, it would perhaps be useful some adjunctive information about the number of ADHF patients in which, using any of these biomarkers (NGAL, TnT and / or BUN) you could acquire additional prognostic information, such as in the case of patients with high levels of NGAL that are subsequently found affected by renal damage even in the presence of e GFR > 50ml/min / m2 and serum creatinine <1.4 mg/dl at hospital admission. However, this could only happen in case a proper clinical and instrumental follow-up had been planned. Please highlight this shortcoming in the section "Study limitations".

Other remarkable mistakes emerged from the careful reading of the text.

-Abstract: "The correlation... was evaluated by Receiver Operating Characteristics (ROC) curve..."

Please consider that a correlation can be identified by means of a regression model, whereas a ROC curve measures the diagnostic accuracy of a parameter. Please rework the text accordingly.

- In the methods of the Abstract: Please provide the measure unit for each biomarker. This information has been omitted across the entire text of the abstract.

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Methods

- “Patients with these laboratory values below the cut-offs were defined as HF (n=120) and patients with values above the cut-offs were defined as CRS (n=126)”.

Please rework the sentence. This is necessary since the patients whose GFR values exceed 50ml/min/1.73 m2 shouldn't be labeled CRS patients; by contrast, they would be comprised within the CRS category according to your definition, which erroneously collates under the CRS label all patients whose values are "above the cut-offs".

- Laboratory analysis. If I am not mistaken, the sentence should be corrected as follows: " For these products..." instead of: " These products..."

- Statistical analysis. Please correct: " Continuous variables were expressed as mean ± standard deviation if normally distributed, and as median plus interquartile range(IQR) if non-normally distributed. Normality was assessed by
the Kolmogorov–Smirnov test. Continuous variables were compared using t-test for independent groups. "At this point you should specify what method was used for possible comparison between not normally distributed variables.

- Results

The term "significant increase" should be removed and the sentence should be rewritten as follows: "BNP was significantly higher in CRS group compared with HF group" and so on...

- Discussion row 263: please correct: "... could help to timely diagnose and understand the pathophysiological..."

row 345: please correct: “underlying”

row 354: please correct: "pattern"

- Figures. Figure 2 Please add the corresponding AUC (area under the curve) value under each of the four ROC plots in order to provide adequate information about the diagnostic accuracy of each investigated parameter. Pointing out even in each figure caption that a GFR<50 ml/min/1.73 mq was adopted as gold standard for CRS would also be appropriate.

- Tables Table 3 The table 3 doesn't demonstrate any correlation between NGAL and eGFR. This would contradict the important role which is attributed to NGAL as a marker of renal dysfunction; alternatively, this would mean that NGAL is an exclusive marker of tubular injury, so as to not be increased due to a mere isolated deterioration of glomerular filtration. Please comment on this issue within the text, if possible.

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests