Reviewer A

General remarks:

This article reports an original MRI-based approach to differentiate between kidneys from CKD patients from those with preserved renal function. In order to achieve this, they compared associations between quantitative histogram parameters obtained by SLEEK and different categories of renal function impairment (none-mild-moderate/severe).

The work is interesting and contains a sufficient number of patients. It is interesting as clinicians have suspected since a long time that there is more heterogeneity in signal intensity in the cortex in patients with CKD than in controls, and is nice to see this quantified. The technique is quite close to what is performed with renal T1 mapping.

Histograms obtained in control patients differed from those with renal insufficiency (RI), especially in the cortex. The authors state correctly in their introduction that eGFR is not sensitive to identify early kidney damage. Nevertheless, their main analysis consist of comparing histograms with eGFR. It would be more informative if the authors had been able to collect hard renal endpoints and follow-up creatinine values in order to construct eGFR slopes over time. In that case, they could have assessed if some histogram profiles (skewness. Kurtosis) better predict fast decline in renal function than baseline eGFR. But this may be part of their future studies.

Besides, it is a shortcoming that they did not collect data on albuminuria as other marker of renal damage, and that they rely on one creatinine value to assess renal function, making it impossible to differentiate between AKI and CKD.

It is also difficult to appreciate the data due to the lack of information on other clinical variables, such as the presence of diabetes, medical history, cause of CKD and use of concomitant medication.Intake of loop diuretics may influence the signal, for example. Lack of information on the reason why MRIs were performed is also strangely lacking. Could the authors add this information?

Moreover, the interpretation of the findings is difficult, as there is no comparison with histology.In this context, the discussion is hypothesis generating, but should be interpreted with caution (especially line 260-282).

Finally, I wonder if the histogram of signal intensities would not be a way to differentiate cortex from medulla, instead of depending of the human eye to differentiate between cortex and medulla.

Detailed remarks:

Introduction:

[rev A, comment 1] I do not agree with the phrase that many techniques such as DWI, T1 mapping and BOLD-MRI did not differentiate between cortex and medulla, as many did. The problem is that in more advanced CKD, placement of the ROIs becomes difficult as the human eye cannot reliably distinguish between cortex and medulla any more.

Reply to Rev. A, comment 1. Thank you for this important comment. It is a fact that as chronic kidney disease progresses, the boundary between cortex and medulla becomes blurred. Previous study demonstrated noncontrast-enhanced SSFP MRI with a spatially selective IR pulse using optimal TI can improve the visibility of renal corticomedullary differentiation even in patients with renal insufficiency (Noda Y, Ito K, Kanki A, Tamada T, Yamamoto A, Kazuya Y et al. Measurement of renal cortical thickness using noncontrast-enhanced steady-state free precession MRI with spatially selective inversion recovery pulse: Association with renal function. J Magn Reson Imaging (2015) 41:1615-21). Therefore, we would like to distinguish renal cortex and medulla on SLEEK images as much as possible.

Methods:

MRI:

[rev A, comment 2] As this is a retrospective study in patients in who underwent an MRI for clinical reasons, it would be useful to know for what indications the MRIs were performed.

Reply to Rev. A, comment 2. Thank you very much for this important comment. We have explained the indications for the MRI examinations in the article (Page 4, line 88-91) (The most common clinical diseases for patients undergoing abdominal SLEEK examination were hypertension with suspected renal artery stenosis, atherosclerotic renal artery stenosis, chronic renal insufficiency, and various glomerular diseases)

[rev A, comment 3] If I understand correctly, identification of the cortex and medulla was based on freehand placement of ROIs around cortex and medulla, according to the interpretation of the radiologists who were not aware of the eGFR of the participants. It is therefore not impossible that some cortical ROIs contain medulla and vice versa.

Reply to Rev. A, comment 3. Thank you for your comment. We drew the cortical ROIs along the edge of the kidney as much as possible, and drew the medullary ROIs as far away as possible from the cortex. This may have a selection bias, but we will use the twelve-layer concentric objects (TLCO) method as much as possible to delineate the cortex and medulla ROIs in the future study.

[rev A, comment 4] It is not very clear to me how cortical thickness was measured. Was it derived from the ROI or by measuring thickness at predefined points (for ex next to the pyramids?).

Reply to Rev. A, comment 4. Thank you very much for your comment. Two radiologists used the ruler tool in the Report Information System (RIS) to measure the cortical thickness at the upper pole, hilar level, and lower pole of the bilateral kidney, and the average value of the six measurements was taken as the final result for statistical analysis. We have made corresponding changes in the article (Page 6, line 144-147).

[rev A, comment 5] Postprocessing was extremely fast and needed only 3-4 minutes per exam.

Reply to Rev. A, comment 5. Thank you for your comment. The post-processing for each patient included delineating the cortex and medulla ROI and measuring the cortical thickness. The subsequent data and statistical analysis were not included in this process, so the image post-processing for each patient was about 3-4 minutes. We have made corresponding changes in the article (Page 4, line 148-149).

[rev A, comment 6] Clinical variables:

CKD staging was apparently based on one single measurement of creatinine. How did the authors distinguish CKD from acute kidney injury?

Reply to Rev. A, comment 6. Thank you very much for your comment. Patients with acute kidney injury were not included in our study.

Results:

[rev A, comment 7]-Interobserver agreement: the authors report ICC's between 0.739 and 0.99. as for the lower ICC (kurtosis), this would correspond to moderate, not

excellent agreement according to the pre-defined cut-offs mentioned under 'statistical analysis'.

-How were ICC's for patients with moderate to severe renal insufficiency? As differentiating cortex from medulla with the human eye becomes more difficult, ICCs may be lower here.

-Besides, how was the ICC for cortical thickness measurement?

Reply to Rev. A, comment 7. Thank you very much for your comment. We have modified it in the article (Page 8, line 184). The ICC's for patients with moderate to severe renal insufficiency ranged from 0.822 to 0.998. We may need to adopt more accurate methods to delineate the cortical and medullary ROIs in the future research. The ICC of cortical thickness was 0.934 (95% CI, 0.906-0.953).

[rev A, comment 8]-It is interesting to see that differences in histogram parameters were larger in the cortex than medulla. However, Table 3 is not clear in its current layout, as numbers and their SD's are not on the same line.

Reply to Rev. A, comment 8. Thank you very much for your comment. We have modified Table 3.

Discussion

[rev A, comment 9] The authors try to explain why heterogeneity of the signal was larger in the cortex than in the medulla, but it remains an intriguing finding to me, as fibrosis and inflammation is not limited to the cortex in CKD, but also occurs in the medulla.

In this context, I am not sure that using BOLD-MRI is of much help to study pathological changes in the medulla (line 258-260), as previous research demonstrated that cortical R2* values were stronger predictors of renal function decline than medullary R2* values.

I lack a word on compartmental analysis (for ex doi: 10.1097/RLI.0b013e318234e75b), a technique that was used in the past to compare cortical and medullary distribution of the BOLD-signal. This technique also obtained histograms, and seems rather close to the technique used by the authors in this paper.

Reply to Rev. A, comment 9. Thank you very much for your comment. Cortex and medulla histogram parameters all showed significant differences between the three groups in our study. It's just that there were more significant cortical parameters than medullary parameters. We speculated that more glomeruli were distributed in the cortex. There were some controversies in the assessment of renal function by renal BOLD-MRI, which may be due to differences in scanning

equipment, scanning parameters, and research subjects. The majority of renal blood supplies to the cortex and only 6% contribute to the renal medulla so that the medulla may be more sensitive to ischemia and hypoxia. But this is only our conjecture and needs to be confirmed by further research in the future.

[rev A, comment 10] Among the limitations the authors should mention, as stated above, the lack of information on clinical variables, histology and AKI status.

Reply to Rev. A, comment 10. Thank you very much for your comment. We have made corresponding changes in the article (Page 13, line 307-308).

References:

[rev A, comment 11] Some overlap (occur twice), for example reference 10 and 36.

Reply to Rev. A, comment 11. Thank you very much for your comment. We have modified these two duplicate references. (Page 12, line 280)

<mark>Reviewer B</mark>

The authors have performed a retrospective analysis of T1 weighted MRI of kidneys to evaluate cortical thickness and histogram analysis both in cortex and medulla. Even though they used SLEEK which is used for non-contrast MR angiography, I believe the findings may be relevant to other T1 weighted sequences. Even though, there is no strong rationale to study histogram of MRI signal intensities, the authors do find several textural measures especially in the cortex to be different with severity of CKD. This when combined with the known reduction in cortical thickness can also be exploited to improve the differentiation.

There are a few limitations of the study that the authors should include in the discussion. Analyzing histograms of MRI signal intensities is not preferred because it varies with the scanner hardware and probably body size and composition. The approach may be ideally useful with parametric maps. It appears that only one slice was used for the analysis. Further, not all of the medulla is sampled. In fact, performing histogram analysis on the whole kidney may be a better approach here, knowing the changes in cortico-medullary contrast on MRI along with reduction in cortical thickness in CKD. SLEEK is not a common sequence used in the clinic and it is not clear how translatable are the findings to other T1 weighted sequences. Lack of histological evidence of fibrosis is a limitation, if as hypothesized the inhomogeneity parameter is related to presence of fibrosis.

Specific comments:

[rev B, comment 1]Authors should include a brief description of the method for cortical thickness measurement. Also what is "minimal cortical thickness", a term used in the manuscript.

Reply to Rev. B, comment 1. Thank you for your comment. We have made corresponding changes in the article (Page 6, line 144-147). (Two radiologists used the ruler tool in the Report Information System (RIS) to measure the cortical thickness at the upper pole, hilar level, and lower pole of the bilateral kidney, and the average value of the six measurements was taken as the final result for statistical analysis). Our statement is misleading and we want to show the average value of six measurements of renal cortex thickness as accurately as possible. We have made corresponding changes in the article (Page 8, line 178-182; Page 9, line 213-215; Page 14, line 311; Page 21, line 473-474, 483).

[rev B, comment 2] It appears that Table 5 appears truncated.

Reply to Rev. B, comment 2. Thank you for your comment. This may be a problem displayed by the system and we will re-upload a new table.