Author's response to reviews

Title: Shear wave elastography in chronic kidney disease: a pilot experience in native kidneys

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Author's response to reviews: see over
Title: Shear wave elastography in chronic kidney disease: a pilot experience in native kidneys

Version: 1 Date: 10 March 2015

Reviewer: Kenichiro Asano

Reviewer’s report:

Review comments

The overall composition of this manuscript is concordant with standard guidelines for clinical research articles. Several concerns of the reviewer are described below;

1. With regard to tissue elastography of human organs, there are only a few reports which include discussion on ethnicity. In the 4-variable MDRD equation for calculation of eGFR, a coefficient is supplemented for women and people of black race origin, respectively, to modify the effect of innate muscle mass volume. The reason why the Hispanic cohort was set separately in this study should be explained. (Major Compulsory Revision)

It was not our intention to examine the Hispanic subjects separately in this study. We had 4/25 CKD subjects and 2/20 control subjects identify as Hispanic race – these numbers are reflective of the general population of the catchment area of the study site. We make no distinguishing comments in the Results or Discussion in our manuscript with respect to Hispanic race except to analyze it as a potential confounder. A sentence under the Patient Population heading of the Methods section has been added to clarify that gender, race, and ethnicity was not factored into patient recruitment.

2. In reference 13, the description on the relationship between fibrosis and tissue stiffness in the kidneys is derived from studies which were performed under a condition with no blood circulation. One of the major problems for discussion is that whether the results of elastography which were obtained in the kidneys without blood flow are truly applicable to the kidneys in vivo. Although this issue is yet to be clarified, there are two articles which imply a larger effect of blood flow on kidney stiffness in vivo (1. Gennisson JL, et al., Ultrasound Med Biol 2012, 38:1559-1567. 2. Asano et al., J Ultrasound Med 2014, 33:793-801.). (Discretionary Revision)

In our manuscript, we did reference the paper by Gennisson in the Discussion section with regards to the potential impact of renal blood flow on shear wave measurements. The second paper by Asano suggests that those with a higher ankle-brachial blood velocities have a decreased renal blood flow due to atherosclerotic disease. These authors do not feel that this is a physiologically
established relationship and therefore have chosen not to include this paper in our discussion.

3. It is a little confusing that diabetes and hypertension are included in both the Cause of CKD and Other Medical History categories in Table 1. Did the author intentionally make the Diabetes/Hypertension cohort because it is frequently hard to distinguish the main cause of renal tissue damage in diabetic patients who are complicated with hypertension? Is the gout category in Table 1 defined as gouty attacks or hyperuricemia?(Mamor Compulsory Revision)

Very few of the participants in this study had biopsy-proven etiologies of CKD. We used the etiology of CKD listed by the treating nephrologist of each participant. As the reviewer suggested, due to the difficulty of distinguishing the predominant factor in those with both diabetes and hypertension, we included the two diagnoses together in our results. Those identified with gout had been diagnosed by their treating providers as per local standard of care. We have added a sentence in the Methods section to clarify this fact.

4. Shear wave elastography was performed by a single radiologist. This should be included in the limitations of this study since inter-observer variance was not verified. The labels of the attached figures are inconsistent with those in figure legends.(Minor Essential Revision)

We have added this limitation to the Discussion as per the reviewer’s recommendation. We apologize to the reviewer for the difficulty with Figure labeling and will ensure these are labeled properly prior to publication.

5. In cases of chronic liver disease, the non-invasive evaluation of tissue fibrosis has a clinical advantage for determining risk of developing hepatocellular carcinoma and esophageal varices. In cases of CKD, however, the evaluation of tissue fibrosis has a smaller significance. The risk or severity of harmful events which occur in CKD can be evaluated by evaluating blood and urine samples, chest X-ray, and other methods which are used in daily practice. Even though the degree of interstitial damage is evaluated in kidney biopsy specimens, the main reason to perform this invasive examination is the identification of the primary disease which caused CKD excluding those who have renal allografts. Unfortunately, studies which were performed on shear wave elastography in the kidneys remain experimental. Application of this non-invasive method for CKD patients and interpretation of the test results should be considered carefully after reviewing future studies.(Discretionary Revision)

As stated in the Discussion, we believe the amount of fibrosis does have significant prognostic and therapeutic implications in CKD. Often those with advanced renal fibrosis will be deemed not to be candidates for immunosuppressive therapies for various causes of CKD (lupus nephritis, for example). Our hope is that growing evidence for the potential role of SWE in CKD will help move this technology from experimental to an approved and commonly-used non-invasive diagnostic test in CKD.

Level of interest: An article of importance in its field
Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.
Reviewer: Wen-Ping Wang

Version: 1 Date: 13 March 2015

Reviewer's report:

Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)

1. In introduction, the authors only listed the potential benefit of contrast-enhanced ultrasound quantitative analysis, which is also a promising technique to quantify CKD severity.

Contrast enhanced ultrasound is another experimental ultrasound technique currently under investigation for clinical use (including a paper published by this Reviewer after our manuscript was submitted for review to BMC Nephrology). It holds potential as a non-invasive measure of renal perfusion, rather than fibrosis. Because we believe this technique has a different measurement goal than SWE, we did not include it in our Introduction section.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

P value should be described as “P” in italic script but not “p” in the manuscript.

These authors have seen both capital and lowercase “P” in BMC nephrology publications and can find no clear instructions on the Instructions for Authors homepage as to the Journal’s preference. We defer to the Editorial staff but have used lowercase “p” in our manuscript.

Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

1. The authors aim was to explore whether SWE-derived estimates of tissue stiffness may serve as a non-invasive biomarker that can distinguish normal and abnormal renal parenchymal tissue. However, CKD in the early stage may be quite difficult to be differentiated from healthy ones. The authors need to make clear classify of their patients included, and compare the difference of SWE between CKD (stage I~II) and advanced CKD (stage III~V) in the statistical analysis.

We agree with this Reviewer that there may be differences in SWE measurements in early (stage I/II) and late (stage III-V) CKD. We had only a single patient with CKD stage I/II and thus are unable to perform a stratified analysis comparing the two subgroups. To help address this issue, we performed a sensitivity analysis excluding this patient from the primary analysis and did not see a significant difference in our results. We have added a sentence to the Results section to reflect this.
2. Inclusion criteria for healthy control subjects need to be more detailed classified. As the authors did not mention eGFR standard of those healthy controls, but only some common medical conditions. In Table 1, we reported a median creatinine of 0.9 and an eGFR of >60 mL/min for the healthy control population. We have added a line to the Methods section to highlight that the healthy control subjects had normal renal function.

3. In methods, authors mentioned “SWE measurements were obtained in the position offering the shortest distance to either kidney” “in a single region of interest (minimum 21 diameter 6 mm) an area of renal parenchyma at least 1 cm deep of the capsule”. Were the regions of interest in the same depth between different patients? Including only the renal cortex or the whole parenchyma? As we known, in the quantitative analysis, the size and depth of region of interest always had direct relations to the results, authors should describe in details in methods, make certain statistical analysis and discuss about various influential factors during SWE measurements.

We thank the reviewer for these methodological comments.

**Region of interest placement.** The region of interest was placed peripherally within the renal parenchyma. We attempted to place the region of interest > 1 cm deep to the capsule wherever possible to minimize artifacts. We avoided placing the ROI on pyramids, where these could be visualized. We did not attempt to differentiate the renal cortex and medulla, as these often cannot be differentiated reliably in patients with renal parenchymal disease.

**Region of interest depth.** We have clarified in the Methods section as well as in Table 1 that the “kidney depth” refers to the distance from the skin to the the region of interest of the probe. We included this number in analysis for confounding and have highlighted this in the limitations section of the Discussion.

We hope these two important changes will clarify our methods to the readers.

Most of cases were male (64%) and most of controls were female (75%). However, the authors found in the results that “among cases, estimated tissue YM was associated with female gender (p= 0.03)”, it seemed the gender distribution of cases and controls was not comparable, and why YM of cases will be associated with female gender (only 36%)

While this observation is true – that there was a higher proportion of female participants in the control arm – we did not observe that gender was a significant predictor in the control arm, nor was it shown to be a confounder in a large prior study of SWE performed in the renal cortex. We have highlighted this point in the Discussion, and acknowledge that this remains a point for future study.

**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:
I declare that I have no competing interests.