Reviewer’s report

Title: Effects of fluid overload on heart rate variability in chronic kidney disease patients on hemodialysis

Version: 2 Date: 13 December 2013

Reviewer: pelagia koufaki

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Effects of fluid overload on heart rate variability in chronic kidney disease patients on haemodialysis

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BMC Nephrology

Research article

REVIEWER’S REPORT

Major/compulsory revisions

(PK) Overall, this is a potentially interesting study to those with closely related research interests. However, I feel it needs to be re-written to clearly address the research question/s and the answers to those questions. As I read through the manuscript I felt that firstly, the research questions and hypotheses needed to be more clearly presented and justified and secondly, it was confusing which particular questions/hypotheses all the various data analyses really addressed. Moreover, the potential clinical or otherwise importance of the study findings needs to be discussed. How can these reported significant relationships between random indices of cardiac autonomic HRV and fluid overload be translated into clinical or research practice? How can clinicians/researchers/health professionals use these data?

(AUTH): The effects of changes in central volumes on ANS is of great interest also from a physiological point of view and many studies has been performed on this topic. We added the following brief explanation for who has not a background on this: “The effects of changes in central volumes on ANS is of great interest also from a physiological point of view and many studies has been performed on this topic [14,15]. The hypothesis of the work are based on the fact that autonomic nervous system (ANS) is known to be affected by variations of central volumes as it proved from different physiological studies, e.g. protocols of lower body negative
pressure (LBNP) [16], rest-tilt [17] and head down tilt bed rest [18]. In fact, a critical decrease in left ventricular filling, due to the decreased venous return, induce a sympathetic activation mediated by cardiopulmonary baroreceptor [19], and the sympathetic activations is also influenced by the hydration status [15].”

However, the motivation of the work is clearly stated in the introduction. Heart rate variability (HRV), which reflects the ability of the sinoatrial node to change heart rate in response to sympathetic and parasympathetic inputs, is a well-known tool for assessing heart rate control and also allows quantification of autonomic activity. Although alterations of the autonomic nervous system (ANS) and high fluid overload (FO) are each recognized risk factors for increased cardiovascular mortality in HD patients, the effects of fluid overload on the ANS in the ESRD population have not yet been fully elucidated.

This work wants to investigate this relationship.

(PK) What you state above is correct and well proven but none of the above is a clear research question. Basic human physiology demonstrates that variations in blood volume affect acute and chronic cardiovascular regulation (HRV is one index of many complex CV interactions), so by performing the comparisons you performed what did you expect to find and why? In other words, what is the particular new research question and hypothesis that you are testing here? Also you have not explained how the reported indices and relationships between measured variables can be used in real life and for what purpose. I feel my concerns have not really been clearly addressed.

Essential Revisions

ABSTRACT

1st para: It is now considered good reporting practice to avoid labelling individuals with their disease or disability. E.g. “chronic haemodialysis patients”. A more appropriate description could be “patients on chronic HD”. Please consider changing throughout.

The abstract lacks specificity and as currently reads, cannot stand on its own to clearly convey the rationale, methodology, analysis and main important findings from this observational study. The conclusion given is not supported by any reported data in the results section.
For example, the statement “Various HRV indices … were significantly correlated with FO in a way that indicates an association……” is not considered accurate scientific writing. Normally, reporting of the correlated variables, actual correlation coefficient and confidence intervals is expected.

Does not fully comply with instructions to authors as posted on line.

(AUTH) Thank you for the suggestion, we modified accordingly

(PK). I do not think you have done so. Some examples that highlight poor attention to detail and insufficient revision quality include:

- You state that you aim is to study the relationship between FO and abnormalities in the ANS? I really think the choice of wording is not self explanatory. Which specific “abnormalities” of the ANS are you referring to?
- In this observational prospective study” : I doubt that following up 5 random patients for 3 months can justify the term “prospective”
- No statistical analysis information in the abstract
- “showed a significant negative correlation with FO”. With regards to my original comments, no mention of the correlation coefficient and significance level.
- “these results indicates”: should have been these results indicate.

The above list is not exhaustive, it just aims to highlight my concern that a thorough revision has not been performed.

BACKGROUND

1st para: It is not immediately obvious how refs 1-2 support the first couple of statements. In fact throughout, the references cited do not always directly support statements made. Please review again. It is slightly discouraging that the most current research evidence used is one paper dated 2011, when there is enough recent research specific to your study population and research outcomes that would add context and rationale to your study aims and research questions.

(AUTH) The HRV analysis is a well-known tool, however clinical trials in patients on chronic HD are very limited. Our work represents a step further in this field, which is still not completely explored.

This is the first study, which takes into account fluid overload and ANS assessed through HRV analysis. No previous paper has been published before.

However, we added further details in the background, which better delineate the motivation of this study, as described above.

(PK) I feel I have to respond by saying that my original comments have not been addressed satisfactorily. I am still of the belief that refs 1 and 2 have no relevance to the statement made about FO and mortality risk in patients on HD. Ref 1 is even on patients with CKD 2-4 and the second paper is a purely
methodological paper on classification of LVH. This is concerning as I have doubts about the accuracy of the claims made throughout. I will not of course check all your references and associated claims as I really think this is something that the authors should do by allowing no doubts in anyone’s mind that their claims are not well justified.

On another note, although, there may not be a manuscript that compares directly low and high plasma volumes and HRV outcomes in CKD, this does not mean that there are no studies with similar rationale and outcomes to yours that refer to the same underlying physiological mechanisms, i.e. volume changes that affect acute BP/HR regulation. I did a 5 minute pubmed search and as I said previously, I found a number of studies that I thought would add context and comparison data. For example (however not the only ones):


2nd para: You state that the aim is: “to investigate the relationship between fluid overload and alterations of the ANS...” What is the rationale and the hypothesis? What is meant by “alterations” and how these alterations of ANS in the current study are achieved? Explain the negative effect of diabetes on ANS the related research question and hypothesis that this study addresses and finally explain what is meant by “the robustness of results is tested……” what is the specific research question linked to this?

As stated in the introduction, alterations of ANS in this population can be the sympathetic overactivity and parasympathetic blunting [11, 12], and these are assessed quantitatively by means of spectral decomposition of HRV signal. We added here a brief sentence, which explain this.

A small group of non-diabetic patients was used to verify if the low HRV and high sympathetic activity can be reversed by reducing FO. We modified this sentence. (PK) The information you added does not constitute a clear hypothesis and research question but rather some background information that should be used to justify your specific but clearly stated hypotheses. So what were you hypotheses? You performed 3 different analyses; what were the specific questions that the analyses addressed and what were you expecting to find? Moreover, the statement “the results were tested in a small …..” does not make
clear sense. Your response above makes much more sense than what appears in the manuscript. Also, why just in non diabetic patients?

Finally, I was hoping that a second chance to look at your manuscript again would prompt you to correct the grammatical mistakes and errors found throughout. The level of revisions made to your manuscript does not provide me with confidence that you have tried to pay at least the minimum amount of attention to detail. Some indicative examples (but not exhaustive) include:

- You start the Background with the abbreviation ESRD which you have not spelt out before.
- Your first statement is not referenced. Which studies demonstrate that CVD in CKD is more prevalent compared to “the healthy population”?
- P5, 2nd para: “many studies has been performed” should have been “have been”
- “the hypothesis of the work are based” should have been “is based”

I am sorry but overall, your revisions do not adequately address my concerns.

METHODS

3rd para: You mention here that patients had hydration status determined retrospectively. However, later on you state that hydration status was assessed before each HD treatment. The assumption is that the research team assessed patients and did not use retrospective classification. Please clarify. How many times patients were assessed? Were patients included in the analysis attending different dialysis shifts? The procedures for data collection need to be clearly stated. The methods section as it currently reads, could not be reproduced by other investigators. For example, how were BP data collected? HRV measures are highly influenced by internal and external environment factors, eg. Diet, sleep, temperature, noise, physical and mental state etc. How were the assessment procedures and conditions standardised?. It is also important for all readers, that the research variables used for analyses are defined and clear explanation as to what they represent, in terms of human physiology, is given. For example, what do the measures of SDANN, LF, LF%, LZC etc reflect? Not just how they are computed but which aspect of autonomic regulation do they actually represent and how can they be interpreted?

The BCM measurements were performed before HD treatment, but the clinicians in charge of the dialysis unit prescribed the HD treatment according the clinical routine.

The classification of the patients was performed retrospectively. The ECG recording and BCM measurement were performed once in the middle week HD treatment. The entire population was recorded once. Only the subgroup of 5 patients was monitored for 3 months with the BCM device. Nurses collected routinely BP by sphygmomanometer. However, BP was not the key parameter in this study.
Spectral analysis of HRV is a technique that was proposed by Akselrod in 1981 [Science 1981: Vol. 213 no. 4504 pp. 220-222]. This paper paved the way for a large amount of studies to investigate the relationship between HRV and autonomic nervous system in healthy and pathological conditions [Pagani et al. J Hypertens Suppl. 1984;2(3):S383-5; Cooke et al., 1999 The Journal of Physiology, 517, 617-628; Eckberg et al.1987; The American Journal of Cardiology, 59(4)]. The methodological procedures and standards for the ECG recording, as well as the standard indices computation are clearly explained in the referential work “Heart Rate Variability. Standards of Measurement, Physiological Interpretation, and Clinical Use. Task Force of the European Society of Cardiology the North American Society” [Circulation, 1996; 93: 1043-1065].

A clear explanation of that was reported in the introduction and in the discussion [“...HF power reflects vagal and respiratory mediated changes in heart rate, whereas LF power is mostly a marker of sympathetic modulation”]. In the methods section we illustrated only the straightforward description of the methodology used.

(PK) Again you response and revisions do not adequately address my comments. The use of the words retrospectively and prospectively indicates lack of understanding of the meaning. What exactly is meant by “medical records of study participants were reviewed prospectively”? prospective evaluation indicates repeat follow up assessment points over a period of time in the future. Retrospective indicates using information collected in a date in the past. You statement in the manuscript still reads that “the patients were retrospectively classified “

Did you classify patients in different FO groups according to measurements taken prior to the dialysis session that you measured HRV indices or not?

You have not addressed my other question about standardisation of testing procedures for HRV or BP. You response above does not address my feedback and more importantly you do not seem to acknowledge anywhere in the manuscript the well reported pitfalls of HRV measurements under non standardised conditions, in recordings of varying length and especially when you perform between subject and not within subject comparisons .

BP measurements collected routinely by nurses is not an acceptable response, especially since you have included this as an outcome in the 5 patients you followed as part of a research investigation and BP changes also forms part of
your conclusions in the discussion. A meaningful interpretation of research data needs to rely on valid and reliable evaluation outcomes and not on some random numbers collected by various people under variable conditions on individuals who present with inherent huge physiological variability anyway. At the very least an attempt to acknowledge the potential huge weaknesses of the measurement outcomes and study settings should have been made obvious.

Statistical analyses:

This is a slightly confusing section. Please clearly list all analyses performed linked to the particular research questions they address and describe the statistical tests performed. If, as you have mentioned, HRV measure may be influenced by diabetes, dialysis vintage etc, then why have not controlled for these factors in your analyses? For example perform a partial correlation?

The first reviewer assessed the statistics in her report.

We know that the statistical analyses may appear complex, but they are standard.

The objective was to investigate possible correlations between HRV indices and FO indices. Diabetes has a well-known effect on HRV and thus on HRV indices, indeed diabetic autonomic neuropathy induces the blunting of HRV and of sympathetic response ability, for this reason the analyses were performed in two subgroups: patients affected by diabetes and patients without diabetes.

We excluded partial correlation because the factors are not statistically different among the groups (high vs low FO).

(PK). No obvious attempt in the revised manuscript to incorporate my feedback. No clear link between the research questions and associated analyses performed to answer the questions. It would have been nice to see the expert’s statistical review that you are referring to. Not clear what you mean by “factors are not statistically different”. I find myself guessing what you mean to say.

RESULTS

Limited and inconsistent presentation of research variables. Select some HRV indices that reflect the different components of the ANS modulation and report them consistently throughout to provide an accurate overall picture.

The tables are difficult to follow independent of text, as they do not all include the statistical analyses annotations.

I did not find that the included figures add any additional information, or highlight any important findings.

We modified table 3 by adding additional parameters. The table 1 and table 3 show all the p-values, in table 2 significant parameters are identified by means of
symbols, which are explained in the footnote of the table. We removed figure 4 as the data were reported in the text. In our opinion the other figures are useful as they clearly show the distribution of the values in the entire population, which cannot be guessed from the simple median value.

(PK) The presentation and interpretation of Tables in the revised version confirm the lack of attention to detail required at this second stage of review. Typos: Table 1 and 3 “the values are expresses” instead of values are expressed “Dry body weight” instead of body weight Table 5: “obatined” instead of obtained, “the” instead of “the” Table 3: no n numbers for groups….. in post hoc comparisons for one way ANOVA, which group was different from which one? The asterisk is linked to a level of significance not to where the significant differences lie….. a p value equal to 0.03 should be marked as significant according to your definition in the stats section but is not highlighted at all in table 3, one way ANOVA, 3rd row…. Table 5: where are the statistical annotations on this table? A p value of 0.06 does not indicate significant increases or changes as stated in the “post study follow up” section or as implied in your discussion. So your conclusions are not really supported by your stats!! Overall, I am very uncomfortable with the level of revision quality to this point, which has not increased my confidence in the data you report and its interpretation.

DISCUSSION

I felt the discussion was rather unfocused and did not draw attention to the importance and relevance of the findings from the current investigation. Thank you for the comment. According also to the previous suggestions, we tried to improve this section by adding the following considerations about the possible translation into clinical or research practice: “The obtained results and the observations collected in the post study follow-up reinforce the opinion that chronic volume overload is an important parameter to be carefully monitored as it is associated to mortality risk and to alteration of ANS, such symphatetic overactivity and low HRV. The effect of normalizing FO on ANS can be evaluated by means of a
low cost examination, such as an ECG holter. As a matter of fact a more appropriate

(PK). The request for re-focusing and re-writing the discussion has not been met. Your data do not support your statements and conclusions (e.g. p 15 end of 1st paragraph: “this is in line with the hypothesis……” data in table 3 do not support that reduced sympathetic response was achieved as no between groups or within groups changes in LF% or in LF/HF variables are noted. Same with the addition of the last 2 paragraphs, with conclusions not supported by what you report in the results section. There are also 5 lines of added text in page 15, which are completely irrelevant with the previous paragraph or with what comes after.

Level of Interest

As presented in the 2nd stage of review, the written manuscript has reduced my confidence in the way data was collected and interpreted and therefore I would re-classify it as of limited value and interest to those with closely related research interests

Quality of written English

Minor spelling, typographical and grammatical errors which have not been addressed in the 2nd stage of review

Statistical review

Yes, but I do not feel adequately qualified to assess the statistics

Declaration of competing interests

None

**Level of interest:** An article of limited interest

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

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

none