Peer Review File

<mark>Review comments-Reviewer A</mark>

First of all, my major concern regarding this paper is the unclear focus of this study. The conclusion focused on the comparative efficiency of ASL vs. BOLD, but the analyses is the diagnostic performance of two parameters from both ASL and BOLD for kidney graft injury. The authors need to revise the paper including the title to clearly indicate the research focus.

We have modified our text as advised. The focus of our research is: evaluate the effectiveness of renal transplantation function by comparing arterial spin labeling (ASL) and blood oxygen level dependent (BOLD) imaging with the receiver operating characteristic (ROC) curve. Change in the text: page1, line 2-3.

Second, the title did not indicate the clinical research design of this study, i.e., a diagnostic test.

We have modified our text as advised.

Change in the text: page1, line 2-3.

Third, the abstract needs some revisions since it is not adequate. The background did not explain why the combination of ASL vs. BOLD is able to accurately diagnose kidney injury, did not describe their respective limitations, and what the clinical significance of this research focus is. The methods did not describe the inclusion of subjects, the diagnosis of kidney graft injury, and the details of the combination of the parameters, i.e., serial test or parallel test. The results need to briefly describe the clinical characteristics of the study samples and report the sensitivity and specificity of the diagnostic test. The conclusion needs to focus on the diagnostic performance of RBF and R2* and have comments for the clinical implications of the findings strictly based on the current findings.

We have modified our text as advised.

Change in the text: page2, line 32-59.

Fourth, the introduction of the main text needs to have comments on the diagnostic accuracy of ASL alone and BOLD alone, explain why the parameters from the combination of ASL and BOLD can improve the accuracy, and have comments on the knowledge gaps on the diagnostic accuracy of RBF and R2*. Please also clarify the clinical significance and clinical needs for this research focus.

This has been mentioned in the discussion section: Several studies (32,33) have investigated the function of renal allografts using ASL and other functional MRI techniques and have identified renal blood flow and regional renal perfusion as major determinants of oxygen delivery and thus oxygenation of renal tissues. BOLD imaging can detect hypoxic states but may be limited in distinguishing between increased oxygen consumption and decreased blood supply. A strong negative correlation between R2* and RBF was observed in the present study, which may explain the small diagnostic value added by the combination of R2* and RBF

compared to RBF values alone. Previous studies (34,35) have evaluated renal function using ASL and BOLD, and reported an 8.3% reduction in the medullary R2* values of transplant patients during a 2-year follow-up period.

Change in the text: None.

Fifth, the methodology of the main text needs to correctly describe the correct clinical research design, sample size estimation, the assessment of clinical characteristics, details of the generation of RBF and R2*, and the gold diagnosis of kidney graft injury. In statistics, please describe the calculation of other diagnostic parameters such as sensitivity and specificity, report the threshold values of AUC, sensitivity and specificity for a good diagnostic test, and ensure P<0.05 is two-sided. Finally, please consider to cite the below related papers: 1. Wang Z, Wei Y, Chen J, Zhang Q, Tang J, Chang Q. Effect of recombinant human brain natriuretic peptide on acute kidney injury after coronary artery bypass grafting: a retrospective comparative cohort study. Ann Transl Med 2022;10(18):973. doi: 10.21037/atm-22-3727. 2. Dong X, Jia Q, Fu W, Li Y, Lin N, Li W, Ye W, Wen Y, Zhang A. Two unusual cases of autologous HSCT related TMA with kidney injury. Ann Palliat Med 2022;11(4):1546-1553. doi: 10.21037/apm-21-226.

We have modified our text as advised.

Change in the text: page8, line 237-240, page7, line208.

Review comments-Reviewer B

1. Some imaging parameters are lacking in the description of the ASL sequence, such as the labeling plain and its position, type of readout, type of pulse used, how many PLD?

Thank you for your advice.

Changes in the text: page6, line 178-202.

2. Has the data quality been evaluated (such as % perfusion signal in ASL, that can help evaluate the quality of the data)?

Thank you for your advice. This has been described in the article. (line 198).

Changes in the text: None.

3. The method of perfusion quantification is lacking in details (matemathical model and postprocessing), could you, please elaborate.

Thank you for your advice.

Changes in the text: page 6, line 191-197.

4. Were motion artefacts present in any of the datasets since data were acquired during free breathing scan? Was data registration performed?

Thank you for your advice.

Changes in the text: page 6, line 191-192.

5.In methods section, l the value of longitudinal magnetisation is marked as 1650ms, could you explain the value you have chosen. Were T1 maps performed? Reference is lacking.

Thank you for your advice. (Ref. 16)

6. How did you choose ROIs both in ASL and BOLD? Were ROIs the same in both?
Thank you for your advice. This has been described in the article. (line 199-202).
ASL ROIs in the cortical region, BOLD ROIs in the medullary region

7. Have you analysed the donor age and type of donor when you compared allongraft function in two groups?
This article is comparing the difference between ASL/BOLD and eGFR testing of transplant kidney function, not comparing the function of different donor transplant kidneys.

Could you please mark in Figure 1 which image goes to what patient example (regarding good and poor function).
Thank you for your advice. This has been described in the article. (line 510).

9. In Discussion line 251 "the thickening part of the renal medulla consumes the most oxygen", could you find a better terminology?
Thank you for your advice.
Changes in the text: page 8, line 265.

Plus, the language editing is highly recommended.
English Language Editor: L. Huleatt

1. Reference 19 and 34 are the same one. Please check and revise.
Reply: Modified. Reference 34 removed, see line 482.

2. Table 2:
The below contents don't match with Table 2.

259   **##Relationship between ASL, BOLD and eGFR**

260   As Figure 2 and Table 2 show, BOLD was positively correlated to eGFR

261   (r=0.7297, P<0.0001), while the R2* values were negatively correlated to eGFR

Reply: Modified, should be ASL, see line 239.

3. Figure 1:
Please check whether the data in your Figure 1 legend are correct. They don't match with your main text.

547 Figure 1 Normal group(A,B) and injury group(C,D) ASL/BOLD: (A) RBF: 289.32

548 mL/100 g/min, (B) R2*: 21.23/s, (C) RBF: 162.11 mL/100 g/min, (D) R2*: 27.66/s.

549 ASL, arterial spin labeling; BOLD, blood oxygen level-dependent; RBF, renal blood

550 flow.

Reply: This figure is a schematic representation of individual cases in the normal and injury groups, with values within the range of the mean values for each group.
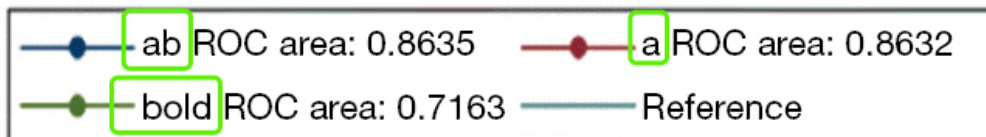
4. Figure 2:

Please check whether the unit for eGFR is correct.

$$0 \quad 20 \quad 40 \quad 60 \quad 80 \quad 100 \quad 120 \quad 140$$

eGFR, $\text{mL·min}^{-1}·(1.73 \text{ m}^2)^{-1}$
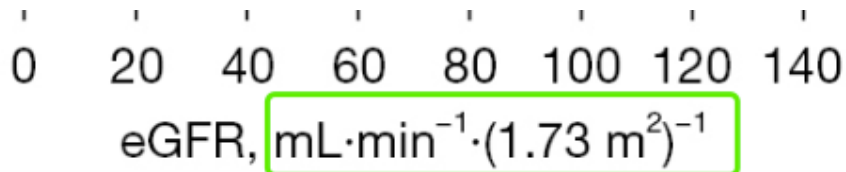
Reply: Confirmed and correct.

5. Figure 3:

What is the meaning of a and b? And please revise 'bold'' to "BOLD".

ab ROC area: 0.8635   a ROC area: 0.8632
bold ROC area: 0.7163   Reference

Reply: Modified, see Figure 3 between lines 526-527.