



Kidney Structural Features from Living Donors Predict Graft Failure in the Recipient

Naim Issa,^{1,2} Camden L. Lopez,³ Aleksandar Denic,¹ Sandra J. Taler,^{1,2} Joseph J. Larson ,³ Walter K. Kremers,^{2,3} Luisa Ricaurte,¹ Massini A. Merzkani,¹ Mariam Priya Alexander,⁴ Harini A. Chakkerla,⁵ Mark D. Stegall,² Joshua J. Augustine,⁶ and Andrew D. Rule ¹

¹Division of Nephrology and Hypertension, ²William J von Liebig Center for Transplantation and Clinical Regeneration, ³Division of Biomedical Statistics and Informatics, and ⁴Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota; ⁵Division of Nephrology, Mayo Clinic Arizona, Scottsdale, Arizona; and ⁶Department of Nephrology, Cleveland Clinic, Cleveland, Ohio

ABSTRACT

Background Nephrosclerosis, nephron size, and nephron number vary among kidneys transplanted from living donors. However, whether these structural features predict kidney transplant recipient outcomes is unclear.

Methods Our study used computed tomography (CT) and implantation biopsy to investigate donated kidney features as predictors of death-censored graft failure at three transplant centers participating in the Aging Kidney Anatomy study. We used global glomerulosclerosis, interstitial fibrosis/tubular atrophy, artery luminal stenosis, and arteriolar hyalinosis to measure nephrosclerosis; mean glomerular volume, cortex volume per glomerulus, and mean cross-sectional tubular area to measure nephron size; and calculations from CT cortical volume and glomerular density on biopsy to assess nephron number. We also determined the death-censored risk of graft failure with each structural feature after adjusting for the predictive clinical characteristics of donor and recipient.

Results The analysis involved 2293 donor-recipient pairs. Mean recipient follow-up was 6.3 years, during which 287 death-censored graft failures and 424 deaths occurred. Factors that predicted death-censored graft failure independent of both donor and recipient clinical characteristics included interstitial fibrosis/tubular atrophy, larger cortical nephron size (but not nephron number), and smaller medullary volume. In a subset with 12 biopsy section slides, arteriolar hyalinosis also predicted death-censored graft failure.

Conclusions Subclinical nephrosclerosis, larger cortical nephron size, and smaller medullary volume in healthy donors modestly predict death-censored graft failure in the recipient, independent of donor or recipient clinical characteristics. These findings provide insights into a graft's "intrinsic quality" at the time of donation, and further support the use of intraoperative biopsies to identify kidney grafts that are at higher risk for failure.

JASN 31: ●●●-●●●, 2020. doi: <https://doi.org/10.1681/ASN.2019090964>

Living kidney donation has become the preferred therapeutic option for patients with ESKD.¹ A thorough clinical evaluation with laboratory testing is required to ensure the donor has healthy kidneys. Despite this, certain live donor clinical characteristics (particularly older age and lower GFR) predict an increased risk of death-censored graft failure in

Received September 24, 2019. Accepted November 27, 2019.

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Andrew D. Rule, Mayo Clinic, 200 1st Street SW, Rochester, MN 55905. Email: rule.andrew@mayo.edu

Copyright © 2020 by the American Society of Nephrology

the recipient.^{2,3} These clinical characteristics may reflect underlying structural features in the kidney that are predictive of longevity of the graft. Indeed, there is substantial variability with respect to nephrosclerosis, nephron size, and nephron number among living kidney donors.^{4–6}

Even if there are subclinical structural features that predict death-censored graft failure, this would not likely affect initial decision regarding living kidney donation. Recipients generally have better outcomes with a kidney from a living donor than from a deceased donor.¹ Consequently, availability of a willing person with acceptable health for kidney donation will generally supersede concerns regarding graft quality. However, long-term death-censored graft failure remains a primary concern,^{7,8} and identifying kidney structural predictors of death-censored graft failure may facilitate enriched strategies to improve graft longevity. Transplantation is also a unique setting to study how kidney structural features contribute to the development of kidney failure because the donated kidney, once placed in the recipient, becomes independent of the nonkidney characteristics of the donor.

We studied donor biopsy specimen structural measures as predictors of death-censored graft failure in a cohort of 2293 living kidney donor-recipient pairs at three transplant centers in the Aging Kidney Anatomy study.^{4–6} We previously found that nephrosclerosis, larger nephron size, and low nephron number modestly predict lower GFR, higher urine albumin, or hypertension in the donor at 4 months after donation.^{5,9} Thus, we hypothesized that these same structural features would be predictive of death-censored graft failure in the matched recipients.

METHODS

Study Sample

This study included living kidney donors at Mayo Clinic Minnesota, Mayo Clinic Arizona, and Cleveland Clinic who donated between May 10, 1999 and December 11, 2017, with a predonation computed tomography (CT) scan and a renal biopsy of the donated kidney during transplantation. The corresponding kidney recipients were followed annually at all three sites. Recipient characteristics and follow-up were standardized using United Network for Organ Sharing (UNOS) Standard Transplant Analysis and Research (STAR) files for each of the three sites, obtained on July 27, 2018.¹⁰ Certain populations at high risk for early death-censored graft failure or death were excluded. Specifically, we excluded kidney recipients with previous or concurrent nonkidney solid organ transplants, ABO-incompatible transplants, and recipients aged <15 years at the time of transplantation. In addition, we excluded recipients with graft failure, death, or lost follow-up within 1 month after transplantation. Early graft loss in those recipients was mainly owing to technical reasons (e.g., renal artery thrombosis or kinking), and their kidney structural features were not significantly different from the remaining cohort.

Significance Statement

The quality of a kidney obtained from a living donor is often inferred from the donor's age, risk factors, and kidney function. Little is known about the influence of a donated kidney's structural features on the risk of death-censored graft failure in the recipient. In an analysis of 2293 kidney donor-recipient pairs, the authors identified subclinical nephrosclerosis, larger nephron size (but not nephron number), and smaller medullary volume as structural predictors of death-censored graft failure that were independent of both donor and recipient clinical characteristics. These findings provide important insights into the factors that define the "intrinsic quality" of the living kidney donor graft at the time of donation, and support use of intraoperative biopsies to identify donor kidneys that are at higher risk for failure.

Recipients with positive crossmatch (including preformed donor-specific antibodies) were not excluded because substantial changes in crossmatch technology over the study period prevented a consistent definition of positive crossmatch. All data were obtained from the medical records of donors and UNOS STAR files for recipients with a waiver of informed consent under institutional review board approval.

Donor Clinical Characteristics

All kidney donors underwent a thorough medical evaluation before donation that included a prescheduled series of tests. The predonation evaluation included serum creatinine to eGFR using the CKD Epidemiology Collaboration equation, urinary iothalamate clearance to measure GFR, 24-hour urine albumin excretion (Mayo Clinic donors only), body mass index (BMI), and office BP. Acceptance criteria for donation varied by site and era, but generally included 24-hour urine albumin excretion <30 mg and GFR normal for age. Mild controlled hypertension in older donors and moderate obesity (BMI of 30–35 kg/m², occasionally up to 40 kg/m² in older donors) were allowed. Patients with diabetes mellitus or cardiovascular disease were not accepted as donors. Hypertension was defined as a preexisting diagnosis of hypertension, an office BP >140/90 mm Hg, or use of antihypertensive medication to lower BP. Living related donors were defined by being a blood relative of the kidney recipient.

Kidney Structural Features

An intraoperative needle core biopsy of the renal cortex was performed at the time of donation. The tissue specimen was fixed in formalin and embedded in paraffin. Unstained sections from the tissue block were sent to Mayo Clinic in Minnesota from the other sites for staining. Two sections (2- to 3- μ m thickness) from the biopsy core were stained, one with periodic acid–Schiff and one with Masson trichrome, and then scanned into high-resolution digital images (Aperio XT digital scanner; Leica Biosystems). Supplemental Appendix 1 describe the stereological measurements and equations used to characterize the microstructural features from these images. Nephron size was characterized by mean nonsclerotic

glomerular volume, cortex volume per glomerulus (inverse of nonsclerotic glomerular volumetric density), and mean cross-sectional tubular area.⁴ Nephrosclerosis on biopsy was characterized by the percentage of glomeruli that were globally sclerotic, the percentage interstitial fibrosis/tubular atrophy (IF/TA), the density of IF/TA foci (per millimeter squared of cortex), and arteriosclerosis severity by percentage luminal stenosis from intimal thickening. In the Mayo Clinic Minnesota subset, detection of any arteriolar hyalinosis was assessed by a pathologist review of all 12 biopsy section slides. Supplemental Appendix 1 describes how CT images were used to determine kidney cortical and medullary volume, presence of any cyst, and surface roughness of donated kidney.⁴ Nephron number was calculated from the product of cortical volume and glomerular density,^{11–13} and single-nephron GFR was calculated from measured GFR divided by nephron number (Supplemental Appendix 1).⁹

Recipient Clinical Characteristics and Outcome

From the UNOS STAR file, the baseline kidney transplant recipients' characteristics considered for prediction of death-censored graft failure were age at transplant, sex, race/ethnicity, height, BMI, primary cause of CKD, diabetes, hypertension, last available calculated panel reactive antibody before transplant (available after December 5, 2007), number of HLA class I mismatches, HLA donor-recipient mismatches, number of previous kidney transplants, pretransplant dialysis, use of depleting immunosuppression induction therapy, maintenance calcineurin inhibitor use, and maintenance steroids use. Delayed graft function was defined by dialysis during the first week post-transplant. Dates of death-censored graft failure and last follow-up were ascertained from the UNOS STAR file. Death-censored graft failure was defined as either retransplantation or initiation of dialysis with follow-up through to August 3, 2018.

Statistical Analyses

The analysis of death-censored graft failure used a Cox proportional hazards model with censoring at death. The relationship between death-censored graft failure and each donor or recipient variable was summarized with a hazard ratio (HR). Missing values of the predictor variables were imputed using multivariate imputation by chained equations.¹⁴ Nine data sets were created, with missing values replaced by different multivariate imputation by chained equations values in each data set. HRs, 95% confidence intervals (95% CIs), and *P* values for each model were obtained by fitting the model on each imputation data set and combining the results using the methods of Rubin¹⁵ and Li *et al.*¹⁶ For each biopsy or CT structural predictor, four models were fit: unadjusted, adjusted for donor characteristics, adjusted for recipient characteristics, and adjusted for both donor and recipient characteristics. To avoid adjusting for an excessive number of clinical covariates, the clinical predictors for death-censored graft failure in this data set were first selected using stepwise

backward elimination (requiring predictive covariates to have *P* value <0.10). The stepwise algorithm was run on each of the imputation data sets, and a covariate was retained in the final set if it was selected in at least five of the nine data sets. The selection process was applied separately to select donor characteristics as covariates and to select recipient characteristics as covariates. To account for differences among the three study sites, all Cox models were fit with site as a stratification factor. The proportional hazards assumption of the models was checked using weighted Schoenfeld residuals.¹⁷

Sensitivity analyses were performed. To address the potential role of early acute rejection as part of the pathway for death-censored graft failure, analyses were repeated with baseline set to 1 year post-transplant with further adjustment for acute rejection during the first year post-transplant. We further assessed the effect of adjusting for donor 24-hour urine albumin. The subset with available arteriolar hyalinosis measures allowed assessment of arteriolar hyalinosis as a predictor of death-censored graft failure. Finally, analyses were repeated adjusting for all donor and recipient clinical covariates, rather than just those identified by backward stepwise selection. All computations and statistical analyses were performed using R version 3.4.2.

RESULTS

Donors and Recipient Clinical Characteristics

After exclusions, there were 2293 living kidney donors studied (Figure 1). This included donor and recipient pairs at Mayo Clinic Minnesota (*n*=1585), Mayo Clinic Arizona (*n*=436), and Cleveland Clinic (*n*=272). Table 1 describes the baseline clinical, CT scan, and biopsy sample characteristics of the donors; Table 2 describes the baseline clinical characteristics of the recipients. During follow-up, 287 (12.5%) recipients experienced death-censored graft failure and 424 (18.5%) recipients died. The mean ±SD time to death-censored graft failure or last follow-up was 6.3 ±3.8 years. The percentage missing data for each characteristic that was imputed for analyses is shown in Supplemental Table 1. Donor clinical characteristics that independently predicted death-censored graft failure (at *P*<0.10) were older age and black race, and higher 24-hour urine albumin (in the subset with 24-hour urine albumin testing) (Supplemental Table 2). Recipient clinical characteristics that independently predicted death-censored graft failure (at *P*<0.10) were younger age, black race, diabetes, cause of CKD, any HLA antigen mismatch, HLA donor-recipient mismatch, previous kidney transplant, pretransplant dialysis, and delayed graft function (Supplemental Table 3).

Structural Features as Predictors of Death-Censored Graft Failure

Each kidney structural feature on biopsy or CT scan at the time of transplantation was evaluated as a predictor of death-censored graft failure. Presence of IF/TA >5% and larger

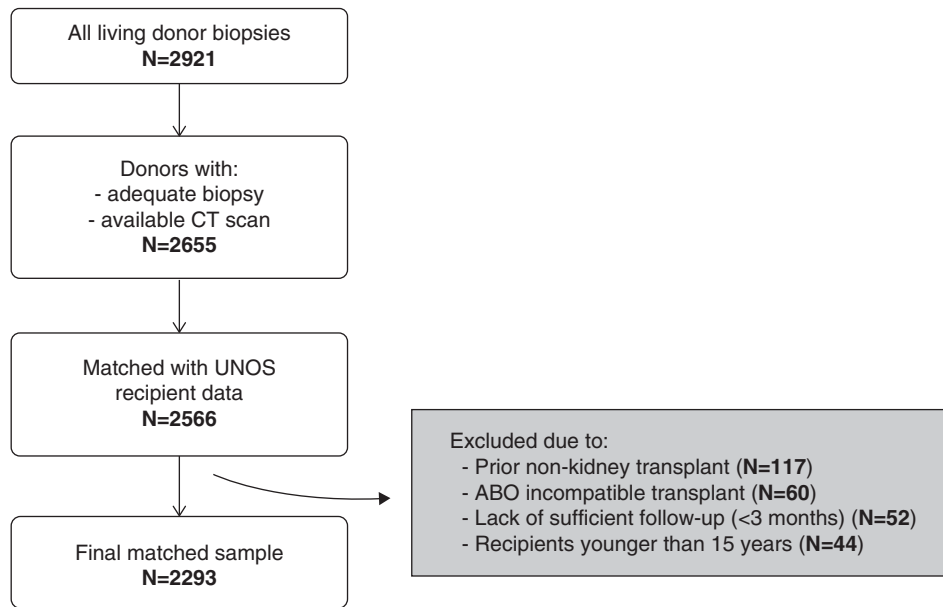


Figure 1. Sampling of matched living donor and recipient pairs. Among 2921 living kidney donors at Mayo Clinic Minnesota, Mayo Clinic Arizona, and Cleveland Clinic who donated between May 1999 and December 2017, 2293 were studied who had a predonation computed tomography (CT) scan and an adequate renal biopsy. These living kidney donors were all paired with their respective recipients using United Network for Organ Sharing (UNOS) Standard Transplant Analysis and Research (STAR) files for each of the 3 sites and excluding recipients with prior non-kidney transplants, ABO incompatible transplants, short follow-up, or pediatric recipients younger than 15 years.

tubular area predicted death-censored graft failure (Figure 2, A and B). After adjusting for both donor and recipient clinical characteristics, death-censored graft failure was predicted by IF/TA >5%, larger glomerular volume, larger tubular area, and smaller medulla volume (Table 3). In analyses adjusting each structural predictor for other structural predictors, only IF/TA >5% versus 0% (HR, 3.62; 95% CI, 1.61 to 8.10) and larger tubular area per SD (HR, 1.18; 95% CI, 1.05 to 1.34) predicted death-censored graft failure independently of each other.

A sensitivity analysis was performed in the 2200 recipients with follow-up beyond 1 year post-transplant. Acute rejection during the first year occurred in 221 (10.0%) and predicted graft loss independent of other recipient characteristics (HR, 2.28; 95% CI, 1.65 to 3.61). The baseline kidney structural predictors for graft loss after 1 year were similar, even after adjustment for donor and recipient characteristics including acute rejection during first year (Supplemental Table 4). Another sensitivity analysis was performed in the subset with donor 24-hour urine albumin available ($n=1705$). The structural predictors of death-censored graft failure had HRs similar to those in the main analysis, although IF/TA >5% and glomerular volume were no longer statistically significant after adjusting for donor and recipient clinical characteristics, including donor 24-hour urine albumin (Supplemental Table 5). Findings in Table 3 were not substantively different with adjustment for all donor and recipient clinical covariates (Supplemental Table 6) and only nonsclerotic glomerular volume per SD was no longer statistically significant (HR, 1.11; 95% CI, 0.99 to 1.24).

Finally, a sensitivity analysis was performed in the 1585 donors that were assessed for arteriolar hyalinosis. Presence of arteriolar hyalinosis was a predictor of death-censored graft failure in unadjusted (Figure 2C) and adjusted analyses (Table 4). The other kidney structural predictors in this subset had HRs similar to those in the main analysis, although IF/TA >5% and glomerular volume were no longer statistically significant after adjusting for donor and recipient characteristics (Table 4). In further analysis of this subset adjusting each structural predictor for other structural predictors, only IF/TA >5% versus 0% (HR, 4.46; 95% CI, 1.85 to 10.74), larger tubular area per SD (HR, 1.17; 95% CI, 1.02 to 1.33), and arteriolar hyalinosis (HR, 1.61; 95% CI, 1.13 to 2.29) predicted death-censored graft failure independently of each other.

DISCUSSION

Biopsy sample features of nephrosclerosis (IF/TA >5% and arteriolar hyalinosis), larger cortical nephron size (larger glomeruli and larger tubules), and smaller medulla at the time of donation were modest predictors of death-censored graft failure in kidneys obtained from living donors. These structure-outcome associations were not explained by the clinical characteristics of either the donor or the recipient. In particular, these associations persisted even after adjustment for gold-standard measures of donor

Table 1. Baseline characteristics of the 2293 donors

Clinical Characteristic	Mean (SD) or n (%)
Age at transplant surgery, yr	43.8 (11.9)
Male	930 (40.6%)
Race	
Black	77 (3.4%)
White	1898 (82.8%)
Other/unknown	318 (13.9%)
BMI, kg/m ²	27.5 (4.8)
Height, cm	170.4 (9.6)
Measured GFR, ml/min per 1.73 m ²	114.7 (24.4)
24 h urine albumin, mg ^a	4.3 (10.9)
Living related donor	1189 (51.9%)
Hypertension	359 (15.7%)
Biopsy features ^b	
Globally sclerotic glomeruli, %	3.3 (6.3)
Glomerulosclerosis above age threshold ^c	138 (6.0%)
%IF/TA	
0%	1670 (72.9%)
>0% to ≤5%	552 (24.1%)
>5%	69 (3.0%)
Number of IF/TA foci per mm ²	0.1 (0.2)
Artery luminal stenosis, %	34.1 (21.0)
Tubular cross-sectional area, per 1000 μm ²	4.2 (1.4)
Nonsclerotic glomeruli volume, per 1000 mm ³	2.5 (1.0)
Cortical volume per glomerulus, mm ³	0.1 (0.0)
Presence of any arteriolar hyalinosis ^d	213 (13.4%)
CT features	
Cortex volume, cm ³	102.3 (21.4)
Medullary volume, cm ³	42.2 (10.6)
Any kidney cyst (>1 cm in diameter)	207 (9.0%)
Kidney surface roughness score	
0	862 (37.6%)
1	557 (24.3%)
2	492 (21.5%)
3	381 (16.6%)
Nephron number, per 1000	929.9 (439.9)
Single-nephron GFR, nl/min	76.1 (47.5)

^aAssessed in 1705 donors.

^bBiopsies used had a mean ± SD cortical area of 6.7 ± 3.0 mm² and number of glomeruli of 19 ± 11 per section.

^cNumber of globally sclerotic glomeruli on biopsy exceeds 95th percentile expected for donor age and number of glomeruli present.³⁰

^dAssessed in 1585 donors.

kidney function (measured GFR and 24-hour urine albumin excretion).

Our results suggest subclinical structural features in the kidney at the time of donation modestly contribute to death-censored graft failure. Low nephron numbers was not a predictor of death-censored graft failure, nor were its surrogates, increased globally sclerotic glomeruli or low cortical volume. Nephron number in donors predominantly reflects physiologic variation at birth and the gradual nephron loss with aging.^{6,18,19} For the recipient, it may be the baseline “health” of the donated nephrons rather than their number that influence outcomes. Larger nephrons and even small amounts of IF/TA may reflect nephrons that are more prone to post-transplant injury (immunologic or

nonimmunologic). Instead, pathologic findings of interstitial fibrosis and arteriolar hyalinosis were predictive of death-censored graft failure. Furthermore, enlarged nephrons, which may have occurred in response to the metabolic milieu of the donor (e.g., as in obesity), predicted death-censored graft failure, despite the different metabolic milieu of the recipient. Thus, subtle subclinical pathology in the living donor kidney may progress and contribute to death-censored graft failure in the recipient.

Nephrosclerosis or arterionephrosclerosis has been described as a process by which arteriosclerosis (artery luminal stenosis from intimal thickening and arteriolar hyalinosis) causes ischemia resulting in global glomerulosclerosis, tubular atrophy, and interstitial fibrosis. In living kidney donors, nephrosclerosis is associated with older age and hypertension.²⁰ With normal aging, donors lose about half their nephrons from age 20 to age 70 years, but the increase in IF/TA with aging is modest.⁹ The findings of IF/TA >5% or arteriolar hyalinosis may be indicative of an injury to the kidney separate from the aging process. Among histologic features at 5 years post-transplant, arteriolar hyalinosis is one of the most important predictors of death-censored graft failure²¹; this study suggests that arteriolar hyalinosis originating from the donor is also important. Once evident, IF/TA also predicts progressive graft dysfunction in the subsequent years.^{22,23}

Table 2. Baseline characteristics of the 2293 recipients

Clinical Characteristics	Mean ± SD or n (%)
Age at transplant surgery, yr	50.4 ± 14.6
Male	1372 (59.8%)
Race	
Black	90 (3.9%)
White	1963 (85.6%)
Other/unknown	240 (10.5%)
BMI, kg/m ²	28.0 ± 6.1
Height, cm	171.5 ± 10.1
Diabetes mellitus	598 (26.1%)
Hypertension	1956 (85.3%)
Cause of CKD	
Glomerular disease	666 (29.0%)
Diabetes	433 (18.9%)
Polycystic kidneys	335 (14.6%)
Hypertension	166 (7.2%)
Other/unknown	693 (30.2%)
HLA-A,B 0 mismatch	226 (9.9%)
HLA-DR 0 mismatch	426 (18.6%)
cPRA, % ^a	10.1 ± 23.9
Previous kidney transplant	292 (12.7%)
Pre-emptive transplant	1014 (44.2%)
Depleting agent for induction	1674 (73.0%)
CNI-based maintenance immunosuppression	2195 (95.7%)
Maintenance steroids use	1728 (75.4%)
Delayed graft function	40 (1.7%)

HLA-DR, HLA donor-recipient; cPRA, calculated panel reactive antibody; CNI, calcineurin inhibitor.

^acPRA was available only in 1224 recipients (during the more recent time period of transplantation).

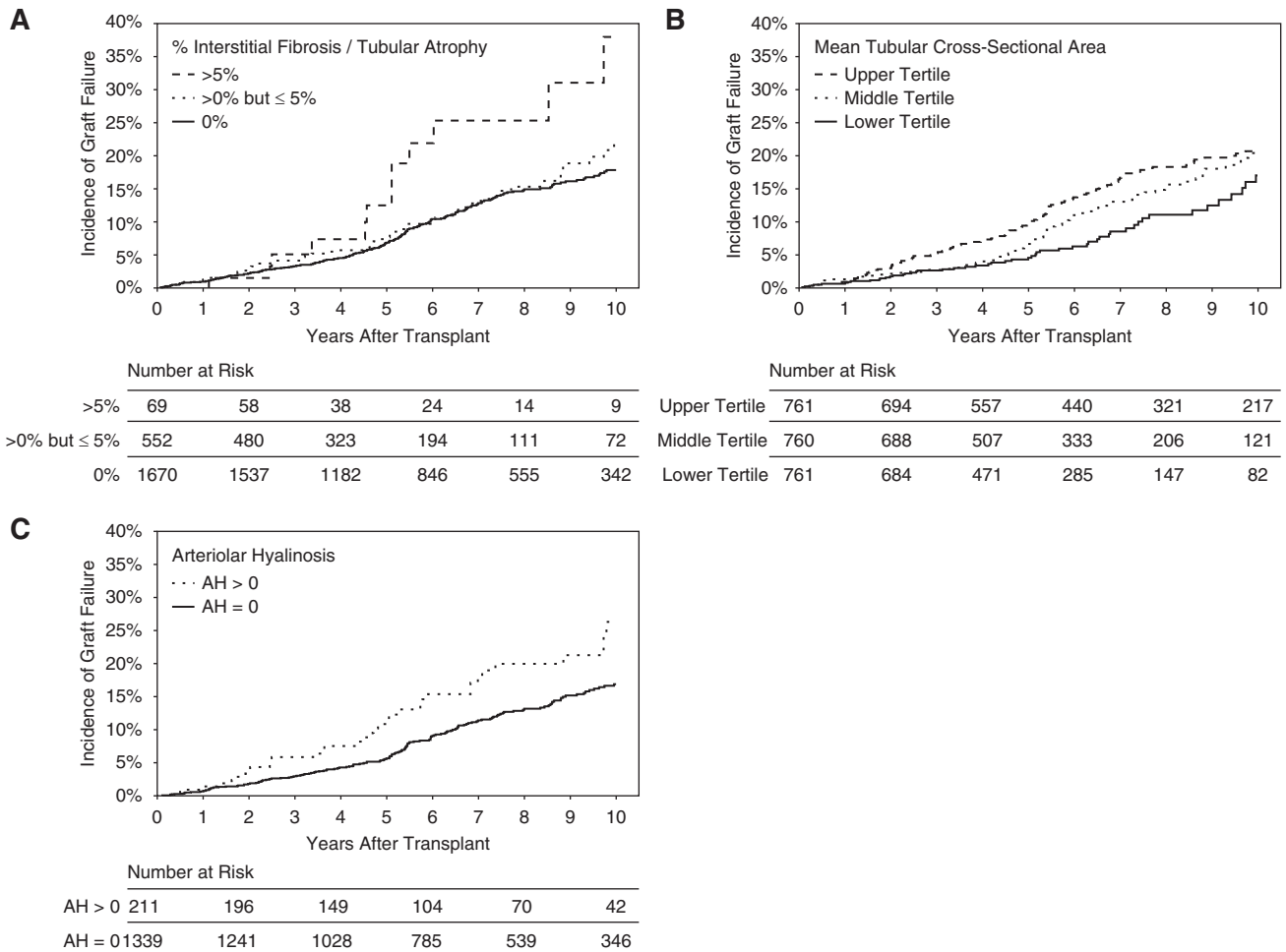


Figure 2. Several histological findings predicted death-censored graft failure. The presence of (A) more than 5% interstitial fibrosis and tubular atrophy, (B) larger mean tubular cross-sectional area, and (C) any arteriolar hyalinosis on the donated kidney biopsy at the time of transplantation predicted death-censored graft failure in the matched recipient.

These findings to compare with those in deceased donor kidneys, where both IF/TA and glomerulosclerosis, at the time of transplant surgery, predict death-censored graft failure.²⁴ In living kidney donors, glomerulosclerosis may be less predictive of death-censored graft failure because the glomerulosclerosis is more reflective of the age-related loss of nephrons than underlying kidney disease. It is plausible that the IF/TA and arteriolar hyalinosis in the transplanted kidney might accelerate the injurious effects of ischemia-reperfusion injury, rejection, or calcineurin inhibitor toxicity to the graft.^{25,26}

Larger nephrons also predicted death-censored graft failure. Larger nephrons occur when metabolic demand for renal function exceeds nephron number, such as with obesity, and are linked to hypertension and albuminuria.^{4,6} Unlike nephrosclerosis,⁹ nephron enlargement may seem benign and reversible, until collapse and sclerosis of the glomerular tuft occurs.²⁷ In this unique setting of transplantation, the clinical characteristics of the donor are no longer linked to the structural features of larger nephrons. Yet the acquisition of a kidney with enlarged nephrons confers an increased risk of

death-censored graft failure. Enlarged glomeruli may make the graft more prone to hyperfiltration that in turn, may potentiate immunologic and nonimmunologic stress to the graft.

In general, less is known about the structural features of the renal medulla. Although smaller cortical volume did not predict death-censored graft failure, there was evidence that smaller medullary volume predicted death-censored graft failure. It is worth noting that the age-related loss of nephrons occurs with superficial glomeruli, not deep glomeruli.²¹ Because medulla is composed of the tubules of deep glomeruli, loss of medulla volume may reflect loss of nephrons that is more pathologic rather than age-related. Further, because lower medullary volume was not predictive of death-censored graft failure after adjusting for IF/TA >5% and larger tubular area, it is likely part of the same pathway as these histologic features.

Intraoperative kidney biopsy has very low risk for harm, with any bleeding easily controlled by the surgeon. These biopsies provide a baseline histologic assessment of the graft, but the clinical importance of this has been unclear.⁴

Table 3. Donor baseline CT and biopsy predictors of death-censored graft failure (n=2293)

Structural Feature ^a	Unadjusted	Adjusted for Donors	Adjusted for Recipients	Adjusted for Both Donors and
	HR (95% CI)	Covariates ^b HR (95% CI)	Covariates ^c HR (95% CI)	Recipients Covariates ^{b,c} HR (95% CI)
% Globally sclerotic glomeruli	1.02 (0.90 to 1.15)	0.99 (0.8 to 1.12)	1.08 (0.96 to 1.22)	1.01 (0.89 to 1.15)
Glomerulosclerosis above age threshold ^d	0.84 (0.49 to 1.44)	0.82 (0.48 to 1.40)	0.95 (0.55 to 1.63)	0.92 (0.53 to 1.58)
%IF/TA				
0%	Reference	Reference	Reference	Reference
>0% to ≤5%	1.16 (0.87 to 1.55)	1.12 (0.83 to 1.50)	1.26 (0.94 to 1.69)	1.15 (0.86 to 1.55)
>5%	2.51 (1.48 to 4.26)	2.33 (1.35 to 4.04)	2.42 (1.42 to 4.14)	1.91 (1.10 to 3.33)
IF/TA foci/mm ²	1.10 (0.98 to 1.24)	1.08 (0.95 to 1.21)	1.12 (1.00 to 1.26)	1.06 (0.94 to 1.20)
% Artery luminal stenosis	1.00 (0.89 to 1.11)	0.96 (0.85 to 1.08)	1.05 (0.94 to 1.18)	0.99 (0.88 to 1.12)
Nonsclerotic glomeruli volume	1.09 (0.98 to 1.22)	1.09 (0.98 to 1.22)	1.12 (1.00 to 1.25)	1.13 (1.01 to 1.26)
Tubular cross-sectional area	1.18 (1.06 to 1.32)	1.18 (1.06 to 1.32)	1.16 (1.03 to 1.29)	1.15 (1.03 to 1.29)
Cortical volume per glomerulus	1.02 (0.92 to 1.14)	1.02 (0.91 to 1.14)	1.05 (0.94 to 1.18)	1.04 (0.93 to 1.17)
Cortex volume	0.98 (0.87 to 1.10)	0.99 (0.88 to 1.12)	0.97 (0.86 to 1.09)	1.01 (0.89 to 1.14)
Medulla volume	0.92 (0.82 to 1.04)	0.91 (0.81 to 1.03)	0.88 (0.78 to 0.99)	0.86 (0.76 to 0.97)
Kidney surface roughness	0.99 (0.88 to 1.12)	0.98 (0.87 to 1.11)	1.01 (0.90 to 1.14)	0.98 (0.87 to 1.10)
Any kidney cyst	0.98 (0.65 to 1.49)	0.93 (0.61 to 1.42)	1.06 (0.70 to 1.62)	0.92 (0.60 to 1.41)
Nephron number	0.98 (0.86 to 1.12)	1.00 (0.88 to 1.14)	0.95 (0.83 to 1.08)	0.99 (0.87 to 1.13)
Single-nephron GFR	1.02 (0.91 to 1.14)	1.02 (0.91 to 1.14)	1.04 (0.93 to 1.17)	1.04 (0.93 to 1.16)

^aAll HR for continuous measures are per SD from Table 1.

^bDonor age and black race.

^cRecipient age, black race, diabetes, cause of CKD, any HLA mismatch, HLA donor-recipient mismatch, previous kidney transplant, pretransplant dialysis, and delayed graft function.

^dNumber of globally sclerotic glomeruli on biopsy exceeds 95th percentile expected for donor age and number of glomeruli.³⁰

Although morphometry is not routinely used to quantify nephrosclerosis or nephron size measures, there may be a role for morphometry in the future, especially if it can be automated with machine learning techniques.²⁸ There are several potential uses of the intraoperative kidney biopsy for clinical management of the recipient. If the biopsy shows arteriolar hyalinosis, IF/TA >5%, or enlarged nephrons, further monitoring of the graft over time with follow-up biopsies may be helpful. These patients may benefit from non-calcineurin inhibitors²⁹ or more aggressive management of BP to prevent worsening arteriolar hyalinosis or nephron enlargement. However, further studies are needed to determine if recipient management benefits from modifications on the basis of the living donor biopsy findings.

There were several potential limitations to this study. The recipient data in our study were limited to UNOS reported

data by the individual transplant centers; however, using UNOS data allowed us to standardize the data from the three different centers. We did not take into account major changes in immunosuppression in the analysis and we did not capture recipients' noncompliance with immunosuppressive medication regimens that can influence allograft survival. The kidney donors and recipients studied were predominantly white, although we were still able to detect a risk of graft loss by race. We did not include donor-recipient sex and size mismatch; however, forcing donor and recipient height into the model did not change our findings (data not shown).

In conclusion, IF/TA, arteriolar hyalinosis, and larger nephrons from the living donor kidney were independent predictors of death-censored graft failure in the recipient. These findings provide important insights into the "intrinsic quality" of the graft at the time of donation. They further support

Table 4. Donor baseline CT and biopsy predictors of death-censored graft failure among donors who had arteriolar hyalinosis assessed on biopsy ($n=1585$)

Structural Feature ^a	Unadjusted	Adjusted for Donors	Adjusted for Recipients	Adjusted for Both Donors and
	HR (95% CI)	Covariates ^b HR (95% CI)	Covariates ^c HR (95% CI)	Recipients Covariates ^{b,c} HR (95% CI)
% Globally sclerotic glomeruli	1.02 (0.89 to 1.16)	0.99 (0.86 to 1.15)	1.07 (0.94 to 1.23)	1.00 (0.87 to 1.16)
Glomerulosclerosis above age threshold ^d	0.69 (0.35 to 1.35)	0.69 (0.36 to 1.35)	0.82 (0.42 to 1.61)	0.80 (0.41 to 1.56)
%IF/TA				
0%	Reference	Reference	Reference	Reference
>0% to ≤5%	1.12 (0.81 to 1.55)	1.09 (0.78 to 1.53)	1.22 (0.87 to 1.70)	1.13 (0.80 to 1.58)
>5%	2.43 (1.38 to 4.29)	2.33 (1.28 to 4.25)	2.27 (1.27 to 4.04)	1.79 (0.97 to 3.28)
IF/TA foci/mm ²	1.05 (0.91 to 1.20)	1.03 (0.89 to 1.19)	1.07 (0.93 to 1.22)	1.01 (0.87 to 1.16)
% Artery luminal stenosis	1.03 (0.92 to 1.17)	1.01 (0.89 to 1.15)	1.08 (0.96 to 1.23)	1.03 (0.90 to 1.17)
Nonsclerotic glomeruli volume	1.09 (0.96 to 1.23)	1.09 (0.97 to 1.23)	1.12 (0.99 to 1.26)	1.13 (1.00 to 1.28)
Tubular cross-sectional area	1.17 (1.04 to 1.31)	1.17 (1.04 to 1.31)	1.16 (1.03 to 1.31)	1.16 (1.03 to 1.31)
Cortical volume per glomerulus	1.03 (0.92 to 1.16)	1.03 (0.91 to 1.16)	1.07 (0.95 to 1.21)	1.06 (0.94 to 1.20)
Cortex volume	0.99 (0.87 to 1.14)	1.01 (0.88 to 1.15)	0.98 (0.86 to 1.12)	1.02 (0.89 to 1.17)
Medulla volume	0.90 (0.79 to 1.03)	0.89 (0.78 to 1.02)	0.86 (0.75 to 0.99)	0.84 (0.73 to 0.96)
Kidney surface roughness	0.99 (0.87 to 1.14)	0.98 (0.86 to 1.13)	1.01 (0.88 to 1.16)	0.98 (0.86 to 1.13)
Any kidney cyst	0.82 (0.49 to 1.39)	0.78 (0.46 to 1.33)	0.90 (0.53 to 1.52)	0.77 (0.45 to 1.31)
Nephron number	0.94 (0.80 to 1.10)	0.96 (0.81 to 1.12)	0.90 (0.77 to 1.05)	0.94 (0.80 to 1.10)
Single-nephron GFR	1.02 (0.90 to 1.15)	1.02 (0.90 to 1.15)	1.04 (0.92 to 1.18)	1.04 (0.92 to 1.18)
Any arteriolar hyalinosis	1.63 (1.16 to 2.31)	1.59 (1.12 to 2.26)	1.71 (1.19 to 2.46)	1.62 (1.12 to 2.34)

^aAll HR for continuous measures are per SD.

^bDonor age and black race.

^cRecipient age, black race, diabetes, cause of CKD, any HLA mismatch, HLA donor-recipient mismatch, previous kidney transplant, pretransplant dialysis, and delayed graft function.

^dNumber of globally sclerotic glomeruli on biopsy exceeds 95th percentile expected for donor age and number of glomeruli.³⁰

the use of intraoperative biopsies to identify kidney allografts that are at higher risk for failure.

ACKNOWLEDGMENTS

Dr. Rule and Dr. Issa designed the study and drafted the paper. Dr. Issa, Dr. Denic, Dr. Rule, Mr. Larson, Dr. Ricaurte, Dr. Chakkera, and Alexander acquired the data. Mr. Lopez, Mr. Larson, and Dr. Kremers performed the statistical analysis. All authors contributed to the interpretation of findings, revision of the paper, and approval of the final version.

We thank Miloš Denić for assistance with computer algorithms for processing of biopsy annotations data.

DISCLOSURES

Dr. Kremers reports grants from the National Institutes of Health, during the conduct of the study; grants from AstraZeneca, grants from Biogen, grants from Roche, outside the submitted work. Dr. Rule reports grants from National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, during the conduct of the study. All of the remaining authors have nothing to disclose.

FUNDING

This study was supported with funding from the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases (R01 DK090358, PI: Dr. Rule).

SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at <http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2019090964/-/DCSupplemental>.

Supplemental Appendix 1. Supplemental methods.

Supplemental Table 1. Percentage of missing data that was imputed.

Supplemental Table 2. Donor clinical characteristics that predict graft failure.

Supplemental Table 3. Recipient clinical characteristics that predict graft failure.

Supplemental Table 4. Structural predictors of graft failure adjusting for acute rejection.

Supplemental Table 5. Structural predictors of graft failure adjusting for 24-hour urine albumin.

Supplemental Table 6. Structural predictors of graft failure adjusting for all donor and recipient clinical covariates.

REFERENCES

- Hart A, Smith JM, Skeans MA, Gustafson SK, Wilk AR, Robinson A, et al.: OPTN/SRTR 2016 annual data report: Kidney. *Am J Transplant* 18 [Suppl 1]: 18–113, 2018
- Davis CL, Delmonico FL: Living-donor kidney transplantation: A review of the current practices for the live donor. *J Am Soc Nephrol* 16: 2098–2110, 2005
- Issa N, Stephany B, Fatica R, Nurko S, Krishnamurthi V, Goldfarb DA, et al.: Donor factors influencing graft outcomes in live donor kidney transplantation. *Transplantation* 83: 593–599, 2007
- Denic A, Alexander MP, Kaushik V, Lerman LO, Lieske JC, Stegall MD, et al.: Detection and clinical patterns of nephron hypertrophy and nephrosclerosis among apparently healthy adults. *Am J Kidney Dis* 68: 58–67, 2016
- Issa N, Vaughan LE, Denic A, Kremers WK, Chakkera HA, Park WD, et al.: Larger nephron size, low nephron number, and nephrosclerosis on biopsy as predictors of kidney function after donating a kidney. *Am J Transplant* 19: 1989–1998, 2019
- Denic A, Lieske JC, Chakkera HA, Poggio ED, Alexander MP, Singh P, et al.: The substantial loss of nephrons in healthy human kidneys with aging. *J Am Soc Nephrol* 28: 313–320, 2017
- Mjøen G, Hallan S, Hartmann A, Foss A, Midtvedt K, Øyen O, et al.: Long-term risks for kidney donors. *Kidney Int* 86: 162–167, 2014
- Muzaale AD, Massie AB, Wang MC, Montgomery RA, McBride MA, Wainright JL, et al.: Risk of end-stage renal disease following live kidney donation. *JAMA* 311: 579–586, 2014
- Denic A, Glasscock RJ, Rule AD: Single-nephron glomerular filtration rate in healthy adults. *N Engl J Med* 377: 1203–1204, 2017
- Data services portal in UNet now includes Center STAR files, 2016. Available at: <https://unos.org/news/data-services-portal-in-unet-now-includes-center-star-files/>. Accessed July 20, 2018
- Weibel ER, Gomez DM: A principle for counting tissue structures on random sections. *J Appl Physiol* 17: 343–348, 1962
- Fulladosa X, Moreso F, Narváez JA, Grinyó JM, Serón D: Estimation of total glomerular number in stable renal transplants. *J Am Soc Nephrol* 14: 2662–2668, 2003
- Lerman LO, Bentley MD, Bell MR, Rumberger JA, Romero JC: Quantitation of the in vivo kidney volume with cine computed tomography. *Invest Radiol* 25: 1206–1211, 1990
- White IR, Royston P, Wood AM: Multiple imputation using chained equations: Issues and guidance for practice. *Stat Med* 30: 377–399, 2011
- Rubin DB: *Multiple Imputation for Nonresponse in Surveys*, New York, John Wiley and Sons, 1987
- Li KH, Raghunathan TE, Rubin DB: Large-sample significance levels from multiply imputed data using moment-based statistics and an F reference distribution. *J Am Stat Assoc* 86: 1065–1073, 1991
- Grambsch P, Therneau T: Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika* 81: 515–526, 1994
- Mañalich R, Reyes L, Herrera M, Melendi C, Fundora I: Relationship between weight at birth and the number and size of renal glomeruli in humans: A histomorphometric study. *Kidney Int* 58: 770–773, 2000
- Luyckx VA, Brenner BM: Low birth weight, nephron number, and kidney disease. *Kidney Int Suppl* [97]: S68–S77, 2005
- Rule AD, Amer H, Cornell LD, Taler SJ, Cosio FG, Kremers WK, et al.: The association between age and nephrosclerosis on renal biopsy among healthy adults. *Ann Intern Med* 152: 561–567, 2010
- Denic A, Ricaurte L, Lopez CL, Narasimhan R, Lerman LO, Lieske JC, et al.: Glomerular volume and glomerulosclerosis at different depths within the human kidney. *J Am Soc Nephrol* 30: 1471–1480, 2019
- Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Chapman JR, Allen RD: Delta analysis of posttransplantation tubulointerstitial damage. *Transplantation* 78: 434–441, 2004
- Chapman JR, O'Connell PJ, Nankivell BJ: Chronic renal allograft dysfunction. *J Am Soc Nephrol* 16: 3015–3026, 2005
- De Vusser K, Lerut E, Kuypers D, Vanrenterghem Y, Jochmans I, Monbaliu D, et al.: The predictive value of kidney allograft baseline biopsies for long-term graft survival. *J Am Soc Nephrol* 24: 1913–1923, 2013
- Norman JT: Protecting the microvasculature: A Tie-ght connection to ameliorating chronic kidney disease? *J Am Soc Nephrol* 17: 2353–2355, 2006
- Zhao H, Alam A, Soo AP, George AJT, Ma D: Ischemia-reperfusion injury reduces long term renal graft survival: Mechanism and beyond. *EBioMedicine* 28: 31–42, 2018
- Hodgin JB, Bitzer M, Wickman L, Afshinnia F, Wang SQ, O'Connor C, et al.: Glomerular aging and focal global glomerulosclerosis: A podometric perspective. *J Am Soc Nephrol* 26: 3162–3178, 2015
- Hermesen M, de Bel T, den Boer M, Steenbergen EJ, Kers J, Florquin S, et al.: Deep learning-based histopathologic assessment of kidney tissue. *J Am Soc Nephrol* 30: 1968–1979, 2019
- Vincenti F, Rostaing L, Grinyo J, Rice K, Steinberg S, Gaitte L, et al.: Belatacept and long-term outcomes in kidney transplantation. *N Engl J Med* 374: 333–343, 2016
- Kremers WK, Denic A, Lieske JC, Alexander MP, Kaushik V, Elsherbiny HE, et al.: Distinguishing age-related from disease-related glomerulosclerosis on kidney biopsy: The Aging Kidney Anatomy study. *Nephrol Dial Transplant* 30: 2034–2039, 2015

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 μm²) 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 μm² 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.