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Postreperfusion Renal Allograft Biopsy Predicts Outcome of Single-Kidney Transplantation: A 10-Year Observational Study in China

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Introduction: Biopsy findings often lead to the discard of many donor kidneys although their clinical value is not fully understood. We investigated the predictive value of postreperfusion biopsy on long-term allograft outcome after single-kidney transplantation.

Methods: We retrospectively evaluated the significance of histologic findings, read by experienced renal pathologists, in 461 postreperfusion biopsy specimens collected from 2010 to 2017 after deceased donor renal transplant; and performed time-to-event analyses to determine the association between histology and hazard of death-censored graft failure. Recipients were followed-up with over a median time of 6.8 (range, 0.2–11.9) years. We assessed specimens using the Remuzzi score (scale of 0–12) and categorized them into low-score (≤3) and high-score (>3) groups. Kappa coefficients were calculated to assess agreement in procurement versus reperfusion biopsies.

Results: High Remuzzi score kidneys came from older donors with a higher incidence of hypertension, higher final creatinine, death from cerebrovascular disease, expanded criteria donor, and a higher kidney donor risk index (KDRI) (all $P < 0.001$). In adjusted analyses, Remuzzi score was independently associated with death-censored graft failure (hazard ratio [HR] 1.389 for each 1 score rise in Remuzzi score, 95% confidence interval 1.181–1.633, $P < 0.001$). Overall histologic agreement (procurement biopsy versus reperfusion biopsy) was kappa = 0.137.

Conclusion: Our findings suggest that postreperfusion biopsy is associated with long-time graft outcomes after transplant from a deceased donor. Agreement between procurement and reperfusion biopsy was found to be low. Prospective trials are necessary to optimize procurement biopsy practices.

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The proportion of deceased donor kidneys procured for transplant but subsequently discarded has been growing steadily in the United States. Internationally, the assessment of preimplant histology has been an important tool for the selection of donor kidneys for many years,[1,2] and their findings are the most frequently cited justification for kidney discard despite conflicting evidence underlying their use.[3,4] Deceased donor kidney procurement biopsies are performed during allocation.[5] Preimplant biopsies are performed before the implantation, and postreperfusion biopsies are performed after opening the end of the anastomosis time,[5] which is largely to inform organ quality assessment by evaluating the degree of chronic histological injury. Although initial studies suggested the usefulness of procurement biopsies in predicting post-transplant outcomes, subsequent research showed that their findings do not correlate with graft survival when accounting for other donor characteristics.[6,7] As a result, the true value of renal histology in predicting long-term outcomes following renal transplantation has

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emerged as a topic of significant interest. Most of the studies that have analyzed the value of histologic findings in predicting long-term post-transplant outcomes have been relatively small cohort studies or have been limited in the biopsy sample features examined.[8,9] In addition, there is a paucity of research attempting to establish a link between histologic findings and subsequent transplant function. Although small studies have demonstrated that the value of histologic findings noted on frozen section before implantation is predictive of outcomes, Wang *et al*. underscored both the poor quality of available data in the literature and the weak association of findings with outcomes in a systematic review of preimplantation biopsies in 2015.[9] The majority of analyses have been confounded by the use of frozen tissue, wedge biopsies versus needle biopsies, and by pathologists with limited expertise in the evaluation of renal tissue, all of which have contributed to the inability to draw definitive conclusions.[9-11]More recent studies suggest that post-reperfusion time-zero biopsies may better represent the impact of donor factors on long-term graft outcomes.[12,13]

In this study, we retrospectively examined post-reperfusion biopsies of renal allografts obtained for protocol purposes in our center, rather than for transplant decisions. We attempt to determine the ability of postreperfusion needle core renal allograft biopsies, optimally processed with formalin-fixed, paraffin embedded sections and read or classified by experienced renal pathologists, to predict post-transplant renal function as well as early and long-term outcomes after single-kidney deceased donor transplantation. We compared the biopsy results of paired kidneys from the same donor and compared the consistency of procurement biopsies and reperfusion biopsies.

METHODS

Study Population

Donor grafts were allocated by the Organ Procurement Organization through the China Organ Transplant Response System, which is an encrypted national network run electronically to ensure fairness and transparency in the organ allocation process.[14] We obtained related statements from the Organ Procurement Organization and the Organ Transplant Ethics Committee (supporting Organ Procurement Organization and institutional review board documents are available for this article online, as Supplementary Material). There were no kidneys procured from prisoners in this study. This research was approved by the ethics committee of the First Affiliated Hospital of Zhejiang University of medicine (Ref: IIT20230409A). Written informed consent was waived because the study design was retrospective and noninterventional. All study activities followed the guidelines of the 2000 Declaration of Helsinki and the 2018 Declaration of Istanbul 2018.

We conducted a retrospective cohort study utilizing data from the Organ Procurement Organization and the transplantation follow-up system of the Kidney Disease Center of the First Affiliated Hospital of Zhejiang University of Medicine. We identified a total of 770 deceased donor kidney transplants at the Renal Disease Center of the First Affiliated Hospital of Zhejiang University School of Medicine between January 1, 2010, and June 30, 2017 and excluded donor organs on which no reperfusion renal allograft biopsy was performed ($n = 33$). Further exclusion criteria (detailed in Figure 1) were as follows: after further excluding the transplants that did not meet the pathological requirements (glomerular < 10, artery < 1, $n = 182$), those for which donor or recipient data were missing ($n = 79$), recipients <18 years old ($n = 7$), those who died with a functioning graft 6 months after transplantation ($n = 6$), those with nephrectomy caused by renal artery embolization ($n = 1$), and pancreas-kidney transplantation ($n = 1$), we analyzed a final cohort of 461 transplants.

Then we utilized a continuous retrospective cohort of all single-kidney deceased donor kidneys transplanted at our center from January 1, 2020 to December 31, 2021, that had both procurement biopsy and postreperfusion biopsy ($n = 89$).

Variable Definition and Data Collection

As part of our data collection process, we obtained recipient and donor demographics (age, sex [male/female], and race), anthropometrics (height, weight, and body mass index), comorbidities (hypertension and diabetes), and the donor terminal creatinine or creatinine at the time of donation. Recipient status at the time of last follow-up was defined as alive with a functioning allograft, alive with a failed allograft, or dead with a functioning allograft. Analyses that used most recent creatinine as an outcome were restricted to those patients who still had an allograft available at the end of the follow-up period. Transplant-specific characteristics, including total number of human leukocyte antigen mismatches and cold ischemia time were obtained. Delayed graft function (DGF) was defined as the need for dialysis within the first week post-transplant.

As recommended by the Organ Procurement and Transplantation Network, the KDRI was calculated for each donor using a 2020 scaling factor.[15] The KDRI is calculated using the following 10 donor-specific clinical

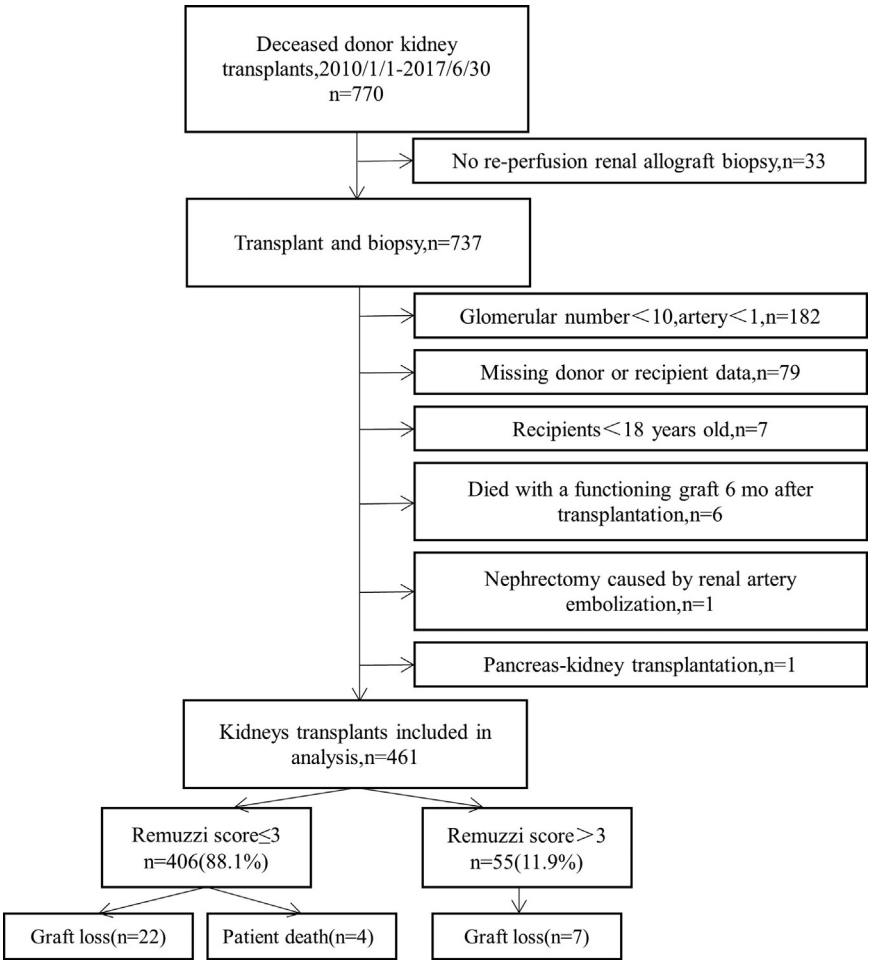

Figure 1. Flow diagram of patient enrollment and follow-up in the study. A total of 461 kidney transplants from 315 deceased donors were performed from 2012 to 2017 in our center. Based on the Remuzzi score, the donors and corresponding recipients were divided into 2 groups: Low score group (≤3, $n = 406$) and high score group (>3, $n = 55$). All enrolled 461 patients were followed-up with for at least 5 years; the median follow-up time was 6.8 (quartiles, 5.7–8.4) years.

characteristics: age, height, weight, ethnicity, history of hypertension, history of diabetes, cause of death, serum creatinine, hepatitis C virus status, and donation after cardiac death status.

Biopsy Variables

Postreperfusion biopsies were performed after opening the clamps at the end of the anastomosis time. Renal biopsies were performed using a 16-gauge Tru-Cut needle to obtain tissue core that were formalin-fixed, paraffin-embedded, and processed according to standard techniques, which included the use of the hematoxylin and eosin, periodic acid Schiff, trichrome, and Jones methenamine silver stains. According to the renal Remuzzi scoring standard,[16,17] the biopsy pathological specimens after renal reperfusion were graded and scored by experienced renal pathologists from our center whose clinical practice exclusively includes renal pathology, with each pathologist examining more than 1000 renal biopsy samples per year. We evaluated specimens for the Remuzzi score (scale, 0–12), degree of glomerulosclerosis, interstitial fibrosis, tubular atrophy, and vascular disease (scale, 0–3). Remuzzi score ≤3 was divided into low score group, and >3 was divided into high score group. Glomerular ≥10 and artery ≥1, were qualified specimens, consistent with the Banff recommendations.[18] We calculated the degree of glomerular sclerosis (%), tubular atrophy (%), renal interstitial fibrosis (%), vessel-wall thickness, renal interstitial nuclear cell infiltration (%), acute tubular injury, renal artery transparency (%), and the number of glomerular thrombi. Others such as glomerulitis (g), mesangial matrix hyperplasia (mm), perivascular capillary vasculitis (t), renal tubulitis (t), renal arteritis (v), tumors, etc., were diagnosed according to pathology and kidney disease diagnostics by routine criteria. They were not included in the statistics.

Procurement biopsies were performed before the implantation in the expanded criteria donor, and the results of these biopsies were compiled retrospectively. Renal biopsies were performed using a 16-gauge Tru-Cut needle to obtain core frozen kidney sections of

biopsies. Information regarding glomerulosclerosis, interstitial fibrosis and tubular atrophy, and vascular disease as reported by the interpreting pathologists was obtained for each biopsy directly from procurement biopsy histology reports. Procurement biopsies were scored using the same scheme as the reperfusion biopsies.

Study Outcomes

Patients were followed up with regularly from the date of transplantation until death, end of study (October 31, 2022), or graft failure. The primary outcome was death-censored graft failure (defined as estimated glomerular filtration rate declining to <15 ml/min per 1.73 m^2 or the return to dialysis or retransplantation). Other outcomes of interest included DGF, 1-year graft creatinine and most recent serum creatinine among kidney transplant recipients with functioning allografts. The secondary outcome of interest was concordance in the overall histologic classification between procurement and reperfusion biopsy.

Statistical Analyses

All analyses were performed using IBM SPSS Statistics for Windows (Version 22.0. IBM Corp., Armonk, NY). Continuous variables were expressed as the mean ± SD or the median with the interquartile range, and comparisons between groups were performed using the *t* test or Mann-Whitney U test. Categorical variables were expressed as counts and percentages, and differences between groups were analyzed using the χ^2 test or Fisher's exact test. Correlation coefficients and Kappa values between procurement and reperfusion biopsy findings were examined using Spearman correlation analysis.

Allograft outcomes were assessed using Kaplan-Meier curves and the log-rank test. We performed univariate and multivariable time-to-event analyses for death-censored allograft failure using Cox-proportional hazards models for the overall cohort. Remuzzi score was analyzed as a continuous variable in the univariate and multivariable analyses. Candidate variables with $P < 0.10$ on univariate analysis were included in multivariable models. In the first adjusted model, only postreperfusion histologic classification and donor KDRI were included. In a second adjusted model, we included postreperfusion histologic classification, donor characteristics (age, sex, body mass index, final creatinine, hypertension status, donation after cerebrovascular disease, and expanded criteria donor), recipient characteristics (age, sex, and body mass index), and transplant characteristics (number of human leukocyte antigen mismatches, cold ischemia time, and warm ischemia time). A *P*-value < 0.05 was deemed to be significant.

RESULTS

After exclusions (detailed in Figure 1), a total of 461 patients were enrolled in this study; 406 in low Remuzzi score group and 55 in high Remuzzi score group. Recipients were followed-up with for a median of 6.8 years (quartiles, 5.7–8.4 years) after kidney transplantation, during which 29 (6.2%) grafts were lost (the causes of graft failure were listed in Supplementary Table S1) and 4 (0.9%) patients from the low score group died. There were 89 patients who finished both procurement and reperfusion biopsies between January 1, 2020, and January 1, 2021, in our center.

All donor, recipient, and transplant variables were included as recorded in the registry (details in Table 1). A direct comparison of low Remuzzi score kidneys versus high Remuzzi score kidneys, demonstrated that high score kidneys come from older donors with a higher incidence of hypertension, higher final creatinine, death from cerebrovascular disease, expanded criteria donor and a higher KDRI (all $P < 0.001$; Table 1). In addition, high Remuzzi score kidneys were more likely to come from extended criteria donors and had higher KDRI scores than deceased donor kidneys with low Remuzzi score histology ($P < 0.05$; Table 1). Recipients of high score deceased donor kidneys were similar to those who received low score kidneys with respect to sex, body mass index, hypertension, and age. Not surprisingly, low Remuzzi score biopsies were associated with lower rates of DGF ($P < 0.001$; Table 1) and better long-term allograft outcomes (the most recent creatinine among kidney transplant recipients with functioning allografts and the graft survival time) ($P < 0.001$; Table 1). In Table 2 and Figure 2, we show the histological distribution in our cohort. As defined, the high Remuzzi score group had higher degree of glomerular sclerosis, tubular atrophy, renal interstitial fibrosis, and renal interstitial nuclear cell infiltration (all $P < 0.001$; Table 2). A distribution of the KDRI score for the cohort is shown in Figure 3. Poor histology was associated with a higher KDRI index. In the univariate and multivariable analysis of pathologic factors affecting DGF, donor final creatinine and reperfusion biopsy score were the main influencing factors ($P < 0.05$, Table 3). In addition, we found that acute tubular injury and glomerular thrombi were not significantly associated with DGF (Table 3) and graft survival (Table 4).

Kaplan-Meier analysis revealed that renal graft survival rates were significantly lower in the high Remuzzi score group than in the low Remuzzi score group ($P = 0.014$; Figure 4). In the univariate Cox regression model,

Table 1. Baseline characteristics of cohorts, 2010–2017 ($n = 461$)

Characteristics	Total	Remuzzi Score		P Value
		Low Score (≤3)	High Score (>3)	
N (%)	461	406 (88.1)	55 (11.9)	-
Donor characteristics				
Age, yr	38.3 ± 13.5	36.9 ± 13.4	48.3 ± 9.6	< 0.001[c]
Male, no. (%)	404 (87.6)	353 (86.9)	51 (92.7)	0.279
Final creatinine, mg/dl	1.13 ± 0.83	1.04 ± 0.77	1.75 ± 0.99	< 0.001[c]
BMI, kg/m^2	22.5 ± 2.9	22.3 ± 2.8	23.7 ± 3.7	0.01[c]
Hypertension, no. (%)	59 (12.8)	37 (9.1)	22 (40.0)	< 0.001[c]
Death from cerebrovascular disease, no. (%)	121 (26.2)	91 (22.4)	30 (54.5)	< 0.001[c]
Expanded criteria donor, no.(%)	38 (8.2)	25 (6.1)	13 (23.6)	< 0.001[c]
KDRI[a]	0.98 ± 0.27	0.94 ± 0.25	1.25 ± 0.27	< 0.001[c]
Recipient characteristics				
Age at transplant, yr	42.1 ± 10.1	41.9 ± 10.1	44.2 ± 9.8	0.117
Male, no. (%)	280 (60.7)	243 (59.8)	37 (67.2)	0.290
BMI, kg/m^2	21.4 ± 2.9	21.4 ± 2.9	21.0 ± 2.5	0.468
Transplant characteristics				
HLA mismatches, no.	3.0 ± 1.2	3.0 ± 1.2	2.9 ± 1.1	0.978
CIT, min	461.5 ± 210.0	468.2 ± 211.5	411.8 ± 189.1	0.054
Transplant outcomes				
Graft survival time, yr (IQR)	6.8 (5.7, 8.4)	7.1 (5.8, 8.4)	5.9 (5.6, 6.8)	< 0.001[c]
DGF, %	15.8	13.3	34.5	< 0.001[c]
1-yr graft creatinine[b], mg/dl	1.3 ± 0.6	1.2 ± 0.5	1.6 ± 0.8	< 0.001[c]
Most recent creatinine[b], mg/dl	1.15 ± 0.42	1.12 ± 0.42	1.37 ± 0.30	< 0.001[c]

BMI, body mass index; CIT, cold ischemia time; DGF, delayed graft function; ECD, expanded criteria donor; HCV, hepatitis C virus; HLA, human leukocyte antigen; IQR, interquartile range; KDRI, kidney donor risk index
[a]The KDRI score was calculated on the basis of the following donor parameters: age, height, weight, history of hypertension, history of diabetes, cause of death (cerebral stroke), serum creatinine at donation, HCV serostatus, and donation after circulatory death status.
[b]For functioning allografts only.
[c]indicates $P < 0.05$.
Continuous variables are shown as mean ± SD; categorical variables are reported as column percentages; median follow-up is reported as median (IQR).

donor death from cerebrovascular disease, KDRI, and Remuzzi score were significantly associated with a higher rate of death-censored graft failure (Table 4). After adjusting for significant covariates, only Remuzzi score was predictive of death-censored graft failure. These results were similar when adjusting for only KDRI and after adjusting for individual donor, recipient, and transplant characteristics (HR 1.389 for each 1 score increase in Remuzzi score; 95% confidence interval 1.181–1.633; $P < 0.001$) (Table 5).

In Table 6, the pathologic parameters at the time of transplantation were associated with long-term outcome (as reflected by serum creatinine). The arteriolar hyalinosis, high Remuzzi score, glomerular sclerosis, interstitial fibrosis, and arterial and arteriolar narrowing were associated with a higher serum creatinine level at last follow-up ($P < 0.05$). Acute tubular injury, glomerular thrombi, and tubular atrophy were not associated with a higher serum creatinine level at last follow-up ($P < 0.05$).

We compared the biopsy results of the left and right kidneys from the same donor ($n = 315$). Reperfusion biopsy categorical agreement was relatively high between the paired kidneys from the same donor (kappa = 0.293, 0.587, 0.673, 0.211, 0.316 for

Table 2. Histologic characteristics of the cohort, 2010–2017 ($N = 461$)

Biopsy findings	Total	Remuzzi score		P-value
		Low score (≤3)	High score (>3)	
N (%)	461	406 (88.1)	55 (11.9)	-
Number of glomeruli, (IQR)	13 (11, 17)	13 (11, 17)	13 (10, 22)	0.634
Number of artery, (IQR)	6 (4, 8)	5 (4, 7.25)	10 (5, 12)	< 0.001[a]
Glomerulosclerosis, %, (IQR)	0 (0, 7.14)	0 (0, 3.26)	20 (10, 36.36)	< 0.001[a]
Glomerular sclerosis score, (IQR)	0 (0, 1)	0 (0, 1)	2 (1, 2)	< 0.001 [a]
Tubular atrophy score, (IQR)	0 (0, 1)	0 (0, 1)	1 (1, 1)	< 0.001 [a]
Interstitial fibrosis score, (IQR)	0 (0, 1)	0 (0, 1)	1 (1, 1)	< 0.001 [a]
Vascular score, (IQR)	0 (0, 0)	0 (0, 0)	1 (0, 3)	< 0.001 [a]
Remuzzi score, (IQR)	1 (0, 3)	1 (0, 2)	5 (4, 6)	< 0.001 [a]

IQR, interquartile range.
[a]indicates $P < 0.05$

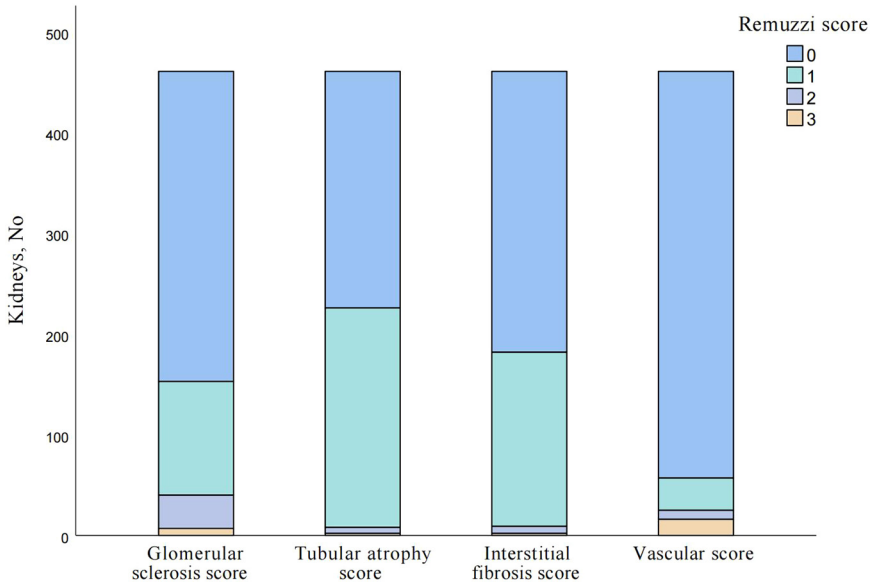

Figure 2. Histological distribution of the cohort ($n = 461$).

glomerular sclerosis score, tubular atrophy score, interstitial fibrosis score, vascular score, and Remuzzi score, respectively; Supplementary Table S2). Categorical agreement between the procurement and reperfusion biopsies was kappa = 0.168, 0.302, 0.432, 0.269, 0.137 for glomerular sclerosis score, tubular atrophy score, interstitial fibrosis score, vascular score, and Remuzzi score, respectively (All $P < 0.05$; Table 7).

When treated as a continuous variable, the overall correlation between procurement and reperfusion glomerulosclerosis was low ($r^2 = 0.11$; Figure 5).

DISCUSSION

Excluding selection bias, sampling techniques (wedge versus core-needle biopsy) and pathologist factors, we evaluated the relationship between reperfusion biopsy

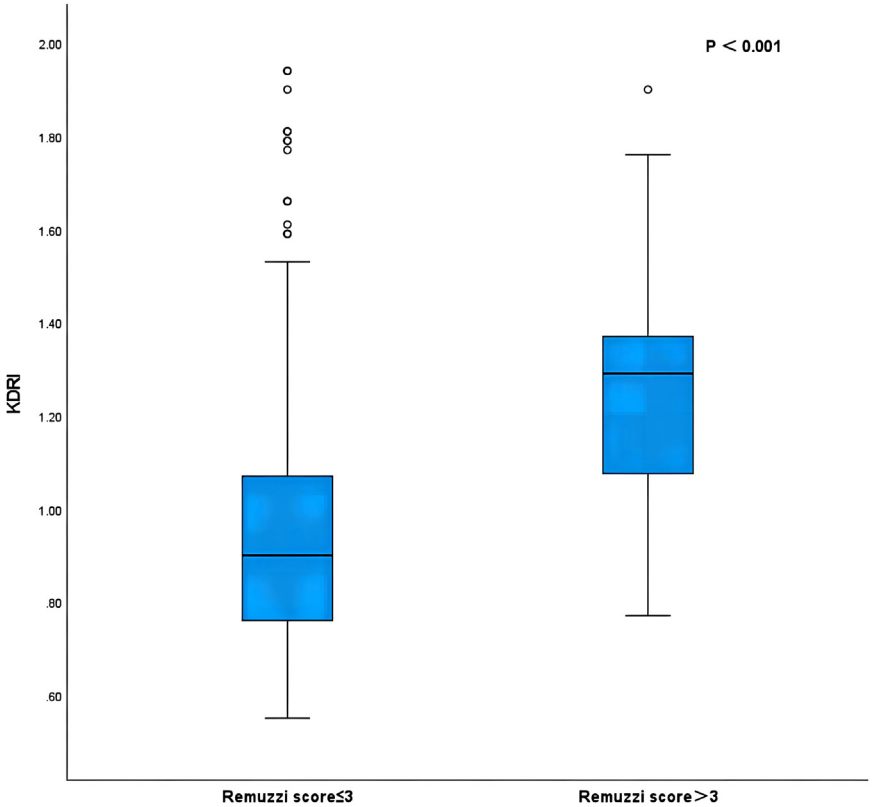

Figure 3. Distribution of the KDRI score between high Remuzzi score and low score group ($P < 0.001$). KDRI, kidney donor risk index.

Table 3. Univariate and multivariable analysis of pathological factors affecting DGF

Variable	DGF (n = 73)	Non-DGF (n = 388)	Univariate P-value	Multivariable P-value
Donor age, yr	41.1 ± 13.8	37.7 ± 13.4	0.05[a]	-
Donor male, no. (%)	67 (91.8)	337 (86.9)	0.246	
Donor final creatini, ,mg/dl	1.7 ± 1.2	1.0 ± 0.7	< 0.001[a]	< 0.001[a]
Donor BMI, kg/m^2	23.1 ± 3.1	22.4 ± 2.9	0.053	-
Donor hypertension, no. (%)	13 (17.8)	46 (11.9)	0.166	
Death from cerebrovascular disease, no. (%)	25 (34.2)	103 (26.5)	0.179	
Expanded criteria donor, no. (%)	8 (11.0)	30 (7.7)	0.358	
KDRI	1.1 ± 0.3	1.0 ± 0.3	< 0.001[a]	-
Recipient age, yr	41.8 ± 9.6	42.2 ± 10.2	0.754	
Recipient male, no. (%)	48 (65.8)	232 (59.8)	0.340	
Recipient BMI, kg/m^2	21.5 ± 2.9	21.3 ± 2.9	0.615	
HLA mismatch, no.	3.1 ± 1.1	3.0 ± 1.2	0.465	
CIT, min	433.8 ± 190.4	466.7 ± 212.8	0.249	
Remuzzi score	2 (0 , 4)	2, (0 , 4)	< 0.001[a]	0.016[a]
Glomerular sclerosis score	0 (0 , 1)	0 (0 , 1)	0.002[a]	
Tubular atrophy score	1 (0 , 1)	0 (0 , 1)	< 0.001[a]	
Interstitial fibrosis score	1 (0 , 1)	0 (0 , 1)	< 0.001[a]	
Vascular score	0 (0 , 1)	0 (0 , 0)	0.144	
Arteriolar hyalinosis, no. (%)	16 (21.9)	51 (13.1)	0.051	-
Acute tubular injury, no. (%)	28 (38.4)	113 (29.1)	0.117	
Glomerular thrombi, no. (%)	2 (2.7)	10 (2.6)	0.936	

BMI, body mass index; CIT, cold ischemia time; DGF, delayed graft function; HLA, human leukocyte antigen; KDRI, kidney donor risk index
[a]Indicates $P < 0.05$.
Factors with $P < 0.1$ were included in the multivariable analysis. Remuzzi Score was analyzed as a continuous variable.

Table 4. Univariate and multivariable analysis for death-censored graft failure

Parameter	No. of Patients	No. of Events	HR	SD Error	P-Value	95% CI
Univariate analysis						
Donor age			1.024	0.014	0.085	(0.997, 1.053)
Donor sex (male)			0.430	0.434	0.052	(0.184, 1.008)
Donor final creatinine			1.152	0.173	0.414	(0.821, 1.616)
Donor BMI			1.088	0.062	0.169	(0.965, 1.228)
Donor hypertension			2.184	0.435	0.072	(0.931, 5.121)
Donor death of CV disease			2.404	0.374	0.019[a]	(1.155, 5.003)
Expanded criteria donor			1.357	0.611	0.618	(0.409, 4.498)
KDRI			3.279	0.590	0.044[a]	(1.032, 10.419)
Recipient age			0.997	0.019	0.860	(0.961, 1.034)
Recipient sex (male)			1.811	0.416	0.153	(0.802, 4.090)
Recipient BMI			1.058	0.060	0.347	(0.941, 1.190)
HLA mismatch			0.955	0.151	0.761	(0.711, 1.284)
CIT			0.998	0.001	0.057	(0.996, 1.000)
Remuzzi Score			1.375	0.083	< 0.001[a]	(1.168, 1.617)
Arteriolar hyalinosis						
absent	394	20				
present	67	9	3.009	0.402	0.006[a]	(1.368, 6.619)
acute tubular injury						
absent	320	20				
present	141	9	1.054	0.402	0.896	(0.480, 2.316)
glomerular thrombi						
absent	449	29				
present	12	0	0.048	5.427	0.576	(0.000, 1993.110)
Multivariable analysis						
Remuzzi Score			1.389	0.083	< 0.001[a]	(1.181 , 1.633)

BMI, body mass index; CI, confidence interval; CIT, cold ischemia time; CV, cardiovascular; HLA, human leukocyte antigen; HR, hazard ratio; KDRI, kidney donor risk index.
[a]indicates $P < 0.05$.
Factors with $P < 0.1$ were included in the multivariable analysis. Remuzzi Score was analyzed as a continuous variable.

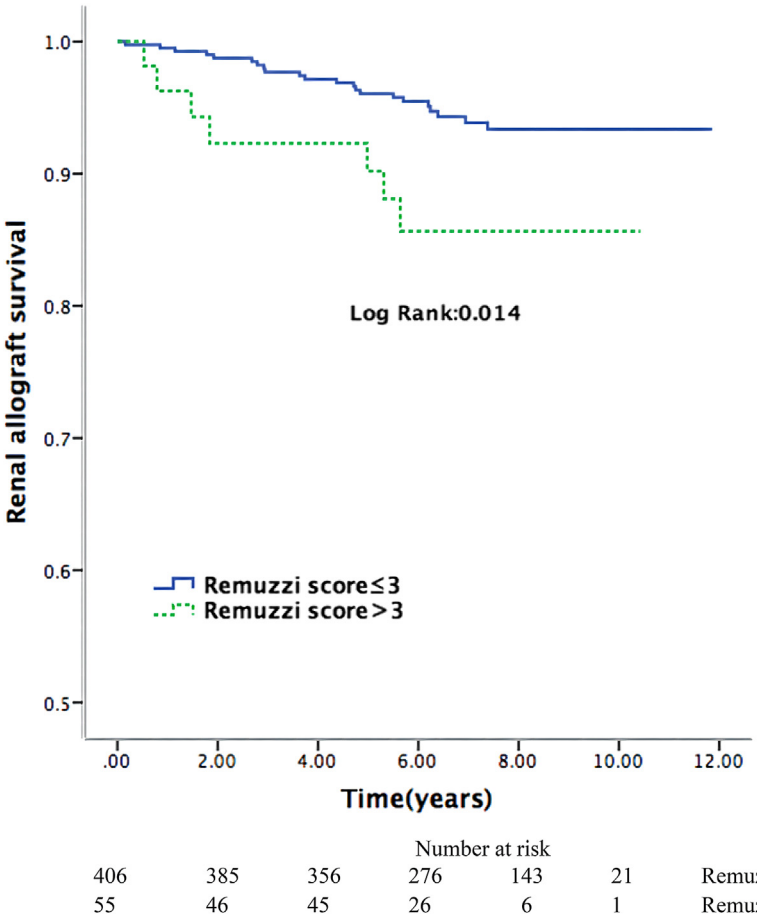

Figure 4. Kaplan–Meier curves for death-censored graft survival showing the difference between high Remuzzi score and low score group.

findings and transplant outcomes. We found that with a median post-transplant follow-up of 6.8 (quartiles, 5.7–8.4) years, reperfusion biopsy findings (HR 1.389 for each 1 score increase in Remuzzi score, 95% confidence interval, 1.181–1.633) were independently associated with graft survival, even after adjusting for KDRI, clinical donor, recipient, and transplant variables. Frozen section procurement biopsy was not correlated well with paraffin-embedded reperfusion biopsy.

Preimplant biopsies are increasingly used in the United States to accept or decline kidneys for transplantation based on the assumption that the results predict post-transplant outcomes. However, this assumption has not been rigorously tested. In contrast, kidney discard in Europe is only rarely based on procurement or preimplantation histology data, because procurement biopsies are not performed or reported for decisions on organ allocation in the Euro Transplant region, not even in expanded criteria donor kidneys.[6] To date, there are no generally accepted protocols for the histologic assessment of organ quality with respect to transplant outcome. Gaber *et al*. suggested in 1995 that a glomerulosclerosis threshold of 20% was associated with adverse outcomes, which has since become a widely used threshold.[19] However, subsequent studies have been highly variable, and no unambiguous case can be made that glomerulosclerosis is independently associated with graft outcomes. There are a number of studies[17,20-25] identifying glomerular or vascular, tubular, and interstitial lesions as well as composite histologic lesion scores as potential

Table 5. Association between Remuzzi score and death-censored graft failure by analytic group

	Unadjusted			Biopsy + KDRI			Adjusted Model[a]		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Remuzzi Score	1.375	(1.168,1.617)	< 0.001[b]	1.375	(1.168,1.617)	< 0.001[b]	1.389	(1.181,1.633)	< 0.001[b]

BMI, body mass index; CI, confidence interval; HR, hazard ratio; KDRI, kidney donor risk index.
[a]Adjusted model includes biopsy, donor age, donor sex, donor final creatinine, donor BMI, donor hypertension status, donation after cerebrovascular disease, expanded criteria donor, recipient age, recipient sex, recipient BMI, number of human leukocyte antigen mismatches, cold ischemia time, and warm ischemia time.
[b]Indicates $P < 0.05$.
Remuzzi score was analyzed as a continuous variable.

Table 6. Most recent serum creatinine among kidney transplant recipients with functioning allografts from 2012–2017

Pathologic finding at time of transplantation	Deceased donor serum creatinine (mg/dl)	Follow-up time f(yr)
ATI absent	1.16 ± 0.43	7.04 ± 2.21[b]
ATI present	1.13 ± 0.34	6.56 ± 2.06
Glomerular thrombi absent	1.15 ± 0.41	6.92 ± 2.17
Glomerular thrombi present	1.11 ± 0.25	6.15 ± 2.23
Arteriolar hyalinosis absent	1.14 ± 0.41[a]	6.96 ± 2.13[b]
Arteriolar hyalinosis present	1.31 ± 0.26	5.64 ± 2.73
Low Remuzzi score (≤3)	1.13 ± 0.41[a]	7.01 ± 2.15[b]
High Remuzzi score (> 3)	1.35 ± 0.30	6.00 ± 2.18
Low glomerular sclerosis score (0 or 1)	1.13 ± 0.41[a]	6.93 ± 2.19
High glomerular sclerosis score (≥2)	1.34 ± 0.32	6.52 ± 1.95
Low tubular atrophy score (0 or 1)	1.15 ± 0.41	6.89 ± 2.19
High tubular atrophy score (≥2)	1.32 ± 0.21	7.08 ± 1.24
Low interstitial fibrosis score (0 or 1)	1.15 ± 0.40[a]	6.89 ± 2.19
High interstitial fibrosis score (≥2)	1.44 ± 0.35	7.05 ± 1.14
Low vascular score (0 or 1)	1.14 ± 0.41[a]	6.95 ± 2.16[b]
High vascular score (≥2)	1.25 ± 0.25	5.71 ± 2.21

ATI, acute tubular injury.
[a]and [b]indicate $P < 0.05$.

predictors of transplant outcome. Various limitations of these investigations have to be considered, such as small study size, short follow-up times, tissue sampling procedures (wedge vs. needle core biopsy), preferential tissue sampling from kidneys of older donors, and, finally, a lack in comprehensive consensus-based protocols for the assessment of preimplant histology. A growing number of studies found that procurement biopsies are poorly reproducible, did not correlate with scores obtained with paraffin-embedded reperfusion biopsies and were not significantly associated with transplant outcomes.[11] There was no association between first biopsy findings and post-transplant outcomes.[26,27] The limitations of procurement biopsies, which should ostensibly provide objective information about organ quality, have been attributed to factors, including oversampling of subcapsular tissue when wedge biopsies are performed, inferior tissue processing and staining for frozen section specimens compared to gold standard formalin-fixed and paraffin embedded biopsies, and time pressured interpretation by pathologists who often lack expertise in kidney pathology. Our analysis uses postimplantation needle biopsies and formalin-fixed, paraffin-embedded tissue sections reviewed by experienced renal pathologists, a process that is different from how preimplantation biopsy samples are currently evaluated. Therefore, the evaluation process used in this analysis represents a "best case scenario" to determine the true value of histology in determining whether a kidney should or should not be utilized for transplantation. Our study aims to provide a more accurate assessment that takes these potential sources of bias into account.

Table 7. Concordance between the reperfusion biopsy and procurement biopsy

	Reperfusion biopsy (n = 89)	Procurement biopsy (n = 89)	Kappa
Glomeruli number, (IQR)	17 (13, 22.5)	15 (11.5, 20)	0.03
Glomerulosclerosis, %, (IQR)	5.9 (0, 12.5)	0 (0, 9.1)	0.056
Glomerular sclerosis score, (IQR)	1 (0, 1)	0 (0, 1)	0.168[a]
Tubular atrophy score, (IQR)	1 (0, 1)	1 (0, 1)	0.302[a]
Interstitial fibrosis score, (IQR)	1 (1, 1)	1 (0, 1)	0.432[a]
Vascular score, (IQR)	0 (0, 1)	0 (0, 1)	0.269[a]
Remuzzi score, (IQR)	3 (2, 4)	2 (1, 3)	0.137[a]

IQR, interquartile range.
[a]indicates $P < 0.05$.
Each of the score were determined by Remuzzi scoring criteria.[16,17]
Changes in each evaluated component of the kidney tissue (vessels, glomeruli, tubules, and connective tissue) received a score ranging from 0 to 3. The sum of these scores was defined as the global kidney score, which could range from 0 to 12. Glomerulosclerosis, interstitial fibrosis, and tubular atrophy were graded on the basis of percentage involvement (0, absent; 1, <20%; 2, 20%–50%; 3, >50%). The degree of vascular disease was a composite assessment of arteries and arterioles, focused on blood vessels with the most severe changes. The vascular score was 1 if the vessel-wall thickness was less than the diameter of the lumen. The vascular score was 2 if the vessel-wall thickness was equal or slightly greater to the diameter of the lumen. The vascular score was 3 if the vessel-wall thickness far exceeded the luminal diameter or the lumen was occluded.

Our study demonstrated that reperfusion biopsy findings was independently associated with graft survival even after adjustment for KDRI, clinical donor, recipient, and transplant variables. In addition, chronic vascular injury scores (cv and ah) were associated with lower most recent serum creatinine. Previous studies have linked postreperfusion to poor post-transplant outcomes. Hofer *et al.*[8] observed lower graft and recipient survival (HR 3.13 and HR 2.4, respectively) in severe injury of preimplantation time-zero biopsies. Cockfield *et al.*[28] analyzed postreperfusion biopsies as in our study and found that arteriolar hyalinosis was independently associated with DGF and graft loss, whereas fibrous intimal thickening was associated with decreased 6-month renal function. However, 89.1% of implantation biopsies were wedge biopsies and the long-term graft function was not described in the study. Using the Columbia University Medical Center database to examine outcomes for 975 transplant recipients included 427 biopsies from living donors and 548 biopsies from deceased donors. Mohan *et al.*[13] found that after adjusting for the KDRI, allograft survival using deceased donor optimal histology kidneys was not significantly better than that with suboptimal histology. Only the percent glomerulosclerosis remained significantly associated with shorter graft survival after adjustment for KDRI. The study was limited to a dichotomous categorization of optimal and suboptimal histology. Comparing with the big United States cohort, our findings have higher quality of grafts: younger donors with a lower incidence of hypertension, lower final creatinine, less expanded criteria donor, lower KDRI, and lower percentage of glomerular sclerosis. It is unclear whether the quality

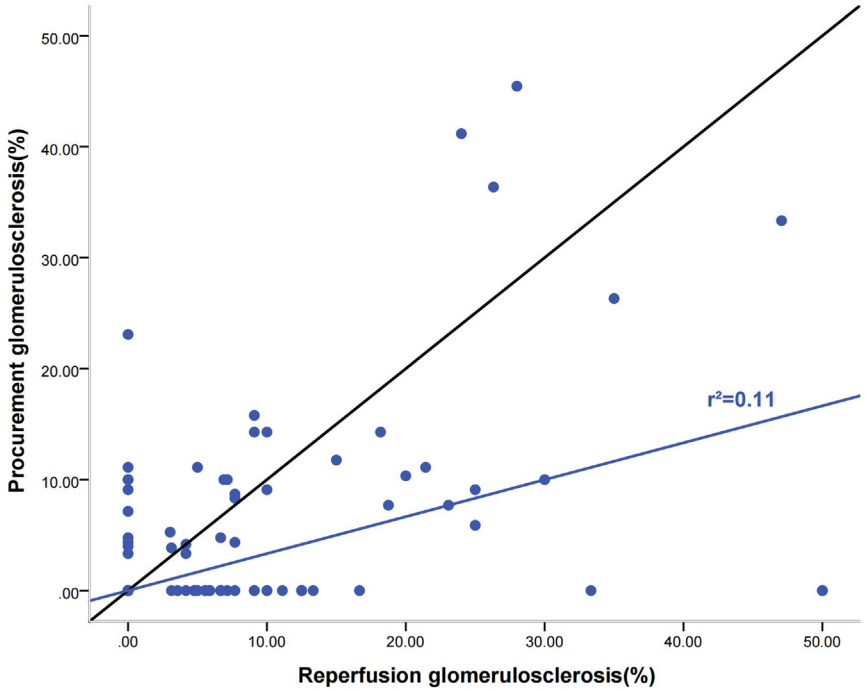

Figure 5. Percent glomerulosclerosis on procurement biopsy versus re-perfusion biopsy. Correlation between procurement and re-perfusion glomerulosclerosis was low ($r^2 = 0.11$). The black line is a reference for concordance.

of donors matter the results. The agreement between paired kidneys from the same donor proved that reperfusion biopsy in our center was reproducible. However, categorical agreement between the procurement and reperfusion biopsies was found to be low. In light of our findings, it is possible that variability in tissue processing and staining are rather the primary factors that influence procurement biopsy accuracy. Frozen samples are harder to interpret due to the technical difficulties of getting good quality sections and staining of tissue, which will all influence the interpretation of the section by the pathologist.[29,30] Almost all procurement biopsies in the United States are frozen section specimens.[27,31] This technique can make the evaluation of features such as interstitial fibrosis and tubular atrophy difficult because of distortion of tubulointerstitial structures, and some centers recommend against the reporting of interstitial fibrosis and tubular atrophy based on these specimens.[32,33] The preparation of formula-fixed, paraffin-embedded specimens is more costly and time consuming; however, these downsides must be weighed against the benefits of more accurate histologic assessments.

In various renal diseases, histopathological lesions from renal biopsy provide prognostic information, even after adjustment for albuminuria and estimated glomerular filtration rate.[34] It is illogical to believe that kidney biopsies are associated with prognosis except for deceased donor kidney transplantation procurement biopsies. Sample processing may account for the lack of correlation between reperfusion and procurement biopsies. Nevertheless, these findings should not lead to the misconception that procurement biopsies are not valuable in predicting graft outcomes. Conversely, independent association between postreperfusion score and graft outcomes highlights the importance of histological evaluation to define whether a graft should be transplanted (either alone or in couple) or discarded. Given the limitations of procurement biopsies, efforts should be made to improve the predictive value of procurement biopsies; and randomized controlled studies are needed to better define the scores to decide what grafts should be transplanted (either alone or in couple) and which ones should be discarded.

A main problem with the studies that evaluated the association between zero-time histology and post-transplant outcome (DGF, estimated glomerular filtration rate, and graft failure) is the fact that in some centers, mainly in the United States, zero-time histology is used for decisions on kidney discard, which leads to heavy selection bias in the studies that evaluate the association between zero-time histology and outcome after transplantation. To identify whether selection bias regarding which kidneys undergo a biopsy and which kidneys are subsequently transplanted influenced the underlying distribution of biopsy findings in the primary analysis, Husain *et al.*[7] examined

the characteristics of all deceased donor kidneys procured for the purpose of transplant during the study period, including those that were discarded. And this study finally illustrated that donor kidney histology assessment during allocation could provide some value in ascertaining organ quality among intermediate-quality kidneys. Similarly, we retrospectively investigated the predictive value of reperfusion biopsy, which in our center was obtained for protocol purposes, not for transplant decisions, on long-term allograft and recipient outcome after single-kidney transplantation, and we found that reperfusion biopsy findings were associated with long-time graft outcomes after deceased donor.

Our study has limitations. Our study population was Chinese; thus, our results may not be generalizable to other populations. Selection and confounding biases may be present and could lead to an overestimation of risks. More studies on post-transplant biopsy and long-term outcomes are needed to confirm the associations found in this study, and to find a better way to get biopsy sampling and read it quickly. Much more data is necessary, and a large-scale multicenter prospective study should be set up, to provide insight in which clinical, histological, biochemical parameters are necessary or sufficient for decisions on kidney acceptance or discard. In the meantime, clinicians should remain very reluctant to use simple histological prejudices for this purpose. Further research is necessary to evaluate whether and in which indications zero-time histology could be used for kidney allocation purposes.

In summary, postreperfusion renal allograft biopsy findings were associated with long-time graft outcomes after deceased donor independent of clinical information. These biopsies reflect baseline donor characteristics, the agonal phase, and periods of cold ischemia. Agreement between procurement and reperfusion biopsy was found to be low. Prospective trials are needed to determine how to optimize procurement biopsy practices for optimal organ allocation.

DISCLOSURE

All the authors declared no competing interests.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

MFW, JYW, and JHL undertook the following: research idea, study design, data analysis, and writing of the manuscript. MFW and JZ performed data acquisition. MFW performed statistical analysis. HPW and MFW examined the renal biopsy samples. All authors discussed and reviewed the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Causes for allograft loss.

Table S2. The comparison of biopsy pathology and allograft outcomes between right and left kidneys from the same donor.

STROBE Statement.

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