

## Original Article

# Assessment of endothelial and renal dysfunction in patients after renal transplantation

Zhe Wang<sup>1\*</sup>, Wei Wang<sup>2\*</sup>, Yihang Jiang<sup>2</sup>, Min Zhang<sup>3</sup>, Hang Yin<sup>2</sup>

Departments of <sup>1</sup>Cardiology, <sup>2</sup>Urology, Chaoyang Hospital, Capital Medical University, Beijing, China; <sup>3</sup>Department of Nephrology, Beijing Rehabilitation Hospital of Capital Medical University, Beijing, China. \*Equal contributors.

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**Abstract:** Objective: Renal failure and cardiovascular events are the most severe complications of renal transplantation, and significantly affect long term survival. A common cause of renal failure is renal fibrosis in which endothelial dysfunction plays an important role. Endothelial dysfunction as an independent factor is directly involved in renal interstitial fibrosis. Thus, the assessment of endothelial dysfunction is very important after renal transplantation. Methods: 79 consecutive patients receiving renal transplantation were recruited from the Urology Clinic between July 2014 and July 2015. RHI was employed to assess endothelial function, blood creatinine, and glomerular filtration rate (eGFR). Mean arterial pressure (MAP) was detected on recruitment and at 12 and 24 months after recruitment. Results: RHI has close relationships with creatinine and eGFR ( $R = -0.881$  and  $0.942$ , respectively;  $P = 0.0001$ ). RHI is also related to the time after renal transplantation ( $R = -0.438$ ,  $P = 0.01$ ). Multivariate linear model analysis shows MAP and eGFR are predictors of RHI after renal transplantation. At 24 months, RHI ( $1.58 \pm 0.29$ ) was significantly lower than on recruitment ( $1.68 \pm 0.31$ ) ( $P = 0.04$ ). The blood creatinine in patients with endothelial dysfunction was significantly higher than in those with normal endothelial function ( $126.3 \pm 45.3$  vs  $98.4 \pm 44.1$ ,  $P < 0.05$ ). The eGFR in patients with endothelial dysfunction was markedly lower than in those with normal endothelial function ( $59.3 \pm 15.2$  vs  $70.1 \pm 16.4$ ,  $P < 0.05$ ). The kidney function remained unchanged in patients with normal endothelial function. Conclusion: At 6 months after renal transplantation, endothelial dysfunction deteriorates over time and has a close relationship with renal dysfunction.

**Keywords:** Endothelial dysfunction, reactive hyperemia index, kidney transplantation, renal fibrosis, renal function

## Introduction

Renal transplantation is the most effective treatment for end stage renal disease (ESRD). Although great progress has been achieved in the transplantation of solid organs in the past 2 decades, the long term survival of recipients is still a challenge due to renal failure. The major cause of renal failure in these patients is renal fibrosis due to the calcineurin inhibitor (CNI; such as cyclosporine and tacrolimus) induced nephrotoxicity.

Traditionally, chronic allograft injury and renal fibrosis are assessed according to the serum creatinine (Cr) and glomerular filtration rate (GFR), but these have limitations in the early assessment of allograft interstitial fibrosis. Although renal biopsy is the golden standard in

the diagnosis of renal allograft interstitial fibrosis, it requires a complex operation and is invasive, and therefore cannot be used routinely as a tool. Studies [1-3] have shown that peritubular capillary (PTC) endothelial lesions and endothelial dysfunction play important roles in the pathogenesis of renal allograft interstitial fibrosis, and both as independent factors are directly involved in renal interstitial fibrosis. However, there is not a non-invasive method for the assessment of endothelial function in studies on renal fibrosis after renal transplantation.

This longitudinal study aimed to investigate endothelial function in patients after renal transplantation. Endothelial function was assessed with reactive hyperemia index (RHI) on recruitment and at 1 and 2 years after recruitment. The correlation between RHI and kidney

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**Table 1.** Clinical characteristics of patients receiving renal transplantation (n = 79)

Parameter	Value
Age (yr)	34.6 ± 6.9
Male, no. (%)	45 (57.0%)
Body mass index, kg/m <sup>2</sup>	24.1 ± 4.3
Smoking status, no. (%)	7 (8.9%)
Hypertension, no. (%)	72 (91.1%)
Diabetes, no. (%)	16 (19.1%)
Coronary artery disease, no. (%)	15 (19.0%)
Glucose, mmol/L	5.89 ± 0.84
Hemoglobin, g%	5.68 ± 0.72
Cholesterol, mmol/L	5.21 ± 1.32
High-density lipoprotein, mmol/L	1.04 ± 0.14
Low-density lipoprotein, mmol/L	2.61 ± 1.13
Triglycerides, mmol/L	1.72 ± 0.30
Time after transplantation, m	21.6 ± 19.7
Anti-Hypertensive Medication	
Alpha Blockers, no. (%)	11 (13.9%)
Beta Blockers, no. (%)	30 (38.0%)
Calcium Channel Blockers, no. (%)	59 (74.7%)
ACE Inhibitors, no. (%)	13 (16.5%)
Angiotensin Receptor Blocker, no. (%)	18 (22.8%)
Statin Therapy, no. (%)	24 (27.8%)

function was further explored. This may provide evidence for endothelial function as a clinical parameter of renal allograft interstitial fibrosis.

### Materials and methods

#### General information

This was a single-center prospective study. A total of 79 consecutive patients were recruited from the Urology Clinic between July 2014 and July 2015. These patients received renal transplantation at least 6 months prior and were no younger than 18 years old. There were 45 males (57.0%) and 34 females (43.0%). The mean age was 34.6 ± 6.9 years, and there were no contradictions. This study was approved by the Ethics Committee of our hospital, and informed consent was obtained from each patient.

#### Methods

The baseline characteristics were collected after recruitment. On recruitment, all of the patients received RHI examination, and then

again at 12 months and 24 months after recruitment. The change in RHI was analyzed. In addition, the serum Cr, GFR, and mean arterial pressure (MAP) were also detected at three time points. Their relationships with RHI and kidney function were further evaluated.

#### Non-invasive detection of endothelial function

RHI was detected with Endo-PAT2000 (Itamar Medical Company) within 1 week after the informed consent was signed. On the day of examination, patients were deprived of food, coffee, tea, and long-acting nitrates for at least 12 hours. They also did not take vitamin C or any vitamin C containing drink, smoke, or exercise strenuously.

The Endo-PAT was developed according to the original plethysmographic biosensor system and can measure the change in finger arterial pulse volume (detection of blood flow). In the measurement, the blood flow of the brachial artery was blocked by cuff inflation, followed by cuff deflation 5 minutes later, then the change in endothelium mediated change in vascular tension was quantified. After cuff deflation, the blood flow may induce the endothelium mediated vascular dilation. This vascular dilation is characterized by responsive congestion which can be recognized by the biological sensor attached to the finger and is reflected as the increased signals. Endo-PAT software was used to calculate the ratio of signal amplitudes before and after blood flow blocking, and the precise RHI was then determined (normally, RHI > 1.67). The higher the RHI, the better the endothelial function is.

The effectiveness of Endo-PAT was evaluated by Bonetti et al. [4] who compared the results from coronary angiography with those from Endo-PAT. Kuvin et al. [5] found that endothelial function determined by Endo-PAT and flow-mediated dilation showed favorable correlation (P < 0.05). Previous studies [6] have shown that this technique is non-invasive, has favorable repeatability, and a short time of detection. Compared to other tools, it is convenient to operate, its operation is independent of the

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**Table 2.** Changes in RHI and kidney function

Parameters	On recruitment	12 months	24 months	P
Reactive hyperemia index	1.68 ± 0.31	1.63 ± 0.38	1.58 ± 0.29	0.45 <sup>1,2</sup> , 0.04 <sup>1-3</sup> ; 0.54 <sup>2-3</sup>
Creatinine (µmol/L)	121.5 ± 48.7	124.1 ± 35.3	127.3 ± 41.2	0.41 <sup>1-2</sup> , 0.09 <sup>1-3</sup> ; 0.21 <sup>2-3</sup>
eGFR (mL/min/1.73 m <sup>2</sup> )	62.2 ± 17.1	59.5 ± 16.7	55.6 ± 18.2	0.35 <sup>1-2</sup> , 0.03 <sup>1-3</sup> ; 0.56 <sup>2-3</sup>
Mean Arterial Pressure (mmHg)	96.9 ± 4.7	97.4 ± 4.3	98.3 ± 3.9	0.12 <sup>1-2</sup> , 0.04 <sup>1-3</sup> ; 0.23 <sup>2-3</sup>

Notes: <sup>1</sup>On recruitment; <sup>2</sup>12 months; <sup>3</sup>24 months.

**Table 3.** Creatinine and eGFR of patients with and without endothelial dysfunction

Parameters	RHI ≤ 1.67 (n = 31)			RHI > 1.67 (n = 48)		
	On recruitment	12 months	24 months	On recruitment	12 months	24 months
Creatinine (µmol/L)	126.3 ± 45.3	134.6 ± 46.7	149.1 ± 44.2*	98.4 ± 44.1**	111.2 ± 41.5	115.6 ± 42.4
eGFR (mL/min/1.73 m <sup>2</sup> )	59.3 ± 15.2	55.4 ± 16.1	50.7 ± 17.2*	70.1 ± 16.4##	68.2 ± 17.1	66.7 ± 16.9

Notes: \*P < 0.05 vs on recruitment; \*\*P < 0.01 vs RHI ≤ 1.67 group; #P < 0.05 vs on recruitment; ##P < 0.01 vs RHI ≤ 1.67 group.

operator, and it can rapidly and automatically determine results.

### Statistical analysis

Statistical analysis was performed with SPSS version 22.0. Qualitative data were compared with Chi-square test. Quantitative data were expressed as mean ± standard deviation and compared with an independent sample t-test. Correlation was evaluated with multivariate linear regression analysis. A value of P < 0.05 was considered statistically significant.

### Results

#### Clinical characteristics of patients at baseline

A total of 79 patients receiving renal transplantation were recruited. The demographics and clinical characteristics are shown in **Table 1**. The mean age was 34.6 ± 6.9 years, and males accounted for 57.0%. There were 7 smokers (8.9%), 72 patients had concomitant hypertension (91.1%), 16 had concomitant diabetes mellitus (DM) (19.1%), and 15 had concomitant coronary heart disease (19.0%). The mean time after transplantation was 21.6 ± 19.7 months. The most common pressure lowering drug was calcium antagonists (74.7%), followed by beta blockers (38%), and statins (27.8%).

#### Changes in RHI, kidney function and MAP

Patients were followed up for 24 months. Normal RHI was defined as > 1.67. On recruitment, the mean RHI was 1.68 ± 0.31, but it was 1.63 ± 0.38 at 12 months and 1.58 ± 0.29

at 24 months. As compared to RHI on recruitment, the RHI at 24 months reduced significantly (P = 0.04). There was no significant difference in serum Cr among three time points. The eGFR at 24 months (55.6 ± 18.2) was significantly lower than on recruitment (62.2 ± 17.1) (P = 0.03). At 24 months, the MAP was 98.3 ± 3.9, markedly higher than on recruitment (96.9 ± 4.7) (P = 0.04) (**Table 2**).

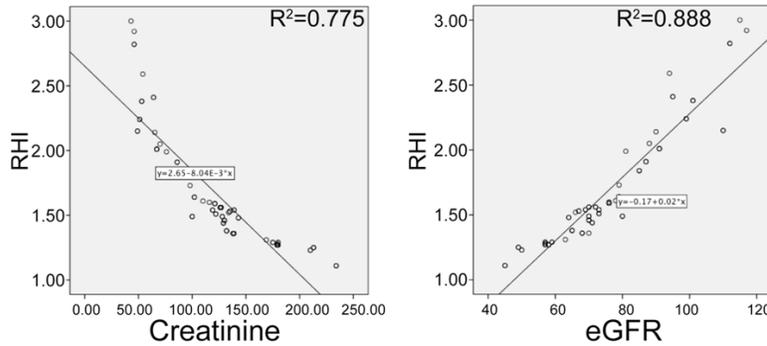
#### Serum Cr and eGFR in patients with normal and abnormal endothelial function

On the basis of RHI on recruitment, patients were divided into an endothelial dysfunction group (RHI ≤ 1.67) (n = 31) and a normal endothelial function group (RHI > 1.67) (n = 48). The RHI, serum Cr, and eGFR were detected on recruitment and at 12 and 24 months after recruitment. Results showed the serum Cr and eGFR in RHI ≤ 1.67 group at 24 months were significantly different from those at recruitment (P < 0.05). In RHI > 1.67 group, the serum Cr and eGFR at 24 months were markedly different from those on recruitment (P < 0.05) (**Table 3**).

#### Correlation of RHI with time after transplantation, serum Cr, and eGFR in patients receiving renal transplantation

RHI was closely related to the time after transplantation (R = -0.438, P = 0.04). RHI was related to serum Cr (R = -0.881, R<sup>2</sup> = 0.775), and the adjusted R<sup>2</sup> was 0.772 (P = 0.0001). RHI was associated with eGFR (R = 0.942, R<sup>2</sup> = 0.888), and the adjusted R<sup>2</sup> was 0.886 (P = 0.0001) (**Figure 1**).

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**Figure 1.** Correlation of RHI with serum Cr and eGFR in patients receiving renal transplantation.

**Table 4.** Multivariate linear regression analysis of RHI in recipients after renal transplantation

	Beta	SE	95% CI <sup>a</sup>	T	P
Age	0.054	0.002	-0.001, 0.008	1.435	0.156
Male	-0.030	0.035	-0.096, 0.043	-0.773	0.442
Coronary artery disease	0.013	0.037	-0.061, 0.088	0.349	0.728
Time after transplantation	-0.028	0.001	-0.003, 0.001	-0.632	0.529
Mean Arterial Pressure	-0.195	0.008	0.003, 0.034	2.379	0.020
eGFR	1.095	0.004	0.021, 0.036	7.513	0.0001
Creatinine	0.016	0.002	-0.003, 0.003	0.090	0.928

Notes: <sup>a</sup>95% Confidence Interval: Lower bound, Upper bound.

### Factors affecting RHI in patients receiving renal transplantation

RHI serves as a dependent variable and multivariate linear regression analysis shows MAP and eGFR are factors affecting RHI ( $\beta = -0.195$ , 95% CI = -0.003-0.034,  $P = 0.02$ ;  $\beta = 1.095$ , 95% CI = 0.021-0.036,  $P = 0.0001$ ). RHI was negatively related to MAP and positively to eGFR (Table 4).

### Discussion

Studies have shown that endothelial dysfunction in recipients after renal transplantation are closely related to renal allograft loss, and endothelial dysfunction is a common event in these patients [7]. This study summarized studies on the assessment of endothelial function with flow-mediated dilation, and analysis was performed with Cox proportional hazards model. Results show renal allograft loss at > 90 days after renal transplantation is closely related to endothelial dysfunction. Malyszko et al. [8] reported that the recipients had endo-

thelial dysfunction after renal transplantation, and the expression of renalase antibody was detectable in endothelial cells. Chronic renal allograft loss is pathologically characterized by renal allograft interstitial fibrosis, and arteriosclerosis in the renal allograft has been accepted as a major cause of renal interstitial fibrosis and renal tubular atrophy [9]. Endothelial dysfunction is characterized by the impairment of vasodilatation and has been a crucial factor causing the occurrence and development of arteriosclerosis [10]. There is evidence showing that endothelial dysfunction plays important roles in the pathogenesis of renal allograft interstitial fibrosis. However, whether RHI related endothelial function may provide evidence for the assessment of kidney dysfunction after renal

transplantation, which then indirectly determines the risk for renal interstitial fibrosis after renal transplantation, is still unclear. This study was to assess long term endothelial function in recipients after renal transplantation and its relationship with renal dysfunction was further evaluated. Our results show RHI is closely related to serum Cr and eGFR ( $R = -0.881$  and  $R = 0.942$ , respectively,  $P = 0.0001$ ), and RHI is associated with post-transplantation renal dysfunction. Multivariate linear regression model reveals that MAP and eGFR are predictors of RHI after renal transplantation.

A study investigated the effects of immunosuppressants on renal allograft fibrosis and kidney function [11], in which 36 patients with stable kidney function were recruited after transplantation and were treated with calcineurin inhibitor and mTORi. All of the patients received renal biopsy and were followed up for 60 months. Results showed that allograft loss was related to the development of renal allograft fibrosis on recruitment and at 60 months ( $r = 0.47$ ,  $P = 0.004$ ;  $r = 0.47$ ,  $P = 0.006$ ). In addition, the

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study also revealed that, at 6 months after transplantation, the serum Cr increased significantly over time but eGFR reduced markedly, regardless of the immunosuppressants, and that calcineurin inhibitor had more potent capability to cause renal dysfunction in comparison to mTORi. Moreover, allograft fibrosis was closely related to eGFR ( $r = -0.47$ ,  $P = 0.004$ ).

Long term monitoring of endothelial function was done in patients after renal transplantation. In this study, patients with stable kidney function were recruited at > 6 months after renal transplantation, and followed up for 12 and 24 months. At 24 months, eGFR ( $55.6 \pm 18.2$ ) was significantly lower than that on recruitment ( $P = 0.03$ ), and the eGFR tended to reduce over time, which was consistent with above mentioned. In addition, at 24 months, the RHI ( $1.58 \pm 0.29$ ) reduced dramatically as compared to that on recruitment ( $1.68 \pm 0.31$ ) ( $P = 0.04$ ). At 24 months, the MAP ( $98.3 \pm 3.9$ ) was markedly higher than that on recruitment ( $P = 0.04$ ). These findings indicate that endothelial dysfunction deteriorates over time after renal transplantation.

In addition, our results also show that serum Cr in patients with endothelial dysfunction is significantly higher than in those with normal kidney function ( $126.3 \pm 45.3$  vs  $98.4 \pm 44.1$ ,  $P < 0.05$ ), but eGFR in patients with endothelial dysfunction is markedly lower than in those with normal kidney function ( $59.3 \pm 15.2$  vs  $70.1 \pm 16.4$ ,  $P < 0.05$ ). At 24 months, the serum Cr and eGFR are significantly different from those on recruitment ( $149.1 \pm 44.2$  vs  $126.3 \pm 45.3$ ,  $P < 0.05$ ;  $50.7 \pm 17.2$  vs  $59.3 \pm 15.2$ ,  $P < 0.05$ ) in patients with endothelial dysfunction. However, in patients with normal endothelial function, the kidney function remains unchanged. This suggests that endothelial dysfunction is closely related to kidney function in recipients after renal transplantation.

The main etiology of chronic allograft nephropathy after renal transplantation is renal fibrosis which is clinically characterized by progressive reduction of kidney function, often with concomitant hypertension and proteinuria. Moreover, endothelial dysfunction plays important roles in renal allograft interstitial fibrosis. Endothelial dysfunction as an independent factor is directly involved in the pathogenesis

of renal interstitial fibrosis. Thus, detection of endothelial function after renal transplantation is of great importance in clinical practice. Endothelial function may be used to evaluate the risk for renal fibrosis and guide the renal biopsy and adjustment of pharmacotherapy to improve renal fibrosis. However, there is still controversy on the diagnostic value of renal biopsy in patients without severe renal interstitial fibrosis [12, 13]. Therefore, it is imperative to identify a factor used for the early prediction of renal interstitial fibrosis. Our results show that endothelial dysfunction after renal transplantation is closely related to renal dysfunction and that endothelial dysfunction deteriorates over time after transplantation. Our findings suggest that endothelial dysfunction is related to renal fibrosis after renal transplantation.

This was a longitudinal study with a relatively long period of follow up, which was to our advantage. In addition, our results provide evidence about the long term influence of renal transplantation on endothelial function. In addition, RHI is employed to evaluate the endothelial function, which is also an advantage of our study. Previous studies [8] have indicated that this was a non-invasive technique, had favorable repeatability, and could be done in a short time. As compared to other tools, it is convenient to operate, its operation is independent of the operator, and it can rapidly and automatically determine results.

This was only a pilot study, and there was no pathological examination. In most studies, biopsy of allograft has been employed in the assessment of chronic renal impairment after renal transplantation [14, 15], especially the interstitial fibrosis, which makes the prediction of allograft loss and/or failure with histological examination possible. The correlation analysis of pathologically proven renal fibrosis and RHI will provide convincing evidence on the use of RHI in the prediction of renal fibrosis after renal transplantation. In addition, the sample size was small in this study. There is an ongoing study with a larger sample size in our department, which investigates the correlation between pathologically proven renal fibrosis and RHI. In the near future, we hope that more clinically important findings will be obtained from this study.

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## Disclosure of conflict of interest

None.

**Address correspondence to:** Hang Yin, Department of Urology, Chaoyang Hospital, Capital Medical University, Beijing, China. E-mail: dr\_yinhang@hotmail.com

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