Original Article

Magnetic resonance diffusion tensor imaging is superior to arterial spin labeling in detecting renal allograft fibrosis

Jiayi Wan[1#], Minmin Jin[1#], Jie Li[2], Jiali Ma[1], Chenqin Que[3], Bin Jiang[1], Yangyang Tian[4], Linkun Hu[4], Yixing Yu[1], Chunhong Hu[1], Jun Wang[1], Mo Zhu[1]

[1]Department of Radiology, The First Affiliated Hospital of Soochow University, Suzhou, China; [2]Suzhou Medical College of Soochow University, Suzhou, China; [3]Xuzhou Medical University, Xuzhou, China; [4]Department of Urology, The First Affiliated Hospital of Soochow University, Suzhou, China

Contributions: (I) Conception and design: J Wan, M Jin; (II) Administrative support: C Hu, M Zhu; (III) Provision of study materials or patients: Y Tian, L Hu; (IV) Collection and assembly of data: J Wan, B Jiang, J Ma, C Que; (V) Data analysis and interpretation: J Wan, J Li, Y Yu, M Jin, J Wang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Jun Wang, MM; Mo Zhu, MD, PhD. Department of Radiology, The First Affiliated Hospital of Soochow University, No. 899 Pinghai Road, Suzhou 215008, China. Email: wangjun19871008@163.com; soochowzhumo@163.com.

Background: Although both magnetic resonance (MR) diffusion tensor imaging (DTI) and arterial spin labeling (ASL) have been demonstrated to be useful for the assessment of renal allograft fibrosis, their diagnostic value for renal allograft fibrosis is rarely compared. In this study, we collected a relatively large sample size to compare the value of DTI and ASL in the assessment of renal transplantation (RT) fibrosis.

Methods: This study included 141 kidney transplant recipients who underwent DTI, ASL, and biopsy. The renal allograft fibrosis was divided into ci0, ci1, ci2, and ci3 fibrosis groups according to the biopsy results. The apparent diffusion coefficient (ADC), fractional anisotropy (FA), and renal blood flow (RBF) were calculated. One-way analysis of variance (ANOVA) was used to compare the differences of functional magnetic resonance imaging (MRI) parameters between different fibrosis subgroups. The area under the receiver operating characteristic curve (AUC) was calculated to evaluate diagnostic performance.

Results: The medullary FA values in ci2 (0.27±0.04, P<0.001) and ci3 (0.21±0.03, P<0.001) groups were significantly lower than those in ci0 group (0.31±0.05). The medullary FA value in ci3 group (0.21±0.03) was significantly lower than that in ci1 group (0.30±0.07, P<0.001) and ci2 group (0.27±0.04, P<0.01). The AUC of DTI was found to be higher than that of ASL in accurately identifying renal allograft fibrosis, and the result was statistically significant in differentiating ci0–2 group and ci3 group (ci0 *vs.* ci1–3, 0.725 *vs.* 0.712, P>0.05; ci0–1 *vs.* ci2–3, 0.787 *vs.* 0.735, P>0.05; ci0–2 *vs.* ci3, 0.945 *vs.* 0.802, P<0.05).

Conclusions: DTI has a higher diagnostic value than ASL in noninvasive identification of the degree of renal allograft fibrosis.

Keywords: Renal transplantation (RT); diffusion; perfusion; fibrosis

Submitted May 22, 2024. Accepted for publication Feb 14, 2025. Published online Mar 14, 2025.
doi: 10.21037/qims-24-1023
View this article at: https://dx.doi.org/10.21037/qims-24-1023

Introduction

Renal transplantation (RT) in patients with end-stage renal disease is the most effective means of renal replacement therapy. Compared with dialysis, RT improves survival and quality of life in end-stage renal disease patients (1). However, a variety of complications after RT are important factors that cause graft dysfunction or even loss of function. Chronic graft rejection is one of the main causes of long-term graft function loss after RT (2-4). In its pathogenesis, antigen-dependent and antigen-independent factors play an important role in causing graft fibrosis. Due to the compensatory nature of the kidney and the lag of clinical features, the progression of fibrosis is often not detected early. The degree of fibrosis is often obtained clinically by renal biopsy and classified according to the Banff criteria (5). However, due to the limitation of the biopsy site, false negative results may occur (6). In addition, poor patient acceptance due to its invasive nature makes repeat biopsy difficult to perform.

In addition to providing morphological features, magnetic resonance imaging (MRI) can also well reflect the complications and functional information of RT (7-9). In addition, MRI has good repeatability, is not affected by the operator, and does not require the use of contrast media and ionizing radiation; it does not cause obvious damage to the function of the transplanted kidney, so it can be used in patients with renal insufficiency and measured repeatedly. For renal allograft fibrosis, MRI can reflect the degree of fibrosis through different sequences according to the different pathophysiological changes caused by fibrosis (10-14). Diffusion tensor imaging (DTI) can measure the diffusion of water molecules in all directions. The extent to which diffusion exhibits orientation preference was evaluated by fractional anisotropy (FA), ranging from 0 to 1. Based on this principle, DTI can well detect the changes of renal structure and microstructure in allograft kidney transplantation, such as renal tubular atrophy and interstitial fibrosis (14). Arterial spin labeling (ASL) is performed by selectively labelling the inflow of blood with a magnetization opposite to that of the tissue in the region of interest (ROI). Tissue perfusion can be measured based on the signal difference between labeled images (tag) and unlabeled images (control) without injection of exogenous contrast agent. However, progressive capillary loss during renal fibrosis leads to reduced blood flow and oxygen delivery to renal parenchymal tubular epithelial cells, thereby driving the progression of fibrosis (14,15). Few previous studies have compared the diagnostic performance of MRI sequences to discriminate the degree of fibrosis. Some researchers have compared the diagnostic efficacy of ASL and intravoxel incoherent motion (IVIM) with reduced field of view (FOV) for fibrosis (16). The aim of this study was to compare the diagnostic efficacy of ASL and DTI for fibrosis. We present this article in accordance with the STROBE reporting checklist (available at https://qims.amegroups.com/article/view/10.21037/qims-24-1023/rc).

Methods

Study population

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the Ethics Review Board of The First Affiliated Hospital of Soochow University on 27 October 2022, with the review number (2022) (No. 412). Informed consent was provided by all individual participants. For participants under 18, informed consent was provided by their legal guardians. Between 21 January 2022 and 31 May 2023, 145 renal transplant recipients who underwent renal allograft biopsy at the urology department of our hospital were recruited. All participants underwent DTI and ASL within 1 week before and after kidney biopsy. The inclusion criteria consisted of RT recipients who met the indications for kidney biopsy, which included abnormal urine examination results, elevated serum creatinine levels, delayed graft function, rejection, and protocol biopsy. The exclusion criteria included claustrophobia, intrauterine device (IUD) placement, and other contraindications to MRI or unavoidable artifacts that severely affected image quality.

MRI data

All participants were subjected to 1.5-T MRI (Ingenia Ambition, Philips Healthcare, Best, the Netherlands) with a 28-channel phased array coil. No special patient preparation was required before MRI examination. Axial T1, T2-weighted, and coronal T2-weighted images were first acquired for morphological evaluation. For DTI, the imaging parameters were as follows: b=0 and 600 s/mm^2, 15 diffusion directions, repetition time (TR) =2,300 ms, echo time (TE) =67 ms, slice spacing =1 mm, slice thickness =5 mm, 20 slices/patient, voxel size =2.5×2.5×5 mm^3, and FOV =320×400 mm^2. For ASL, images with a voxel size of 3.75×3.75×8 mm^3 were obtained using pseudo-continuous

pulsed ASL along with a three-dimensional (3D) gradient and spin-echo scheme. The imaging parameters were as follows: TE =15 ms, TR =3,963 ms, turbo spin echo (TSE) factor =20, echo planar imaging (EPI) factor =15, matrix =64×60, and FOV =240×240 mm^2.

Image post-processing

DTI and ASL data were transferred to a workstation (Intellispace Portal; v10; Philips Healthcare). Images were postprocessed by an abdominal radiologist with more than 10 years of clinical experience who was blinded to clinical and pathological findings. Firstly, DTI data were registered and corrected. FA and apparent diffusion coefficient (ADC) maps of the transplanted kidney were reconstructed. Appropriate cortical and medullary ROIs were delineated on T2 images with high corticomedullary contrast and directly applied to the corresponding FA and ADC maps. At least three image slices near the renal hilum were selected for ROI analysis for each participant. The measured medullary and cortical FA and ADC values were averaged. For ASL, ROI was placed in the renal cortex in a proton density-weighted image and transferred to the obtained renal blood flow (RBF) map to calculate renal perfusion. ROI should be avoided to include cysts, hemorrhage, and areas of heterogeneous signal intensity.

Kidney allograft biopsy

The degree of fibrosis requires the results of renal biopsy as the reference standard. All renal biopsy results were made by a nephropathologist with periodic acid-Schiff (PAS), hematoxylin-eosin (HE), Jones staining, periodic acid-silver methenamine (PASM), and Masson's trichrome staining, according to the Banff 2019 model (17), without knowledge of the MRI results. The interstitial fibrosis score (ci score) was classified as follows: ci0 (no fibrosis, <5% cortical fibrosis), ci1 (mild fibrosis, 5–25% cortical fibrosis), ci2 (moderate fibrosis, 26–50% cortical fibrosis), and ci3 (severe fibrosis, >50% cortical fibrosis). For better statistical analysis, we divided the fibrosis group into no fibrosis (ci0) versus with fibrosis (ci1–3), no or mild fibrosis (ci0–1) versus moderate to severe fibrosis (ci2–3), and non-severe fibrosis (ci0–2) versus severe fibrosis (ci3).

Statistical analysis

The role of functional MRI parameters in identifying the degree of fibrosis of allografts can be evaluated by the receiver operating characteristic (ROC) curve. Previous studies (18,19) showed that the lowest acceptable area under the ROC curve (AUC) for the diagnosis of allograft fibrosis ci0 versus ci1–3, ci0–1 versus ci2–3, and ci0–2 versus ci3 was 0.70, 0.75, and 0.80, respectively. The best cut-off value was determined according to the maximum Youden index (sensitivity + specificity − 1). Comparison of ROC curve results required the use of DeLong test. The correlation between functional MRI parameters and Banff fibrosis score can be explored by calculating Spearman rank correlation coefficient. One-way analysis of variance (ANOVA) was used to compare the differences in functional MRI parameters between different fibrosis subgroups. When ANOVA results were significant, the *post-hoc* Bonferroni test was used to compare the groups.

Results

Clinical results

Of 145 patients recruited, two were excluded because they did not complete the MRI scan and another two were excluded because their data could not be postprocessed. Finally, a total of 141 patients were included (83 males and 58 females; average age was 42±11 years, range, 17–67 years). Biopsy results showed that the number of cases in the ci0, ci1, ci2, and ci3 groups were 64, 32, 31, and 14, respectively. The clinical and laboratory characteristics of the participants are shown in *Table 1*.

Relationships between functional MRI parameters and Banff fibrosis score

Cortical FA was negatively related to Banff fibrosis score (rho =−0.30, P<0.001). Medullary FA was also negatively related to Banff fibrosis score (rho =−0.50, P<0.001). Cortical ADC showed a negative relation to Banff fibrosis score (rho =−0.19, P<0.05). Medullary ADC showed a negative relation to Banff fibrosis score (rho =−0.22, P<0.01). There was a negative relation between RBF and Banff fibrosis score (rho =−0.43, P<0.001) (*Figure 1*).

Differences in functional MRI parameters between fibrosis subgroups

The ci0 group [mean ± standard deviation (SD), 2.13± 0.19 ×10^{-3} mm^2/s], ci1 group (2.13±0.19 ×10^{-3} mm^2/s), ci2 group

Table 1 Clinical features and laboratory characteristics of study participant

Parameters	ci0 (n=64)	ci1 (n=32)	ci2 (n=31)	ci3 (n=14)	Total
Age (years)	39±11	44±11	42±11	46±10	42±11
Sex					
Male	32 [50]	21 [66]	22 [71]	9 [64]	83 [59]
Female	32 [50]	11 [34]	9 [29]	5 [36]	58 [41]
Baseline Scr (μmol/L)	115.3 [83.4–158.4]	131.4 [97.1–172.1]	139.8 [113.1–194.0]	181.5 [124.5–274.4]	131.7 [96.7–177.2]
Baseline eGFR (mL/min/1.73 m^2)	62 [39–88]	52 [40–75]	49 [35–58]	32 [23–52]	54 [34–74]
Urinary protein (g/24 h)	0.40 [0.13–0.52]	0.32 [0.14–1.04]	0.37 [0.14–1.97]	0.80 [0.28–1.60]	0.38 [0.14–1.02]
Albumin (g/L)	41.4±4.1	39.4±3.7	38.8±4.6	37.4±7.1	40.0±4.7
Hemoglobin (g/L)	105.8±21.9	125.9±23.0	121.4±20.5	109.6±15.5	114.1±22.9
Systolic blood pressure (mmHg)	135.3±17.0	132.7±18.7	131.7±17.9	136.5±15.8	134.0±17.4
Diastolic blood pressure (mmHg)	85.5±9.9	82.7±10.7	83.8±11.9	84.5±11.3	84.4±10.6
Height (cm)	167.0±9.0	168.6±9.2	161.9±28.7	164.3±6.5	166.0±15.5
Weight (kg)	59.8±13.2	64.9±14.1	65.1±12.6	59.3±5.0	62.1±12.9
Hypertension (%)	65	88	70	43	69
Diabetes (%)	6	3	3	7	5
Immunosuppressive regimens					
Pre + MMF + Tac	61 [95]	25 [78]	28 [90]	12 [86]	126 [90]
Pre + MMF + CsA	3 [5]	3 [9]	2 [7]	1 [7]	9 [6]
Others	0	4 [13]	1 [3]	1 [7]	6 [4]

Data are presented as mean ± SD, median [Q1–Q3], n [%], or %. ci0, no fibrosis (<5% of cortex occupied by fibrosis); ci1, mild fibrosis (5–25% of cortex occupied by fibrosis); ci2, moderate fibrosis (26–50% of cortex occupied by fibrosis); ci3, severe fibrosis (>50% of cortex occupied by fibrosis). SD, standard deviation; CsA, cyclosporine A; MMF, mycophenolate mofetil; Pre, prednisone; Tac, tacrolimus; eGFR, estimated glomerular filtration rate; Scr, serum creatinine.

(2.09±0.18 ×10^{-3} mm^2/s), ci3 group (2.02±0.15 ×10^{-3} mm^2/s) cortical ADC values decreased with the progression of fibrosis, but there was no statistically significant difference. The cortical FA value in the ci0 group (0.15±0.03, P<0.05) was significantly higher than that in the ci2 group (0.13±0.03) and ci3 group (0.13±0.02). There was no significant difference in cortical FA value between the ci1 group (0.14±0.03) and other groups. The ci0 group (2.09±0.24 ×10^{-3} mm^2/s), ci1 group (2.06±0.19 ×10^{-3} mm^2/s), ci2 group (2.05±0.26 ×10^{-3} mm^2/s), and ci3 group (1.93±0.23 ×10^{-3} mm^2/s) medullary ADC values decreased gradually with the progression of fibrosis, but there was no significant difference. The medullary FA values in the ci2 (0.27±0.04, P<0.001) and ci3 (0.21±0.03, P<0.001) groups were significantly lower than those in the ci0 group (0.31±0.05). The medullary FA value in the ci3 group (0.21±0.03) was significantly lower than that in the ci1 group (0.30±0.07, P<0.001) and ci2 group (0.27±0.04, P<0.01). The RBF value of the ci0 group (228.39±75.12 mL/min/100 g) was significantly higher than that of the ci2 group (165.87±66.75 mL/min/100 g, P<0.001) and ci3 group (127.79±50.36 mL/min/100 g, P<0.001). The RBF value in the ci1 group (196.46±73.91 mL/min/100 g, P<0.05) was significantly higher than that in the ci3 group (127.79±50.36 mL/min/100 g) (*Figures 2-4*).

The role of DTI and ASL in fibrosis staging

In the comparison between ci0 and ci1–3, the diagnostic performance of medullary FA was better than that of RBF, but there was no statistical significance (AUC =0.725 *vs.* 0.712, P>0.05). When 0.268 was used as the cut-off value of

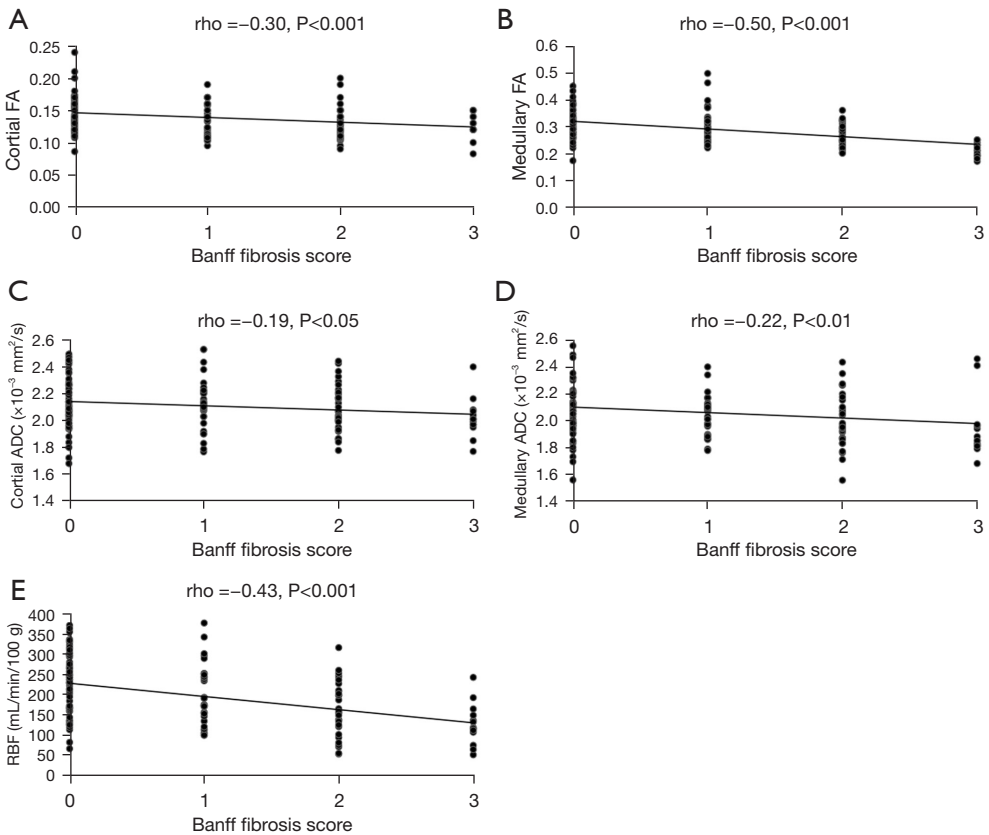

Figure 1 Relations between functional MRI parameters and the Banff fibrosis score. Scatterplots show negative relations between (A) cortical FA and Banff fibrosis score, (B) medullary FA and Banff fibrosis score, (C) cortical ADC and Banff fibrosis score, (D) medullary ADC and Banff fibrosis score, (E) RBF and Banff fibrosis score. MRI, magnetic resonance imaging; FA, fractional anisotropy; ADC, apparent diffusion coefficient; RBF, renal blood flow.

medullary FA, the sensitivity was 84.4% and the specificity was 53.2%. The diagnostic performance of RBF combined with medullary FA was not significantly better than that of medullary FA alone (AUC =0.746 *vs.* 0.725, P>0.05). In the comparison between ci0–1 and ci2–3, medullary FA was better than RBF (AUC =0.787 *vs.* 0.735, P>0.05), but the results were not statistically significant. When 0.266 was used as the cut-off value of medullary FA, the sensitivity and specificity were 79.2% and 66.7%, respectively. The diagnostic performance of RBF combined with medullary FA was not significantly better than that of medullary FA alone (AUC =0.811 *vs.* 0.787, P>0.05). In the comparison between ci0–2 and ci3, medullary FA was significantly better than RBF (AUC =0.945 *vs.* 0.802, P<0.05), the results were statistically significant. When the cut-off value of medullary FA was 0.251, the sensitivity was 81.1% and the specificity was 99.9%. Compared with medullary FA alone, the combination of RBF and medullary FA did not significantly increase the diagnostic efficacy (AUC =0.958 *vs.* 0.945, P>0.05) (*Table 2*).

Discussion

By assessing the degree of fibrosis in renal allograft, it is possible to identify high-risk patients with potential progression to allograft dysfunction in advance, and carry out corresponding clinical intervention. Most previous MRI studies of renal allograft fibrosis have been inadequate due to the limitation of the number of transplanted kidney biopsies (18,20-23). In this study, we collected data from a relatively large sample and compared the value of DTI and ASL in identifying the degree of fibrosis in allografts. We found that DTI had better diagnostic performance than ASL, and the results were statistically different in the

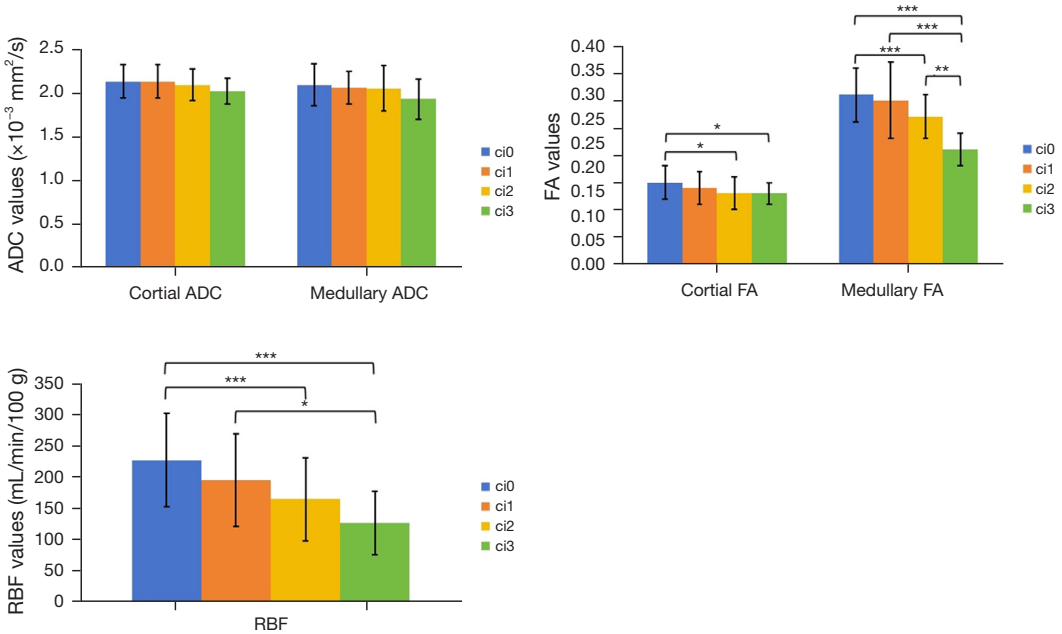

Figure 2 Comparison of functional MRI parameters among fibrosis score groups. *, **, and *** indicate P<0.05, P<0.01, and P<0.001, respectively. ci0, no fibrosis (<5% of cortex occupied by fibrosis); ci1, mild fibrosis (5–25% of cortex occupied by fibrosis); ci2, moderate fibrosis (26–50% of cortex occupied by fibrosis); ci3, severe fibrosis (>50% of cortex occupied by fibrosis). MRI, magnetic resonance imaging; ADC, apparent diffusion coefficient; FA, fractional anisotropy; RBF, renal blood flow.

discrimination between the ci0–2 and ci3 groups.

In the present study, we used the fibrosis results obtained from biopsy as the reference standard, and found that the cortical ADC values decreased with fibrosis progression in each group but did not reach statistical significance. Cortical FA values were significantly higher in the ci0 group (mean ± SD, 0.15±0.03, P<0.05) than in the ci2 (0.13±0.03) and ci3 (0.13±0.02) groups. This may be attributed to the progression of renal fibrosis, which is accompanied by the destruction of cortical microstructure and microcirculation. As a result, it has an impact on cortical DTI. In an earlier study, Razek *et al.* (24) found that the renal cortical parameters obtained by DTI were correlated with laboratory indicators, and the changes of laboratory indicators were closely correlated with the disruption of renal cortical microstructure and microcirculation. For the medullary ADC values, each group also showed a decreasing trend with the progression of fibrosis, but the difference was not statistically significant. For medullary FA values, the ci2 (0.27±0.04, P<0.001) and ci3 (0.21±0.03, P<0.001) groups were significantly lower than the ci0 (0.31±0.05) group, and the ci3 group (0.21±0.03) was significantly lower than the ci1 (0.30±0.07, P<0.001) and ci2 (0.27±0.04, P<0.01) groups, which was consistent with the previous results of Hueper *et al.* (25), which found that medullary FA and ADC values were negatively correlated with the degree of fibrosis, and the results of other researchers (26-29). Similar results were obtained in previously published studies, the diffusion anisotropy of renal allografts was higher in the renal medulla than in the cortex because the longitudinal arrangement of renal tubules within the medulla as well as the glomeruli within the cortex limit the direction of water molecule diffusion (30,31). We found that RBF decreased gradually with the progression of fibrosis. The RBF in the ci0 group (228.39±75.12 mL/min/100 g) was significantly higher than that in the ci2 (165.87±66.75 mL/min/100 g, P<0.001) and ci3 groups (127.79±50.36 mL/min/100 g, P<0.001), and that in the ci1 group (196.46±73.91 mL/min/100 g, P<0.05) was significantly higher than that in the ci3 group (127.79±50.36 mL/min/100 g). Both RBF and diffusion parameter values were negatively correlated with the degree of fibrosis. This is similar to the previous results of Yu *et al.* (16). However, the study by Yu *et al.* suggested that compared with diffusion parameters, ASL has a stronger correlation with fibrosis and a higher diagnostic efficiency for the degree of fibrosis. In this study, we chose DTI to

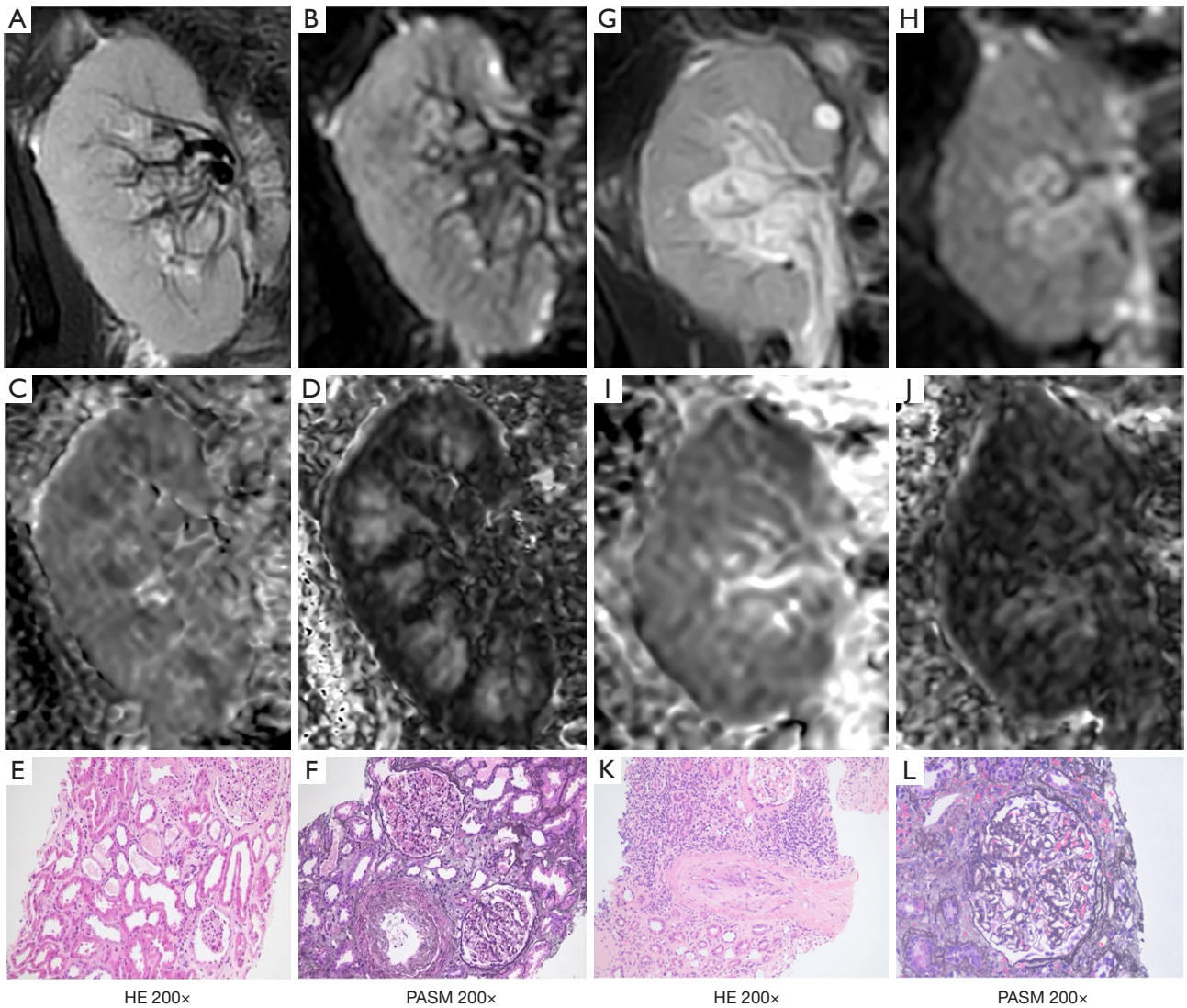

Figure 3 Comparison of DTI and pathological findings. (A-F) A 17-year-old woman with no fibrosis (ci0), while panels (G-L) are from a 42-year-old man with moderate fibrosis (ci2). (A,G) T2-weighted images; (B,H) b0 images; (C,I) ADC maps; (D,J) FA maps; (E,K) hematoxylin-eosin; (F,L) periodic acid-silver methenamine. ci0, no fibrosis (<5% of cortex occupied by fibrosis); ci2, moderate fibrosis (26–50% of cortex occupied by fibrosis). ADC, apparent diffusion coefficient; DTI, diffusion tensor imaging; FA, fractional anisotropy; HE, hematoxylin-eosin; PASM, periodic acid-silver methenamine.

compare with ASL. Compared with IVIM sequence, the FA values obtained by DTI can better reflect the diffusion degree of water molecules in each direction. We found a stronger correlation between medullary FA values and fibrosis scores than RBF. DTI had a higher diagnostic efficiency than ASL in the differentiation of fibrosis stages, and the results were statistically different in the differentiation of the ci0–2 and ci3 groups (AUC =0.945 *vs.* 0.802, P<0.05). This may indicate that the effect of the fibrotic response on the transplanted kidney is more on the direction of water diffusion, possibly due to the accumulation of fibrotic matrix which hinders the diffusion of water molecules. These results indicate that DTI is more sensitive than ASL in noninvasive identification of fibrosis severity in renal allografts. Compared with DTI alone, the combination of DTI and ASL has no significant improvement in the diagnosis of renal allograft fibrosis of different degrees, indicating that the added value of ASL

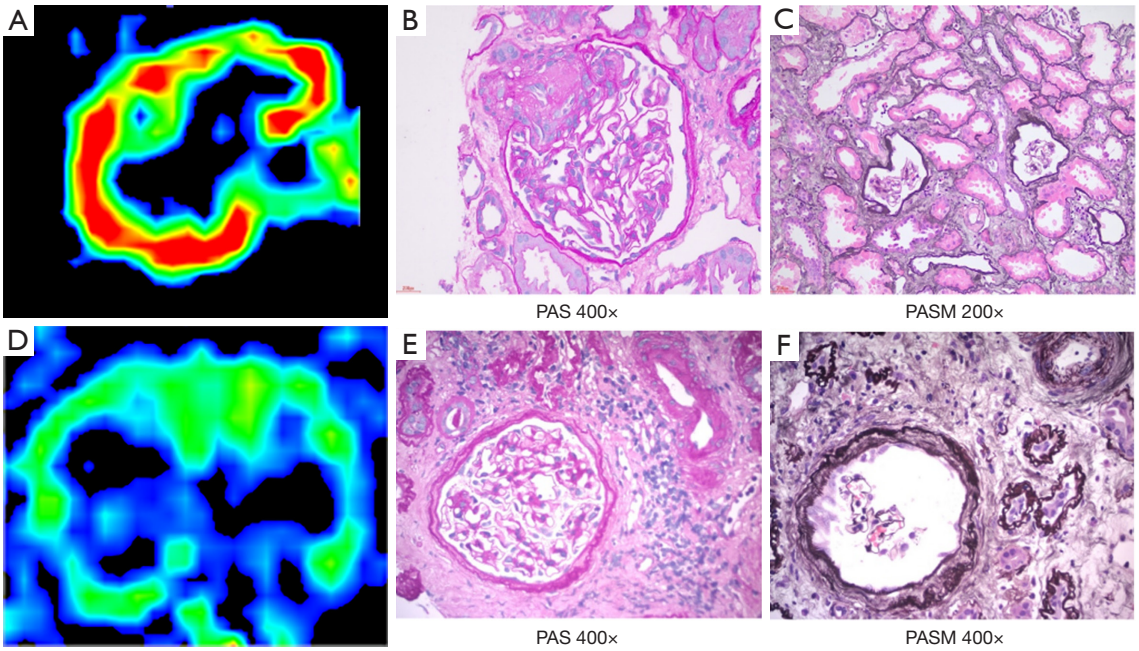

Figure 4 Comparison of RBF derived from ASL and pathological findings. (A-C) A 56-year-old woman with mild fibrosis (ci1); (D-F) a 43-year-old woman with severe fibrosis (ci3). (A,D) RBF maps; (B,E) periodic acid-Schiff; (C,F) periodic acid-silver methenamine. ci1, mild fibrosis (5–25% of cortex occupied by fibrosis); ci3, severe fibrosis (>50% of cortex occupied by fibrosis). RBF, renal blood flow; ASL, arterial spin labeling; PAS, periodic acid-Schiff; PASM, periodic acid-silver methenamine.

Table 2 Discriminative performances of DTI and ASL on diagnosing renal allograft fibrosis degree

Parameters	ci0 *vs.* ci1–3	ci0–1 *vs.* ci2–3	ci0–2 *vs.* ci3
ASL (RBF)			
AUC	0.712	0.735	0.802
P value	<0.001	<0.001	<0.001
Cut-off	203.53	166.50	149.93
Sensitivity	0.641	0.719	0.740
Specificity	0.714	0.667	0.786
DTI (medullary FA)			
AUC	0.725	0.787	0.945
P value	<0.001	<0.001	<0.001
Cut-off	0.268	0.266	0.251
Sensitivity	0.844	0.792	0.811
Specificity	0.532	0.667	0.999

RBF is in the unit of mL/min/100 g. ci0 *vs.* ci1–3, no fibrosis *vs.* with fibrosis; ci0–1 *vs.* ci2–3, no or mild fibrosis *vs.* moderate to severe fibrosis; ci0–2 *vs.* ci3, non-severe fibrosis *vs.* severe fibrosis. DTI, diffusion tensor imaging; ASL, arterial spin labeling; AUC, area under the curve; RBF, renal blood flow; FA, fractional anisotropy.

to DTI is limited. In addition, ASL has its limitations and cannot distinguish the reduction of renal perfusion function caused by fibrosis or other damage factors (32-35).

Our study has certain limitations. First, the sample size in the ci3 group is small, which may have caused selection bias and the results to have certain errors. Second, due to the limitation of MRI technology itself, it is impossible to distinguish the changes of parameters caused by fibrotic and non-fibrotic factors. The actual parameter values may have not only been affected by fibrosis. In addition, the samples obtained by biopsy have limitations and may not accurately reflect the true situation of fibrosis in the transplanted kidney.

Conclusions

DTI has a higher diagnostic value than ASL in noninvasively differentiating the degree of fibrosis in RT. The FA value obtained by DTI can well reflect the degree of diffusion of water molecules in all directions. With the progression of fibrosis, FA value can show statistically significant changes. Compared with the RBF value obtained by ASL, the medullary FA value has a stronger correlation with fibrosis

score, and the diagnostic efficiency is higher.

Acknowledgments

None.

Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://qims.amegroups.com/article/view/10.21037/qims-24-1023/rc

Funding: This work was supported by Undergraduate Training Program for Innovation and Entrepreneurship Soochow University (No. 202310285070Z) and Basic Research Pilot Program of Suzhou City (No. SSD2024049).

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://qims.amegroups.com/article/view/10.21037/qims-24-1023/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the Ethics Review Board of The First Affiliated Hospital of Soochow University (2022) (No. 412). Informed consent was provided by all individual participants. For participants under 18 years, informed consent was provided by their legal guardians.

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Cite this article as: Wan J, Jin M, Li J, Ma J, Que C, Jiang B, Tian Y, Hu L, Yu Y, Hu C, Wang J, Zhu M. Magnetic resonance diffusion tensor imaging is superior to arterial spin labeling in detecting renal allograft fibrosis. Quant Imaging Med Surg 2025;15(4):3211-3221. doi: 10.21037/qims-24-1023

