

Kidney structural features from living donors predict graft failure in the recipient - Supplement

Table of Content

Supplementary Methods.....	2-4
Supplementary Table 1. Percentage of missing data that was imputed.....	5
Supplementary Table 2. Donor clinical characteristics that predict graft failure.....	6
Supplementary Table 3. Recipient clinical characteristics that predict graft failure.....	7
Supplementary Table 4. Structural predictors of graft failure adjusting for acute rejection.....	8
Supplementary Table 5. Structural predictors of graft failure adjusting for 24 h urine albumin.....	9
Supplementary Table 6 Structural predictors of graft failure adjusting for all donor and recipient clinical covariates.....	10
References.....	11

Supplementary Methods

1. Biopsy adequacy

The biopsy sections had to have at least 2 mm² of cortex and 4 glomeruli per section to be used in this study.

2. Biopsy morphometry

The following direct measurements were obtained from the two consecutive renal biopsy sections that were scanned into the high resolution image files used for analyses:

1. The area of cortex on the periodic acid-Schiff (PAS) stained sections.
2. The number and total area of complete non-sclerotic glomeruli (NSG) on the PAS stained section.
3. The number and total area of partial NSG on the PAS stained section. These are glomeruli on the section edge that have been transected by the biopsy needle. We counted partial NSG as 0.5 complete NSG (the average area of partial NSG was approximately half the average area of complete NSG).
4. The number of globally sclerotic glomeruli (GSG) on the PAS and trichrome stained sections.
5. Area of non-tubular regions within 5 consecutive circles (totaling 1,000,000 μm^2) along the sectioned cortex. The excluded non-tubular area included glomeruli and vessels.
7. The number of complete tubules within these 5 circles.
8. The number of partial tubules within these 5 circles (partial tubules were counted as half a complete tubule).
9. Luminal boundary and intimal-media boundary of the largest and most orthogonal artery (if present on the TRI stained section).

A) Arteriolar hyalinosis:

The severity of arteriosclerosis was determined by the percentage of luminal stenosis by intimal thickening in the small-medium artery (if any) most orthogonal to its axis. Prior work had determined that detection of any arteriolar hyalinosis in donors required manual review of the 12 consecutive biopsy section slides by renal pathologists, data only available at Mayo Clinic, Rochester, Minnesota.

B) Morphometric measures of nephron size:

All measures of nephron size were based on one section (PAS stained). During morphometric analysis of all donor biopsy sections, we did not find a single glomerulus with segmental glomerulosclerosis. Thus, we assumed sectioned profiles of glomeruli that appeared to be NSG were truly NSG and sectioned profiles that appeared to be GSG were truly GSG.

The total number of NSG was obtained by summing the numbers of complete and partial NSG (counted as 0.5 NSG). Mean area of NSG was obtained by dividing a total area of both complete and partial NSG by a total number of NSG per biopsy section. NSG area density was determined by the total number of NSG divided by the area of cortex. We then used stereological models by Weibel and Gomez to characterize three-dimensional structures from these two-dimensional measurements to calculate Mean NSG volume (**Eq. 1**), NSG density (**Eq. 2**) which was inverted into cortex volume per glomerulus (**Eq. 3**). The mean profile tubular area estimates the average cross-sectional area of a tubule by counting the number of tubules in 1,000,000 μm^2 defined area of cortex after excluding all non-tubular structures (**Eq. 4**).

$$\text{(Eq. 1) Mean NSG volume (mm}^3\text{)} = \frac{1.382 \times (\text{Mean area of NSG})^{\frac{3}{2}}}{1.01}$$

$$\text{(Eq. 2) NSG volumetric density (NSG per mm}^3\text{ of cortex)} = \frac{1}{1.382} \times \sqrt[2]{\frac{\left(\frac{\text{Total number of NSG}}{\text{Area of cortex}}\right)^3}{\frac{\text{Total area of NSG}}{\text{Area of cortex}}}}$$

$$\text{(Eq. 3) Cortex volume per glomerulus (mm}^3\text{)} = \frac{1}{\text{NSG volumetric density}}$$

$$\text{(Eq. 4) Mean profile tubular area (}\mu\text{m}^2\text{)} = \frac{1,000,000 - \text{area of nontubular structures}}{\text{Number of complete tubules} + 0.5 \times \text{Number of partial tubules}}$$

C) Morphometric measures of nephrosclerosis:

Due to the relative infrequency of GSG, the GSG number was averaged between the PAS and trichrome stained sections (Mean GSG number). The mean GSG number was then used to estimate % globally sclerotic glomeruli (Eq. 5). % Luminal stenosis was the area of intima relative to the area of intima and lumen (Eq. 6).

$$\text{(Eq. 5) \% Globally sclerotic glomeruli} = \frac{\text{Mean number of GSG}}{\text{Total number of NSG} + \text{Mean number of GSG}}$$

$$\text{(Eq. 6) \% Luminal stenosis} = \frac{\text{Intima to media boundary area} - \text{intimal to luminal boundary area}}{\text{Intima to media boundary area}}$$

The percentage of interstitial fibrosis and tubular atrophy (IF/TA) (0%, 1 to 5%, 6 to 10%, >10 %) was estimated by a renal pathologist based on visual inspection under 10x to 40x magnification of the percentage of total cortex area that was IF/TA on the Mason's trichrome stained section. The same pathologist also counted the number of distinct IF/TA foci on a PAS stained section.

D) Donor kidney macrostructural findings

The CT images from the angiogram/cortical phase were downloaded onto a workstation for processing. The kidney cortical and medullary volumes were segmented in a random order and blinded to other donor characteristics using a semi-automated algorithm (ITK-SNAP software, version 2.2; University of Pennsylvania, Philadelphia, PA). The donated kidney cortex volume, medulla volume, and the diameter of the largest parenchymal cyst (if present) were measured.¹

E) Estimation of nephron number and single nephron GFR (snGFR)

The total nephron number per kidney was calculated by multiplying the summed cortical volume of both kidneys (mm^3) by the non-sclerotic glomerular volumetric density (NSG density) (**Eq. 2**), dividing by 2 (per kidney), dividing by 1.43 (tissue volume shrinkage due to formalin fixation and paraffin embedding), and dividing by 1.268 (volume shrinkage due to loss of tissue perfusion pressure).² The snGFR was calculated from measured GFR (in ml/min) divided by two times nephron number (for both kidneys).³

Supplementary Table 1. The percentage of missing data that was imputed for each donor and recipient variable. The Table also provides both counts and percentages of patients in the cohort who are missing data for at least one donor variable, at least one recipient variable, or at least one of any of the donor or recipient variables.

Variable	Imputed
Donor:	
Measured GFR	5.3%
Related versus unrelated	1.0%
Number of IF/TA foci per mm ²	0.1%
Artery luminal stenosis	10.9%
Tubular cross-sectional area	0.5%
Cortex volume	5.5%
Medulla volume	5.5%
Kidney surface roughness score	0.04%
Missing at least 1 donor variable	23.1%
Recipient:	
Body mass index	0.4%
Diabetes	0.2%
Hypertension	2.8%
HLA-A,B 0-mismatch	0.6%
HLA-DR 0-mismatch	0.6%
Pre-emptive transplant	0.1%
Missing at least 1 recipient variable	4.1%
Missing at least 1 of any of the donor or recipient variables	26.0%

Supplemental Table 2. Donors' clinical characteristics that predicted graft failure.

Donor clinical characteristics	Univariate		Multivariate		Backwards Stepwise Selection†	
	OR (95% CI)	p	OR (95% CI)	p		p
Age per 10 years	1.09 (0.99-1.20)	0.09	1.10 (0.98-1.24)	0.08	1.11 (1.00-1.22)	0.05
Male	0.95 (0.75-1.20)	0.67	0.98 (0.76-1.26)	0.87		
Black	2.15 (1.31-3.54)	0.02	2.35 (1.42-3.89)	0.008	2.30 (1.39-3.80)	0.01
BMI						
20 – 30 kg/m ²	ref	0.29	ref	0.26		
<20 kg/m ²	1.46 (0.54-4.00)		1.61 (0.59-4.41)			
>30 kg/m ²	1.17 (0.88-1.57)		1.15 (0.84-1.57)			
Hypertension	1.14 (0.85-1.53)	0.39	1.04 (0.75-1.42)	0.83		
Measured GFR, per 20 ml/min/1.73 m ²	0.98 (0.89-1.08)	0.71	0.99 (0.88-1.11)	0.88		
Unrelated to the recipient	0.98 (0.89-1.08)	0.71	0.99 (0.88-1.11)	0.88		
24h urine albumin*	1.09 (1.00-1.19)	0.04	1.11 (1.02-1.21)	0.02	1.11 (1.02-1.21)	0.02

* 24h urine albumin was only available in 1705 donors

† Backwards stepwise selection keeping variables with p < 0.10.

Supplemental Table 3. Recipients' clinical characteristics that predicted graft failure.

Recipient clinical characteristics	Univariate		Multivariate		Backwards stepwise selection*	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Age per 10 years	0.79 (0.73-0.85)	<0.0001	0.82 (0.75-0.89)	<0.0001	0.81 (0.75-0.89)	<0.0001
Male	0.98 (0.78-1.24)	0.88	1.05 (0.76-1.45)	0.76		
Black	2.20 (1.38-3.49)	0.01	1.84 (1.14-2.97)	0.04	1.85 (1.15-2.97)	0.03
BMI category						
Normal	ref	0.52	ref	0.36		
Underweight	0.59 (0.26-1.35)		0.47 (0.20-1.10)			
Overweight	0.87 (0.65-1.16)		1.03 (0.76-1.38)			
Obese	0.88 (0.66-1.17)		1.01 (0.75-1.37)			
Diabetes mellitus	1.34 (1.04-1.73)	0.02	1.63 (1.08-2.44)	0.02	1.55 (1.03-2.32)	0.04
Hypertension	0.78 (0.56-1.07)	0.12	0.95 (0.68-1.32)	0.74		
Etiology of CKD						0.0009
Glomerular disease	ref	<0.0001	ref	0.0008		ref
Diabetes	1.10 (0.79-1.53)		0.79 (0.49-1.28)		0.82 (0.51-1.34)	
Polycystic disease	0.17 (0.08-0.36)		0.22 (0.10-0.46)		0.22 (0.10-0.45)	
Hypertension	0.69 (0.41-1.18)		0.71 (0.41-1.24)		0.72 (0.42-1.25)	
Other/unknown	1.30 (0.98-1.71)		1.04 (0.75-1.45)		1.02 (0.74-1.41)	
Any HLA-Antigen mismatch	2.22 (1.30-3.81)	0.004	1.72 (0.95-3.14)	0.07	1.78 (0.99-3.20)	0.05
HLA-DR mismatch	1.60 (1.15-2.24)	0.006	1.40 (0.97-2.03)	0.07	1.41 (0.97-2.03)	0.07
Previous kidney transplant	1.68 (1.39-2.04)	<0.0001	1.25 (0.97-1.60)	0.08	1.26 (0.99-1.62)	0.06
Prior dialysis	1.98 (1.55-2.55)	<0.0001	1.54 (1.19-1.99)	0.001	1.52 (1.18-1.97)	0.001
Induction immunosuppression						
Depleting	ref	0.02	ref	0.16		
Non-depleting	0.63 (0.43-0.91)		0.79 (0.54-1.17)			
Other/None	1.16 (0.66-2.03)		1.41 (0.78-2.54)			
CNI-based immunosuppression	1.09 (0.68-1.72)	0.73	1.27 (0.78-2.05)	0.34		
Steroid maintenance	0.86 (0.59-1.25)	0.44	0.88 (0.59-1.30)	0.51		
Delayed graft function	3.96 (2.26-6.93)	<0.0001	3.06 (1.71-5.46)	0.0002	3.07 (1.72-5.47)	0.0001

* Backwards stepwise selection keeping variables with $p < 0.10$.

Supplemental Table 4. Baseline donor CT and biopsy predictors of graft failure beyond 1 year post transplant adjusting for acute rejection in the first year (n=2200).

Structural feature*	Unadjusted	Adjusted for Donors Covariates ‡	Adjusted for Recipients Covariates **	Adjusted for both Donors and Recipients Covariates ‡**
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
% Globally sclerotic glomeruli	1.03 (0.91-1.16)	1.00 (0.88-1.14)	1.09 (0.97-1.23)	1.03 (0.91-1.17)
Glomerulosclerosis above age threshold†	0.85 (0.49-1.49)	0.83 (0.47-1.45)	0.95 (0.54-1.66)	0.92 (0.52-1.62)
%IF/TA				
0%	ref	ref	ref	ref
>0%-≤5%	1.16 (0.86-1.58)	1.13 (0.83-1.53)	1.25 (0.92-1.71)	1.16 (0.85-1.58)
>5%	2.79 (1.65-4.73)	2.63 (1.51-4.58)	2.71 (1.58-4.66)	2.14 (1.21-3.77)
IF/TA foci/mm ²	1.12 (1.00-1.26)	1.10 (0.97-1.25)	1.13 (1.00-1.26)	1.07 (0.94-1.21)
% Artery luminal stenosis	0.99 (0.88-1.11)	0.95 (0.85-1.07)	1.05 (0.94-1.18)	0.99 (0.88-1.12)
Non-sclerotic glomeruli volume	1.10 (0.98-1.23)	1.10 (0.98-1.23)	1.14 (1.01-1.28)	1.15 (1.03-1.30)
Tubular cross-sectional area	1.19 (1.06-1.33)	1.18 (1.05-1.32)	1.14 (1.01-1.28)	1.14 (1.01-1.28)
Cortical volume per glomerulus	1.00 (0.88-1.13)	0.99 (0.87-1.12)	1.02 (0.90-1.15)	1.01 (0.90-1.14)
Cortical volume	1.00 (0.89-1.13)	1.02 (0.90-1.15)	0.99 (0.88-1.12)	1.04 (0.92-1.17)
Medullary volume	0.94 (0.83-1.06)	0.93 (0.82-1.05)	0.90 (0.79-1.02)	0.87 (0.77-0.99)
Kidney surface roughness	1.03 (0.91-1.16)	1.02 (0.90-1.16)	1.05 (0.93-1.19)	1.02 (0.90-1.15)
Any kidney cyst	0.09 (0.63-1.52)	0.93 (0.60-1.46)	1.09 (0.70-1.69)	0.94 (0.60-1.47)
Nephron number	1.01 (0.89-1.16)	1.03 (0.90-1.18)	0.99 (0.86-1.13)	1.03 (0.90-1.18)
Single nephron GFR	0.99 (0.87-1.12)	0.98 (0.86-1.12)	1.00 (0.88-1.14)	1.00 (0.89-1.13)

*All Hazard Ratios (HR) for continuous measures are per Standard Deviation (SD). ref: reference.

†Number of globally sclerotic glomeruli on biopsy exceeds 95th percentile expected for donor age and number of glomeruli.

‡ Donor age and black race

** Recipient age, black race, diabetes, etiology of CKD, any HLA mismatch, HLA-DR mismatch, previous kidney transplant, pre-transplant dialysis, delayed graft function, and acute rejection during first year

Supplemental Table 5. Baseline donor CT and biopsy predictors of graft failure among donors who had 24h urine albumin testing (n=1705).

Structural feature*	Unadjusted	Adjusted for Donors Covariates‡	Adjusted for Recipients Covariates **	Adjusted for both Donors and Recipients Covariates‡**
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
% Globally sclerotic glomeruli	1.02 (0.89-1.17)	1.00 (0.86-1.15)	1.08 (0.94-1.24)	1.01 (0.88-1.17)
Glomerulosclerosis above age threshold†	0.80 (0.42-1.51)	0.82 (0.44-1.56)	0.94 (0.50-1.79)	0.94 (0.50-1.79)
%IF/TA				
0%	ref	ref	ref	ref
>0%-<5%	1.04 (0.74-1.46)	1.05 (0.74-1.49)	1.11 (0.79-1.56)	1.05 (0.74-1.49)
>5%	2.65 (1.53-4.58)	2.50 (1.39-4.48)	2.41 (1.37-4.21)	1.78 (0.98-3.22)
IF/TA foci/mm ²	1.08 (0.94-1.23)	1.06 (0.92-1.22)	1.08 (0.95-1.23)	1.02 (0.89-1.17)
% Artery luminal stenosis	1.05 (0.92-1.18)	1.02 (0.90-1.16)	1.10 (0.97-1.25)	1.05 (0.92-1.20)
Non-sclerotic glomeruli volume	1.09 (0.96-1.23)	1.07 (0.95-1.21)	1.11 (0.98-1.26)	1.10 (0.97-1.25)
Tubular cross-sectional area	1.19 (1.06-1.34)	1.17 (1.03-1.32)	1.18 (1.04-1.33)	1.15 (1.01-1.31)
Cortical volume per glomerulus	1.05 (0.94-1.18)	1.03 (0.91-1.16)	1.09 (0.97-1.22)	1.06 (0.94-1.20)
Cortical volume	0.98 (0.86-1.12)	0.96 (0.84-1.10)	0.97 (0.85-1.10)	0.98 (0.86-1.13)
Medullary volume	0.88 (0.77-1.01)	0.86 (0.75-0.99)	0.85 (0.74-0.97)	0.81 (0.70-0.94)
Kidney surface roughness	1.00 (0.87-1.15)	1.00 (0.87-1.15)	1.03 (0.90-1.18)	1.01 (0.88-1.16)
Any kidney cyst	0.71 (0.39-1.26)	0.66 (0.37-1.19)	0.80 (0.45-1.44)	0.68 (0.38-1.23)
Nephron number	0.93 (0.80-1.09)	0.96 (0.82-1.12)	0.89 (0.76-1.05)	0.95 (0.81-1.11)
Single nephron GFR	1.04 (0.93-1.17)	1.03 (0.92-1.17)	1.07 (0.95-1.19)	1.05 (0.93-1.19)

*All Hazard Ratios (HR) for continuous measures are per Standard Deviation (SD). ref: reference.

†Number of globally sclerotic glomeruli on biopsy exceeds 95th percentile expected for donor age and number of glomeruli.

‡ Donor age, black race, and 24h urine albumin

** Recipient age, black race, diabetes, etiology of CKD, any HLA mismatch, HLA-DR mismatch, previous kidney transplant, pre-transplant dialysis, and delayed graft function

Supplemental Table 6. Baseline donor CT and biopsy predictors of graft failure adjusting for all the donor and recipient clinical covariates listed in Supplemental Tables 2 and 3 (without exclusions based on backwards stepwise selection).

Structural feature*	Adjusted for Donors	Adjusted for Recipients	Adjusted for both Donors and
	Covariates	Covariates	Recipients Covariates
	HR (95% CI)	HR (95% CI)	HR (95% CI)
% Globally sclerotic glomeruli	0.98 (0.80, 1.20)	1.12 (0.93, 1.35)	1.00 (0.82, 1.23)
Glomerulosclerosis above age threshold†	0.81 (0.47, 1.39)	0.92 (0.53, 1.58)	0.87 (0.50, 1.50)
%IF/TA			
0%	ref	ref	ref
>0% -≤5%	1.14 (0.85, 1.53)	1.29 (0.96, 1.73)	1.20 (0.89, 1.63)
>5%	2.42 (1.39, 4.22)	2.48 (1.44, 4.26)	1.97 (1.11, 3.52)
IF/TA foci/mm ²	1.08 (0.96, 1.21)	1.13 (1.01, 1.26)	1.07 (0.95, 1.21)
% Artery luminal Stenosis	0.97 (0.84, 1.13)	1.07 (0.93, 1.23)	1.01 (0.87, 1.17)
Non-sclerotic glomeruli volume	1.09 (0.97, 1.21)	1.10 (0.98, 1.22)	1.11 (0.99, 1.24)
Tubular cross-sectional area	1.12 (1.04, 1.21)	1.10 (1.01, 1.19)	1.09 (1.01, 1.19)
Cortex volume	0.99 (0.91, 1.07)	0.98 (0.93, 1.04)	1.00 (0.93, 1.08)
Medulla Volume	0.89 (0.78, 1.01)	0.89 (0.80, 1.00)	0.82 (0.71, 0.93)
Kidney surface roughness	0.99 (0.88, 1.10)	1.02 (0.91, 1.14)	0.99 (0.89, 1.11)
Any kidney cyst	0.95 (0.62, 1.45)	1.08 (0.70, 1.64)	0.95 (0.61, 1.46)
Nephron number	1.01 (0.87, 1.17)	0.95 (0.82, 1.10)	1.01 (0.87, 1.17)
Single nephron GFR	1.02 (0.90, 1.16)	1.03 (0.91, 1.17)	1.03 (0.90, 1.17)

References

1. Denic, A, Alexander, MP, Kaushik, V, Lerman, LO, Lieske, JC, Stegall, MD, Larson, JJ, Kremers, WK, Vrtiska, TJ, Chakker, HA, Poggio, ED, Rule, AD: Detection and Clinical Patterns of Nephron Hypertrophy and Nephrosclerosis Among Apparently Healthy Adults. *Am J Kidney Dis*, 68: 58-67, 2016.
2. Denic, A, Lieske, JC, Chakker, HA, Poggio, ED, Alexander, MP, Singh, P, Kremers, WK, Lerman, LO, Rule, AD: The Substantial Loss of Nephrons in Healthy Human Kidneys with Aging. *J Am Soc Nephrol*, 28: 313-320, 2017.
3. Denic, A, Mathew, J, Lerman, LO, Lieske, JC, Larson, JJ, Alexander, MP, Poggio, E, Glassock, RJ, Rule, AD: Single-Nephron Glomerular Filtration Rate in Healthy Adults. *N Engl J Med*, 376: 2349-2357, 2017.