Renal Histologic Analysis Provides Complementary Information to Kidney Function Measurement for Patients with Early Diabetic or Hypertensive Disease

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ABSTRACT

Background Patients with diabetic or hypertensive kidney disease rarely undergo kidney biopsy because nephrologists commonly believe that biopsy-related risk outweighs the potential benefits of obtaining histologic information to guide clinical decisions. Although kidney function is acutely regulated, histologic changes such as interstitial fibrosis, tubular atrophy, and glomerulosclerosis may represent chronic kidney damage, and thus might provide additional information about disease severity. However, whether histologic analysis provides information complementary to clinically used kidney function measurements, such as eGFR and proteinuria, is unclear.

Methods We performed a standardized semiquantitative histologic analysis of 859 nephrectomies obtained from individuals with or without diabetes mellitus or hypertension and varying degrees of kidney dysfunction. Changes in glomeruli, tubules, interstitium, and the vasculature were scored using 17 descriptive parameters in a standardized manner. We used multivariable linear and logistic regression analyses and unbiased, hierarchical clustering to assess associations between histologic alterations and clinical variables.

Results At CKD stages 3–5, eGFR correlates reasonably well with the degree of glomerulosclerosis and interstitial fibrosis and tubular atrophy (IFTA). In patients with CKD stages 1–2, the degree of histologic damage was highly variable and eGFR poorly estimated the degree of damage. Individuals with diabetes mellitus, hypertension, or Black race had significantly more glomerulosclerosis and IFTA, at the same eGFR level. Inclusion of glomerulosclerosis improved the kidney function decline estimation, even at early disease stages.

Conclusions Histologic analysis is an important complementary method for kidney disease evaluation, especially at early disease stages. Some individuals present with relatively severe structural damage despite preserved eGFR.

CKD is defined by functional changes, reduction of eGFR, proteinuria, or by structural kidney damage (glomerulosclerosis [GS] and interstitial fibrosis [IF] and tubule atrophy [TA]).[1] Diabetes mellitus (DM) and hypertension (HTN) are responsible for most (70%) CKD. Histopathological characterization of diabetic kidney disease dates back more than six decades.[2] More recently, the diagnosis of diabetic and hypertensive CKD is established by clinical parameters, such as eGFR and proteinuria.

Kidney biopsies in DM and HTN are usually restricted for patients with atypical presentations because nephrologists commonly believe the risk of the kidney biopsy outweighs the potential benefit of obtaining histologic information for clinical decision making.[3] Research biopsy samples have only been obtained in the Pima Indian population[4] or in a small subgroup of patients with type 1 diabetes;[5] however, the generalizability of these findings is unclear, given small study sizes, ethnic differences, and that most patients in our clinics have type 2 not type1 diabetes. Recent studies have almost exclusively relied on clinically indicated biopsies that are biased toward patients with unusual presentations and advanced disease stages.[6] These studies indicate the dominance of chronic changes, such as GS, IF and tubular atrophy (IFTA), and vascular sclerosis.[7–9] In single-center collections of clinically obtained kidney biopsies that were heavily biased toward patients with glomerular diseases, GS and IFTA correlated with eGFR.[10–12]

Given the observed correlation between eGFR and fibrosis, it remains unclear whether histologic alterations represent an independent domain of disease severity from eGFR.[10–18] In several scenarios, the eGFR is a poor estimator of kidney damage and prognosis. Acute changes in eGFR make it difficult to evaluate disease severity in deceased kidney donors, or after the initiation of treatment with inhibitors of the angiotensin converting enzyme inhibitor, angiotensin receptor blocker, or sodium glucose cotransporter inhibitor. It is also challenging to estimate kidney damage in subjects with extreme age, extreme body size (obesity and cachexia), and severe liver disease. A histologic damage–based CKD staging system might be useful in these scenarios for risk stratification.

Baseline kidney function strongly predicts CKD progression at CKD stages 3–5.[19] In contrast, kidney function is a poor predictor of progression in subjects with eGFR $\geq$60 ml/min per 1.73 m^2.[2] Sethi and colleagues proposed that histologic damage could be a better predictor of kidney function decline than eGFR,[13] because it represents chronic irreversible events.[14] The role of fibrosis in kidney function decline prediction has not been formally tested because most histologic studies do not adjust for baseline kidney function, which is a key determinant of progression, and/or analyze samples with advanced CKD. Two recent reports indicated that inclusion of GS and/or IFTA can improve prediction of kidney function decline even after adjusting for baseline kidney function when applied to clinically indicated biopsy samples, which again were significantly biased toward patients with glomerular disease.[12,17] Multiple other studies did not replicate this observation.[11,18]

In this study we analyzed the precision of eGFR and other clinical parameters in estimating histologic abnormalities in subjects with and without DM and HTN, and varying degrees of CKD, with the aim of clarifying the potential role of histologic information in clinical assessment of diabetic and hypertensive CKD in an unbiased collection of

Significance Statement

CKD is defined by both functional changes (such as in eGFR and proteinuria) and renal histologic alterations. Although kidney function is acutely regulated, histologic changes such as interstitial fibrosis, tubular atrophy, and glomerulosclerosis could represent chronic damage, thus might provide additional information about disease severity. In an analysis of 859 kidney tissue samples, the authors found that the relationship between histologic changes and eGFR is not linear. At CKD stages 3–5, eGFR correlates with interstitial fibrosis/tubular atrophy and glomerulosclerosis reasonably well, whereas at earlier disease stages, eGFR poorly estimates histologic damage. Patients with diabetes, hypertension, or Black race had more severe histologic damage at the same eGFR. The inclusion of glomerulosclerosis significantly improved the kidney function decline estimation.

tissue samples. In addition, we also examined the role of histologic damage in predicting kidney function decline.

METHODS

Study Population

This study consisted of a cross-sectional evaluation of 1007 human subjects undergoing clinically indicated nephrectomies for renal neoplasia across six academic medical centers. Demographic, clinical information and laboratory data were obtained from medical records by an "honest broker," as established by Cooperative Human Tissue Network. The study protocol was approved by the institutional review board of the University of Pennsylvania and no informed consent was obtained because the study was considered exempt.

Subjects were included in the analysis if they were $\geq$18 years old, had serum creatinine measurement at time of enrollment, and had a tumor-free renal cortex. eGFR values were calculated using the CKD Epidemiology Collaboration equation[20] on the basis of serum creatinine, age, sex, and self-reported or clinician-determined race, as obtained from medical charts. The final sample size was 859 and Supplemental Figure 1 shows the study flow chart. For a subset of samples, we were able to obtain longitudinal kidney function measurements. The longitudinal cohort included 280 subjects with $\geq$3 months follow-up, of which 167 had complete clinical information after nephrectomy. On average, we collected three kidney function measurements (serum creatinine) over a period of at least 1 year, spanning pre- and postnephrectomy.

Sample Procurement and Histopathological Analysis

Deidentified human kidney tissues were collected from the non-neoplastic portion of surgical nephrectomies at least 2 cm away from the cancer margin. Kidney samples were formalin fixed, paraffin embedded, and stained with periodic acid–Schiff. Samples were scored in an unbiased manner by

a specialized renal pathologist. On average, we scored 129 (mean SD 123) glomeruli per sample. More than 93% of samples had >10 glomeruli, an important advantage of using nephrectomy samples, compared with the limited number of glomeruli present in biopsy specimens.

In total, 17 light microscopy (LM) parameters were selected for semiquantitative scoring to describe the tubulointerstitium, glomeruli, and renal vasculature. Definitions of these LM descriptors are detailed Supplemental Table 1, and are adapted from the Neptune digital pathology protocol,[21] the Oxford classification of IgA nephropathy,[22] and the Renal Pathology Society classification of diabetic nephropathy.[23] Glomerular descriptors included global GS, segmental sclerosis, Kimmelstiel–Wilson (KW) nodules, glomerular wall–thickening, pericapsular fibrosis, glomerular hypoperfusion, mesangial matrix, and mesangial cellularity. Vascular descriptors included arteriolar hyalinosis and arterial intimal fibrosis. Tubulointerstitial descriptors included acute tubular injury,[24] TA, tubular reabsorption droplets, IF, interstitial eosinophils, lymphocytes, and plasma cells.

Statistics

Statistical analyses were performed using RStudio v3.6.2 (R Development Core Team, Vienna, Austria) and STATA version 16.0 (Statacorp LP, College Station, TX). Descriptive statistics (*i.e.*, mean, median, and proportion) were used to describe clinical and demographic characteristics across all subjects. Continuous variables were compared using Kruskal–Wallis test for non-normally distributed variables and ANOVA for normally distributed variables. Categorical and binary variables were compared using chi-squared test. Spearman correlations coefficients (rho) were estimated eGFR and histopathological variables.

We analyzed the following covariates at the time of nephrectomy: age, sex, presence of DM, presence of HTN, race, body mass index (BMI), BP, serum albumin, and urine dipstick. Race/ethnicity was reported as Black (*e.g.*, African American), White (*e.g.*, Caucasian), Asian, Hispanic, multiracial, and other, and was recategorized as Black and non-Black, as used in the CKD Epidemiology Collaboration equation. Variables with >20% missingness were not included into the primary regression model but were assessed secondarily for effect.

Continuous histopathologic variables including IFTA and GS, were each log transformed (with constant, *i.e.*, 1, added to account for scores of zero) for the linear regression analyses. The relationship between histopathologic variables and eGFR was modeled as a polynomial function of eGFR (*e.g.*, $eGFR^2$ and $eGFR^3$) and adjusted for age, sex, race, BMI, diabetes, and/or HTN status. To obtain adjusted means for log-transformed variables on the original scale, we exponentiated the model predictions and subtracted 1 (given constant of 1 added to samples before log transformation), and then used

smearing adjustment to decrease bias introduced by the retransformation.[25] Stepwise regression with a combination of forward and backward selection was used to guide variable selection[26] in the construction of primary regression models for histopathologic dependent variables. Independent variables tested were age, sex, race, eGFR, BMI, diabetes, and HTN. Several variables were binarized, using the following criteria: any segmental sclerosis, >1+ glomerular hypoperfusion, >1+ mesangial cellularity, >1+ mesangial matrix, >1+ glomerular wall thickening, >1+ pericapsular fibrosis, any acute tubular injury, any tubular reabsorption, >1+ interstitial lymphocytic infiltrate, any interstitial plasmocytic infiltrate, any interstitial eosinophils, >1+ intimal fibrosis, and >1+ arterial hyalinosis. Logistic regression was used to test binary outcomes. The significance level for removal from the model was set at ≥0.2 and significance level for addition to the model was set at <0.05. Robust standard errors were used and an alpha level <0.05 was considered statistically significant. Model fitness was determined by Akaike Information Criterion and likelihood ratio testing.

Longitudinal eGFR was modeled using linear mixed modeling in which subject-specific eGFR slopes were estimated by regressing repeated eGFR values on the time variable (random effects), which was added to the population-average model (fixed-effects model) with the overall model fit estimated *via* maximum likelihood.[27]

To identify unbiased subgroups on the basis of LM descriptors, we used hierarchical clustering methods on scaled data.[28] The average silhouette method was used to determine the optimal number of clusters with data shown as a cluster dendrogram.[29] Radar plots were used to show differences between clusters in terms of LM. To compare the continuous and categorical clinical, laboratory, and pathologic parameters between groups, Kruskal–Wallis and chi-squared tests were used, respectively.

RESULTS

Clinical and Histologic Characteristics of Study Participants

Kidney tissue samples were obtained from the non-neoplastic portion of tumor nephrectomies at six academic medical centers. Supplemental Figure 1 shows the inclusion and exclusion criteria leading to our final sample size of 859, with available histopathological and clinical information (Table 1). The mean subject age was 61 (SD±13) years, 64% were male, and 20% were of Black race. In total, 35% of subjects had a history of DM and 69% had a history of HTN. Mean BP was 136/77 mm Hg in the cohort. The median BMI was 29 kg/m^2. The mean eGFR was 65 (SD±26.7) ml/min per 1.73 m^2 and 55% of subjects had no detectable proteinuria by dipstick analysis. As expected, some of the clinical variables showed relatedness. Kidney function (eGFR) correlated with age, HTN, systolic BP,

Table 1. Clinical and histopathological characteristics of subjects in our multi-institutional kidney tissue biobank

Clinical and Histopathological Characteristics (*n*=859)	Result
eGFR, mean (SD), ml/min per 1.73 m^2	65 (26.7)
Age, mean (SD), yr	61 (13)
DM, *n* (%)	304 (35.4)
HTN, *n* (%)	595 (69)
Sex, M, *n* (%)	548 (63.8)
Race, Black, *n* (%)	176 (20.4)
BMI, median (IQR), kg/m^2	29.4 (25.7–34.2)
Systolic BP, mean (SD), mm Hg	136 (20)
Diastolic BP, mean (SD), mm Hg	77 (12)
Serum albumin, median (IQR), g/dl	4.05 (3.7–4.4)
Dipstick proteinuria (%)	
Negative	54.9
Trace	9.2
30 mg/dl	14.9
100 mg/dl	12.5
300 mg/dl	1.3
>1000 mg/dl	7.6
Tubulointerstitial	
Acute tubular injury, median (IQR) (%)	0 (0–2)
TA, median (IQR) (%)	5 (2–10)
Tubular reabsorption 0–3 (%)	
0	81.4
1	16.1
2	2.1
3	0.5
Interstitial fibrosis, median (IQR) (%)	5 (2–15)
Interstitial eosinophils 0–3 (%)	
0	78.8
1	19.6
2	1.4
3	0.2
Interstitial lymphocytic infiltrates 0–3 (%)	
0	25.7
1	48.3
2	19.7
3	6.3
Interstitial plasmocytic infiltrates 0–3 (%)	
0	65.4
1	28.3
2	5.4
3	0.9
Glomerular	
Segmental sclerosis, median (IQR) (range) (%)	0 (0) (0–29)
GS, median (IQR) (%)	6.7 (2.7–16.1)
Wall thickening 0–3 (%)	
0	88.9
1	7.2
2	2.9
3	1
Hypoperfused 0–3 (%)	
0	33.7
1	53.7
2	9.8
3	2.8
Mesangial matrix 0–3 (%)	
0	78.2
1	13.2

Table 1. Continued

Clinical and Histopathological Characteristics (*n*=859)	Result
2	4.4
3	4.2
Mesangial cellularity 0–3 (%)	
0	83.1
1	9.4
2	4.3
3	3.1
KW nodules present (%)	3.5
Pericapsular fibrosis 0–3 (%)	
0	43.7
1	41.7
2	14.5
3	0.1
Vascular	
Arteriolar hyalinosis 0–3 (%)	
0	67.3
1	23.6
2	6.3
3	2.8
Intimal fibrosis 0–3 (%)	
0	13.7
1	41.2
2	32.4
3	12.7

IQR, interquartile range; M, male.

BMI, and proteinuria. Diabetic subjects were older (median age 66 versus 60 years, $P<0.001$), had a significantly lower median eGFR (65 versus 69 ml/min per 1.73 m^2, $P<0.001$) and the majority had HTN (88.2% versus 59.4%, $P<0.001$) (data not shown).

Histopathological abnormalities were scored semiquantitatively for 17 descriptors on formalin-fixed, paraffin-embedded, and periodic acid–Schiff-stained slides. The median (interquartile range) of combined IFTA was 5% (2%–15%). Glomerular analysis showed a median of 6.7% (2.7%–16.1%) global GS. In our collection of approximately 1000 samples, we identified 170 samples with focal GS, defined as the presence of segmental sclerosis involving <50% of total glomeruli. Assessment of blood vessels indicated that 45.1% of subjects had at least moderate (2+) intimal fibrosis.

As expected, histologic alteration reflecting chronic damage showed important relatedness. The correlation between the tubulointerstitial changes, such as interstitial fibrosis and tubular atrophy, was the highest at 0.94 (Spearman's rho). The correlation between tubular and glomerular abnormalities was also strong, at 0.66 (Spearman's rho) between IF and GS. The correlation between vascular changes (intimal fibrosis) and GS and IF were lower (rho 0.44, and 0.48, respectively). Acute injury parameters, such as tubule injury and reabsorption droplets, did not correlate with chronic changes.

Table 2. The relationship between histologic alterations and clinical and demographic characteristics

Dependent Variables	F	Adjusted r^2	Probability>F	Independent Variable	Beta	Robust SE	95% Confidence Interval		P Value
GS (%) Glomerular	54.37	0.29	<0.001						
				DM	0.306	0.079	0.151	0.462	<0.001
				HTN	0.122	0.083	−0.042	0.286	0.14
				eGFR	−0.018	0.002	−0.021	−0.014	<0.001
				Age	0.010	0.003	0.004	0.017	<0.01
				BMI	−0.007	0.005	−0.017	0.003	0.09
	Wald Chi^2	Pseudo r^2	Prob > chi^2						
KW nodules (yes/no)	24.67	0.21	0.001						
				DM	1.945	0.505	0.955	2.934	<0.001
				eGFR	−0.037	0.497	−3.507	−1.558	<0.001
Mesangial cellularity (yes/no)	68.4	0.22	<0.001						
				DM	1.693	0.341	1.025	2.361	<0.001
				Female	0.412	0.309	−0.193	1.017	0.18
				eGFR	−0.033	0.006	−0.045	−0.021	<0.001
				HTN	0.743	0.552	−0.340	1.830	0.18
Mesangial matrix (yes/no)	69.64	0.24	<0.001						
				DM	2.030	0.340	1.368	2.700	<0.001
				Female	0.403	0.292	−0.169	0.975	0.17
				eGFR	−0.033	0.005	−0.044	−0.022	<0.001
				Black race	0.528	0.340	−0.138	1.200	0.12
Glomerular wall thickening (yes/no)	29.88	0.24	<0.001						
				DM	1.460	0.442	0.592	2.324	<0.01
				eGFR	−0.050	0.010	−0.069	−0.031	<0.001
Pericapsular fibrosis (yes/no)	83.25	0.18	<0.001						
				Black race	0.745	0.252	0.015	1.238	<0.01
				DM	0.606	0.234	0.148	1.064	<0.01
				BMI	−0.023	0.018	−0.058	0.012	0.20
				eGFR	−0.037	0.005	−0.046	−0.028	<0.001
Tubulointerstitium TA (%)	58.32	0.30	<0.001						
				DM	0.314	0.079	0.159	0.469	<0.001
				Black race	0.274	0.093	0.092	0.456	<0.01
				HTN	0.197	0.085	0.029	0.364	0.02
				eGFR	−0.021	0.001	−0.024	−0.018	<0.001
				BMI	−0.012	0.005	−0.021	−0.002	0.02
Interstitial fibrosis (%)	59.15	0.30	<0.001						
				Black race	0.285	0.088	0.112	0.458	<0.01
				DM	0.230	0.078	0.077	0.383	<0.01
				HTN	0.237	0.084	0.073	0.402	<0.01
				eGFR	−0.020	0.001	−0.023	−0.017	<0.001
				BMI	−0.013	0.005	−0.023	−0.004	<0.01
	Wald Chi^2	Pseudo r^2	Prob > chi^2						
Interstitial lymphocytic infiltrate (yes/no)	113.68	0.16	<0.001						
				DM	0.615	0.200	0.223	1.001	<0.01
				HTN	0.419	0.243	−0.057	0.894	0.09
				Black race	0.301	0.216	−0.122	0.723	0.16
				eGFR	−0.033	0.004	−0.041	−0.026	<0.001
				BMI	−0.027	0.015	−0.054	0.001	0.06
Interstitial plasma cell infiltrate (yes/no)	98.34	0.13	<0.001						
				HTN	0.433	0.207	0.028	0.838	0.04
				DM	0.422	0.181	0.066	0.778	0.02
				eGFR	−0.030	0.004	−0.037	−0.023	<0.001
				BMI	−0.023	0.013	−0.048	0.002	0.07
Vessels									

Table 2. Continued

Dependent Variables	F	Adjusted r^2	Probability>F	Independent Variable	Beta	Robust SE	95% Confidence Interval		P Value
Intimal fibrosis (yes/no)	107.97	0.12	<0.001						
				HTN	0.720	0.187	0.354	1.085	<0.001
				Age	0.039	0.007	0.026	0.053	<0.001
				eGFR	−0.016	0.003	−0.022	−0.009	<0.001
				BMI	−0.015	0.011	−0.037	0.007	0.19
Arterial hyalinosis (yes/no)	31.12	0.11	<0.001						
				HTN	0.731	−0.030	1.629	0.458	0.11
				DM	0.655	0.093	1.217	0.287	0.02
				eGFR	−0.019	−0.030	−0.008	0.006	<0.001

Models are organized by dependent variables, that is, glomerular, tubulointerstitial, or vascular abnormality. Linear regression was used for continuous variables (GS, IF, and TA after log transformation) and logistic regression for binary variables. Prob, probability.

Modeling Histologic Abnormalities Using Clinical and Demographic Variables

To understand the extent clinical variables can estimate the observed histologic abnormalities in kidney biopsies, we modeled histologic abnormalities as dependent variables using stepwise linear regression models. Only samples with complete clinical and demographic information were included in the analyses ($n=750$, Supplemental Table 2).

Several clinical parameters correlated with glomerular, tubulointerstitial, and vascular histological abnormalities (Table 2). We were able to estimate GS on the basis of DM and HTN status, Black race, age, and eGFR. Mesangial matrix expansion, mesangial cellularity, and pericapsular fibrosis showed associations with eGFR, DM, and female sex, and/or Black race. KW nodules showed association with eGFR and DM status. Tubulointerstitial alterations such as IFTA and lymphocytic infiltrate were best modeled by Black race, presence of DM, HTN, eGFR, and BMI. Vascular changes, including intimal fibrosis, showed association with eGFR, age, HTN, and BMI. In general, there was a significant overlap between HTN- and DM-associated lesions except KW nodules, which were highly specific for DM. Overall, DM showed better association with glomerular lesions, whereas HTN also correlated with vascular lesions. Notably, BMI improved models describing vascular, glomerular, and/or tubular abnormalities, and age improved the estimation of GS and intimal fibrosis, indicating the important role of these clinical variables.

Although we observed a relationship between GS, IFTA (histologic damage), and kidney function, we noted that clinical variables only explained a small fraction of the variance seen by histologic analysis. We hypothesized that this could be explained by a potential nonlinear relationship. Therefore, we generated scatterplots depicting the relationship between eGFR and IFTA and GS (Figure 1A). Local linear polynomial smoothing line highlighted the nonlinear relationship between histologic changes and eGFR.

We next created eGFR sextiles of 143 subjects per group to assess the relationship of GS and IFTA at different eGFR levels (Supplemental Table 3). Figure 1B shows beta-coefficient plots normalized to the highest eGFR quantile (eGFR>91 ml/min per 1.73 m^2). At eGFR levels below 67 ml/min per 1.73 m^2, we observed a stronger association between eGFR and IF, TA and GS than for samples with for eGFR>67 ml/min per 1.73 m^2. Indeed, the Spearman correlation between IF, TA, and eGFR was 0.41 for samples eGFR<60 ml/min per 1.73 m^2 versus 0.17 for those above. The Spearman correlation between GS and eGFR was 0.37 versus 0.12 at CKD stages 3–5 versus 1–2, and between eGFR and intimal fibrosis it was 0.26 versus 0.09, respectively.

In summary, our results indicate that kidney function estimates histologic damage much better in samples with more advanced kidney disease and fairly poorly in early disease (eGFR≥60 ml/min per 1.73 m^2).

How Much IFTA and GS Is Expected at Defined eGFR Levels?

Next, we aimed to define the degree of histologic damage that characterizes each CKD stage using polynomial linear regression, which could model the observed nonlinear relationship. We generated estimates for IFTA and GS for any given eGFR using a polynomial linear regression model, adjusting for covariates previously showing significant effect, namely, age, sex, race, BMI, presence of DM, and/or HTN. Figure 2 shows the relationship between IFTA, GS, and eGFR stratified by DM and HTN (Supplemental Table 4, A–C). We found that subjects with the same eGFR, but with DM and HTN had significantly greater IFTA and GS. For example, eGFR of 60 ml/min per 1.73 m^2 was associated with 6.5% IFTA versus <4% IFTA ($P<0.001$) for subjects with or without DM and HTN, respectively. Similarly, we estimated significantly greater GS in the presence of DM and HTN than absence of DM and HTN (7.5% versus 4.6%, $P<0.001$). Although DM alone was associated with significantly greater GS at a given eGFR than absence of HTN/DM, most samples with DM also had HTN, we therefore could not untangle the DM and HTN interaction.

Our preliminary analysis also indicated an association between race and IFTA, therefore we next stratified samples

Table 3. Clinical and histopathological variables associated with kidney function decline: Clinical and histopathologic characteristics of longitudinal cohort

Clinical and Pathologic Characteristics of Follow-up Data (n=280)	Result
eGFR, mean (SD), ml/min per 1.73 m^2	65.7 (24.2)
Follow up eGFR, mean (SD), ml/min per 1.73 m^2	53.9 (24.8)
Age, mean (SD), yr	62.8 (12)
DM, n (%)	107 (38.2)
HTN n (%)	206 (73.6)
Sex, M, n (%)	168 (60)
Race, Black, n (%)	56 (20)
BMI, mean (SD), kg/m^2	31.5 (7.5)
Systolic BP, mean (SD), mm Hg	137 (22)
Diastolic BP, mean (SD), mm Hg	77 (12)
Follow up eGFR slope, median (IQR) ml/min per 1.73 m^2/yr	−4.8 (−12.2 to −1.3)
Percent eGFR slope change/yr, median (IQR)	−6.7 (−17.4 to −2.0)
Partial/total nephrectomy (%)	29.2/70.8
Tumor size, mean (SD) cm	6.2 (3.5)
Tubulointerstitial	
Acute tubular injury, median (IQR) (%)	0 (0–2)
TA, median (IQR) (%)	5 (2–10)
Tubular reabsorption 0–3 (%)	
0	81.1
1	15.7
2	2.5
3	0.7
Interstitial fibrosis, median (IQR) (%)	5 (2–10)
Interstitial eosinophils 0–3 (%)	81.1/17.9/1.1
0	81.1
1	17.9
2	1.1
3	0
Interstitial lymphocytic infiltrates 0–3 (%)	
0	27.1
1	53.2
2	16.1
3	3.6
Interstitial plasma cell infiltrates 0–3 (%)	
0	69.6
1	26.1
2	3.9
3	0.4
Glomerular	
Segmental sclerosis, median (IQR) (range) (%)	0 (0)
GS, median (IQR) (%)	5.8 (2.8–12.7)
Wall thickening 0–3 (%)	
0	91.4
1	5
2	3.2
3	0.4
Hypoperfused 0–3 (%)	
0	34.3
1	56.4
2	8.6
3	0.7
Mesangial matrix 0–3 (%)	
0	77.7
1	13.3
2	4.5
3	4.4

Table 3. Continued

Clinical and Pathologic Characteristics of Follow-up Data (n=280)	Result
Mesangial cellularity 0–3 (%)	
0	82.5
1	11.8
2	3.2
3	2.5
KW nodules present (%)	2.9
Pericapsular fibrosis 0–3 (%)	
0	42.1
1	46.8
2	11.1
3	0
Vascular	
Arteriolar hyalinosis 0–3 (%)	
0	69.6
1	24.6
2	3.9
3	1.8
Intimal fibrosis 0–3 (%)	
0	12.5
1	45.7
2	35.7
3	6.1

IQR, interquartile range; M, male.

by race (Figure 3). We found that compared with non-Black race, Black race alone was associated with more severe IFTA ($P<0.05$) but not GS, especially at eGFR values ≤30 ml/min per 1.73 m^2 (Supplemental Table 5, A–C). In our study, Black subjects were younger, had lower prevalence of DM, but had significantly higher systolic and diastolic BP and proteinuria on urine dipstick at the time of nephrectomy (Supplemental Table 6). Re-estimation of the eGFR without using the race coefficient showed no statistically significant differences in the degree of IFTA in subjects with Black race compared with non-Black race (Supplemental Table 7). Hence, the differences observed between Black and non-Black participants were eliminated by the removal of race from the eGFR equation.

In summary, we developed estimates for the degree of IFTA and GS for any given eGFR and CKD stage. We found that for the observed eGFR, DM, HTN and Black race were associated with more severe GS and IFTA.

Histology-Based Unbiased Clustering Analysis Highlights Heterogeneity in Structure at Early Disease Stages

To better understand structural heterogeneity in samples with an eGFR≥60 ml/min per 1.73 m^2 (Supplemental Table 8) we performed unbiased cluster analysis, on the basis of histologic descriptors. Our unbiased hierarchical clustering identified three distinct clusters (Figure 4). One group (cluster 2) had a notably greater degree of IFTA, 19% versus 3% (cluster 1) or 6% (cluster 3), which was associated with a lower eGFR (78 versus 84 and 82 ml/min per 1.73 m^2).
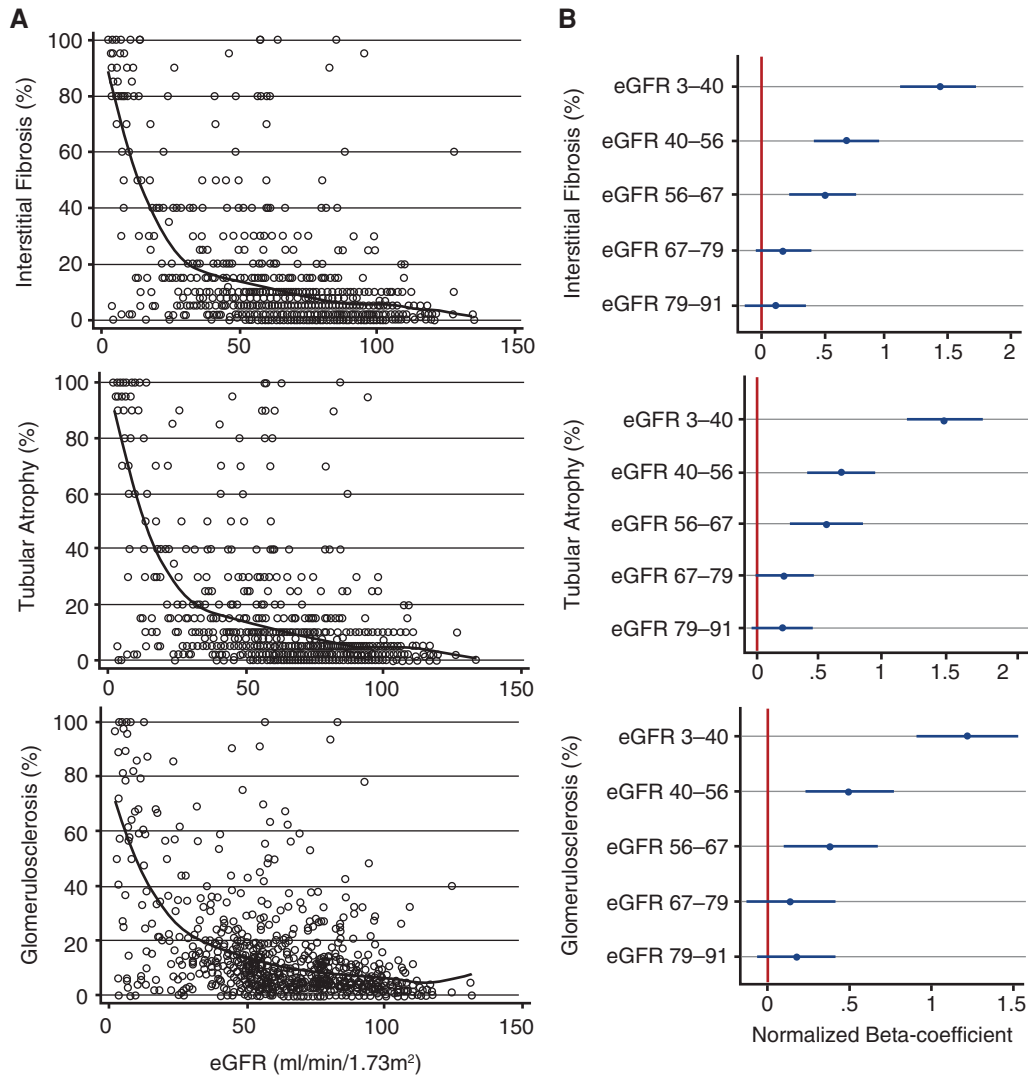
Figure 1. IF, TA, and GS have a nonlinear relationship with eGFR. (A) Local two-degree polynomial smoothing for IF, TA, and GS (black line) overlay on scatterplots of IF, TA, or GS versus eGFR (open circles). (B) Beta-coefficients (with 95% confidence intervals [95% CI]) of six eGFR quantiles in regression models normalized to the highest eGFR sextile (eGFR 91–135 ml/min per 1.73 m^2). Models were adjusted for age, sex, race, diabetes, HTN, BMI, and systolic BP.

Clusters 1 and 3, however, showed no significant differences in eGFR (84±16 and 82±15 ml/min per 1.73 m^2, $P>0.05$). Despite no clear differences in kidney function, cluster 3 had significantly greater IF, 6.4 versus 2.6%, and GS, 7.6 versus 4.5% ($P<0.001$) than cluster 1 samples. We observed no differences in age, sex, race, BMI, or BP. We noted the increased incidence of DM and HTN in cluster 3 with more severe IFTA and GS. Using linear mixed modeling, we also assessed whether histopathology-based clusters were associated with eGFR decline over time. Cluster 2, which had significantly lower eGFR and higher severity of IFTA and GS, was also associated with eGFR decline ($P=0.01$). Importantly, we found that cluster 3 was also associated with significantly faster eGFR decline over time

compared with cluster 1 ($P=0.02$, Figure 4C and Supplemental Table 9), despite no significant difference in baseline eGFR.

In summary, our unbiased histology-based clustering indicates that some samples, especially those with DM and HTN, show severe IFTA and GS on histologic analysis, despite preserved eGFR.

Histologic Alterations Improve Kidney Function Decline Estimation

Next, we aimed to understand whether histologic abnormalities such as IFTA and GS provides complementary information to eGFR and improve kidney function decline estimation in our cohort.

Figure 2. The relationship of histologic alterations and kidney function stratified by HTN and diabetes. (A–C) Adjusted means for percent IF, TA, and GS stratified by absence of HTN and DM (*n*=193, navy lines±shaded 95% CI and navy bars), HTN alone (*n*=290, light blue lines±shaded 95% CI and light blue bars), and presence of HTN and DM (*n*=238, orange lines±shaded 95% CI shaded and orange bars). Percentages of IF, TA, and GS modeled using linear regression as a cubic function of eGFR^3 adjusted for age, sex, race, HTN, diabetes, and BMI. Asterisk denotes $P<0.05$ compared with the "No HTN or DM" group, **$P<0.01$, * <0.05.

We analyzed the relationship between renal structural findings and the rate of decline of eGFR in a cohort of participants with a minimum of 3 months of follow-up data (Table 3). Our longitudinal cohort had a baseline eGFR of 66 (SD±24) ml/min per 1.73 m^2 and a mean follow-up eGFR of 54 (SD±25) ml/min per 1.73 m^2 after a median follow-up time of 3 years (interquartile range, 1.1–5.6 years).

To determine whether histopathological variables improve the precision of the rate of kidney function decline, we used linear mixed models (Supplemental Table 10). We found that GS, age, baseline eGFR, and radical (versus partial) nephrectomy predicted longitudinal eGFR (Table 4). Inclusion of GS into the model significantly

improved the model fitness (likelihood ratio test <0.01 and Akaike Information Criterion 3898 versus 3913).

Clinical parameters are poor estimates of kidney function decline in patients with eGFR ≥60 ml/min per 1.73 m^2,[30,31] therefore we reanalyzed this subgroup in our cohort. We found that even in subjects with eGFR≥60 ml/min per 1.73 m^2, GS significantly improved modeling eGFR changes over time ($P<0.01$, Table 5).

Overall, we found that baseline eGFR is an important predictors of kidney function decline, however the inclusion of histologic variables (GS) improved kidney function decline estimation. The degree of GS improved kidney function decline estimation even in subjects with eGFR≥60 ml/min per 1.73 m^2.
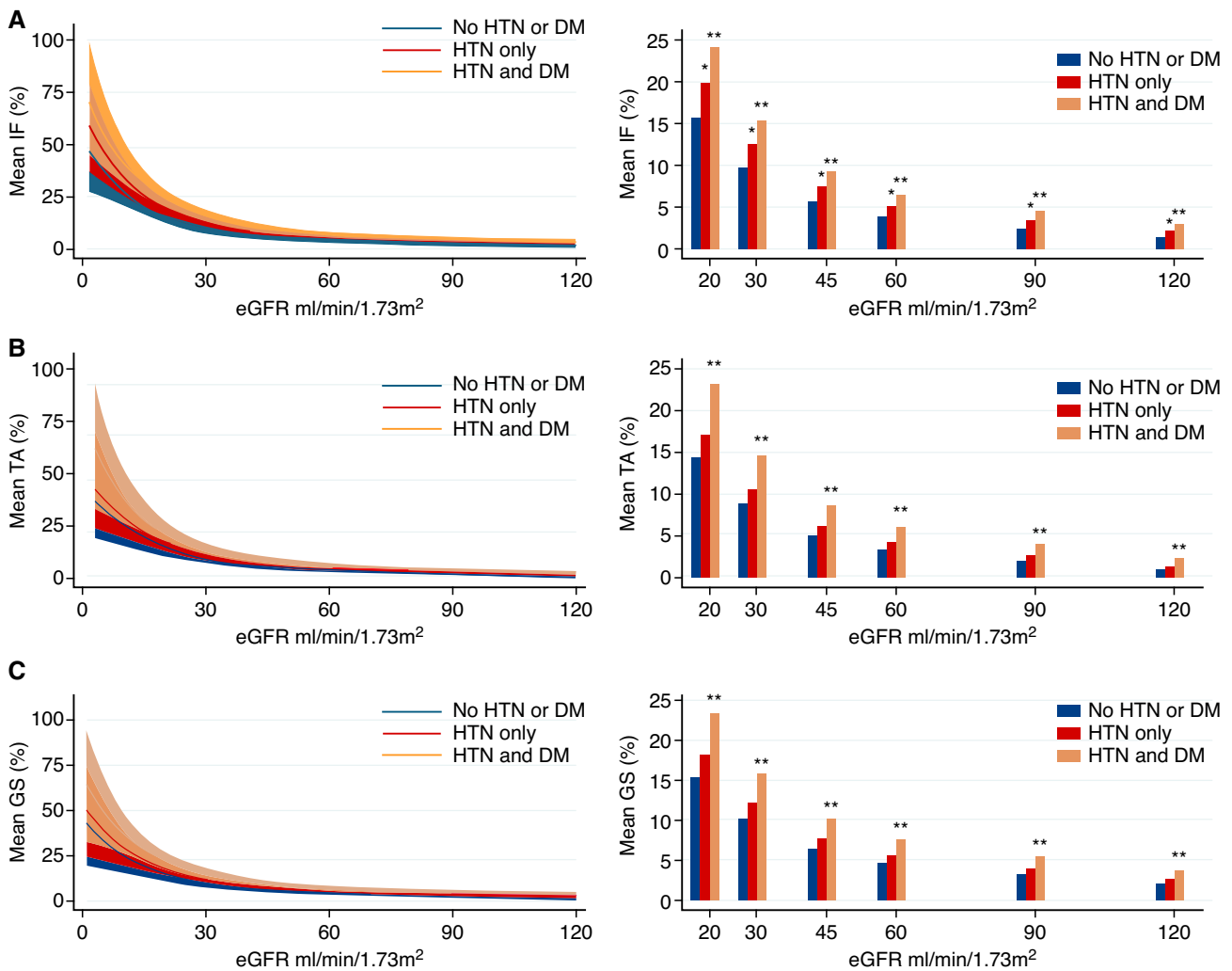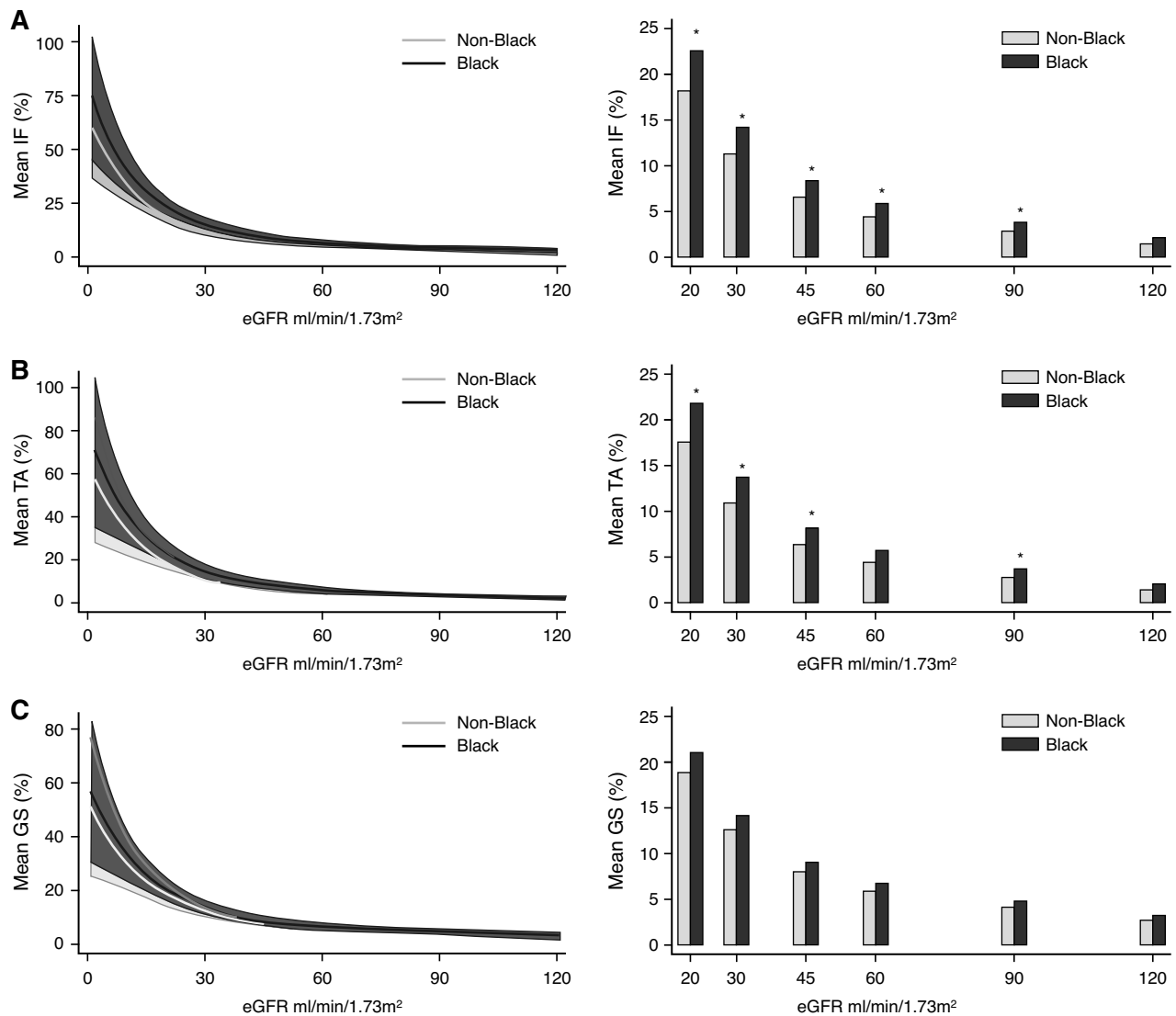
Figure 3. The relationship of histologic alterations and kidney function is altered by race. (A–C) Adjusted means for percent IF, TA, and GS stratified by non-Black race (n=590, gray lines±shaded 95% CI and light gray bars) and Black race (n=160, black lines±shaded 95% CI and black bars). Percentages of IF, TA, and GS modeled using linear regression as a cubic function of eGFR[3] adjusted for age, sex, race, HTN, diabetes, and BMI. *P<0.05 compared with the non-Black race group.

DISCUSSION

In this study, we examined the relationship between functional and structural changes observed in patients with DM, HTN, and varying degree of kidney dysfunction. Given the small but significant risk associated with a kidney biopsy, it is important to understand whether clinical parameters can estimate histologic alterations or histologic alterations provide complementary information. We found that eGFR and clinical parameters estimate IFTA and GS reasonably well in patients with more advanced CKD (stage 3–5). We found that eGFR underestimates the degree of GS and IFTA in patients with DM, HTN, and Black race. Our analysis emphasizes that eGFR alone is an imperfect measure of histologic changes in early CKD stages, given some subjects showed fairly significant damage despite preserved eGFR. Importantly, we found that histologic damage assessment improves kidney function decline estimation, even in subjects with relatively preserved kidney function.

Previous studies assessing the correlation between kidney function and histologic abnormalities have analyzed clinically indicated biopsies with mixed glomerular diseases,[10–12,32] and relied on histologic information from clinical charts. These studies could be biased by practitioners performing a biopsy when suspecting a specific glomerular disease. In addition, histologic reading of biopsies by different pathologists shows modest correlation. In this study, we developed a large,

A

B

C

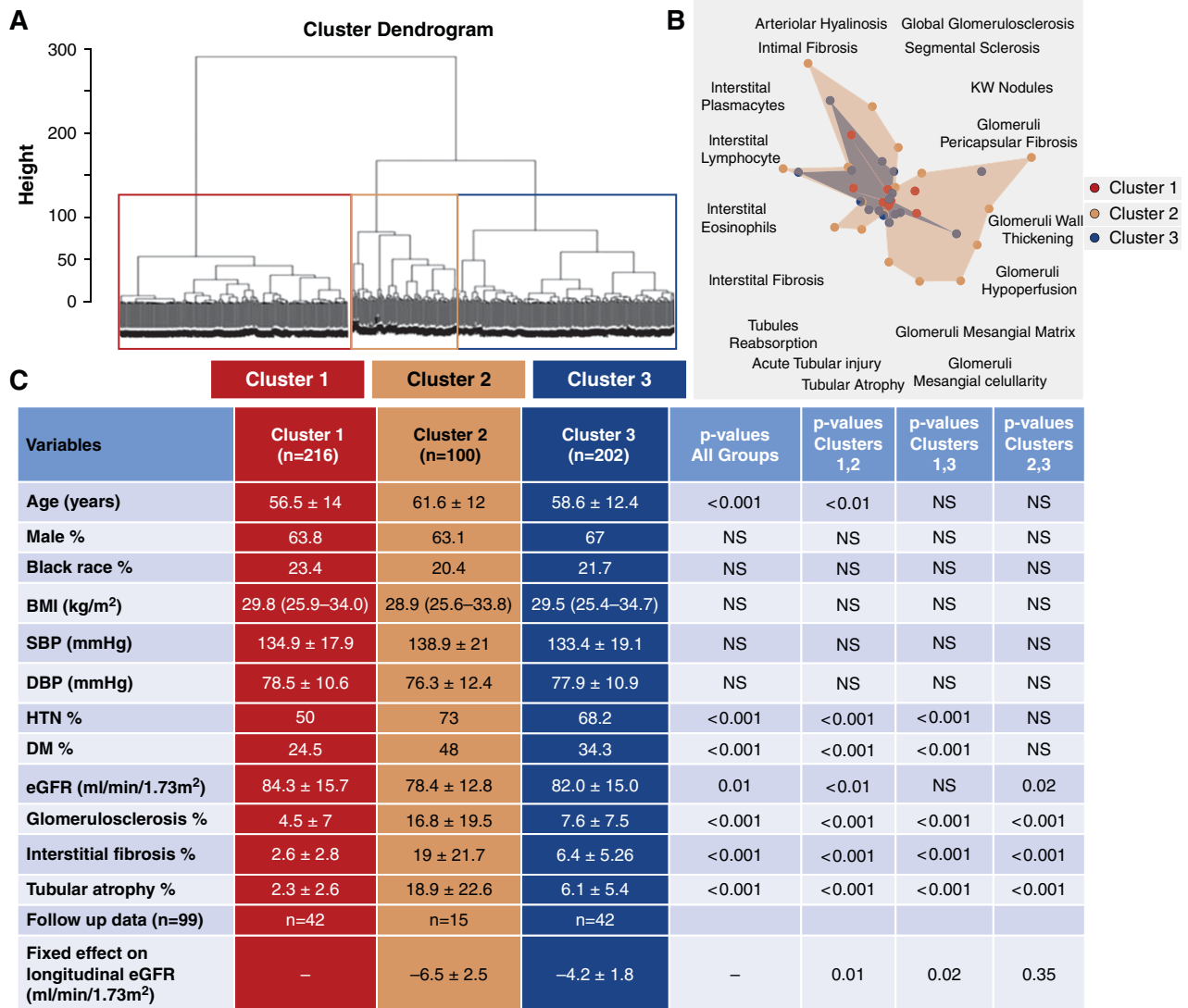| Variables	Cluster 1 (n=216)	Cluster 2 (n=100)	Cluster 3 (n=202)	p-values All Groups	p-values Clusters 1,2	p-values Clusters 1,3	p-values Clusters 2,3
Age (years)	56.5 ± 14	61.6 ± 12	58.6 ± 12.4	<0.001	<0.01	NS	NS
Male %	63.8	63.1	67	NS	NS	NS	NS
Black race %	23.4	20.4	21.7	NS	NS	NS	NS
BMI (kg/m^2)	29.8 (25.9–34.0)	28.9 (25.6–33.8)	29.5 (25.4–34.7)	NS	NS	NS	NS
SBP (mmHg)	134.9 ± 17.9	138.9 ± 21	133.4 ± 19.1	NS	NS	NS	NS
DBP (mmHg)	78.5 ± 10.6	76.3 ± 12.4	77.9 ± 10.9	NS	NS	NS	NS
HTN %	50	73	68.2	<0.001	<0.001	<0.001	NS
DM %	24.5	48	34.3	<0.001	<0.001	<0.001	NS
eGFR (ml/min/1.73m^2)	84.3 ± 15.7	78.4 ± 12.8	82.0 ± 15.0	0.01	<0.01	NS	0.02
Glomerulosclerosis %	4.5 ± 7	16.8 ± 19.5	7.6 ± 7.5	<0.001	<0.001	<0.001	<0.001
Interstitial fibrosis %	2.6 ± 2.8	19 ± 21.7	6.4 ± 5.26	<0.001	<0.001	<0.001	<0.001
Tubular atrophy %	2.3 ± 2.6	18.9 ± 22.6	6.1 ± 5.4	<0.001	<0.001	<0.001	<0.001
Follow up data (n=99)	n=42	n=15	n=42				
Fixed effect on longitudinal eGFR (ml/min/1.73m^2)	–	−6.5 ± 2.5	−4.2 ± 1.8	–	0.01	0.02	0.35

Figure 4. Histopathology-based clustering of subjects with eGFR≥60 ml/min per 1.73 m^2. (A) Hierarchical clustering dendrogram shows three distinct clusters. (B) Radar plot of histopathologic characteristics in cluster 1 (red), cluster 2 (orange), and cluster 3 (blue). (C) Clinical and select histopathologic characteristics of histopathology-based clusters. Clusters with follow-up data are indicated, and associations with longitudinal eGFR are assessed using linear mixed model. Complete fixed effects used in the model include: cluster, age, sex, race (as Black or non-Black), presence of diabetes or HTN, BMI (kg/m^2), nephrectomy status (total versus partial), tumor size (cm), and length of follow-up (years). Random effects include subject-specific eGFR slopes obtained by regressing an average of three eGFR values on the time variable. SBP, systolic BP; DBP, diastolic BP.

multicenter biobank of human kidney tissue samples derived from nephrectomies that included samples with a wide distribution of age, sex, eGFR, race, high prevalence of HTN (69%), and DM (35%) and a subset of follow-up data over 3 years. Our collection is highly valuable because little information is available for subjects with HTN and DM and preserved kidney function. We were able score relatively large kidney specimens in a standardized manner.

We found a nonlinear relationship between histopathologic variables and eGFR. In our dataset, the relationship between eGFR and IFTA and GS was weak, at eGFR≥60 ml/min per 1.73 m^2. Previous studies assessing the relationship between renal structure and function were biased toward samples with advanced CKD, where the relationship between IFTA and GS is much stronger. There are several potential implications of the observed nonlinear relationship. First, eGFR as a functional measurement shows important, sometimes, rapid variability (*e.g.*, volume-depleted state). Fibrosis may not be a limiting or key determinant of kidney function variability at high eGFR values, but seems an important component for more advanced disease. Another important limitation is that creatinine-based

Table 4. Clinical and histopathological variables associated with kidney function decline: Variables (fixed effects) associated with eGFR decline in linear mixed model

Longitudinal eGFR (n=167)	Coefficient	SE	z	P>|z|	95% Confidence Interval	
Age (per 1 year older)	−0.25	0.06	−4.21	<0.001	−0.36	−0.13
eGFR baseline (per 1 ml/min per 1.73 m^2)	0.74	0.03	25.18	<0.001	0.68	0.80
GS (per 1% difference)	−0.25	0.06	−4.35	<0.001	−0.36	−0.14
Radical nephrectomy (yes/no)	−4.32	1.40	−3.10	<0.01	−7.05	−1.58
Tumor size (per 1 cm difference)	−0.01	0.20	−0.03	0.98	−0.39	0.38
Sex, F (yes/no)	−1.68	1.34	−1.26	0.21	−4.30	0.94
Race, Black (yes/no)	−2.64	1.96	−1.35	0.18	−6.49	1.20
BMI (per 1 kg/m^2)	−0.04	0.08	0.47	0.66	−0.20	0.13
Diabetes (yes/no)	0.59	1.26	0.52	0.64	−1.87	3.06
HTN (yes/no)	−0.43	1.48	−0.29	0.77	−3.33	2.48
Length of follow-up time (per 1 year difference)	−0.02	0.12	−0.14	0.89	−0.26	0.22

Variables meeting significance (P<0.05) shown in bold. Coefficients represent fixed effects obtained in linear mixed model using subject-specific eGFR slopes obtained by regressing repeated eGFR values on the time variable (random effects). F, female.

eGFR estimations are less accurate at high eGFR ranges, and we cannot exclude the role of such imprecision in kidney function estimation. Second, our analyses emphasize that the presence of DM and HTN markedly alters the relationship between eGFR and fibrosis, such that the degree of IFTA and GS was approximately 7%–15% in subjects with DM and HTN versus <10% without DM/HTN at CKD stage 3 (eGFR 30–59 ml/min per 1.73 m^2). The simplest interpretation is that underlying hyperfiltration plays a role in determining eGFR in patients with DM.[2] It is important to note that two of the most commonly used drug classes, inhibitors of angiotensin converting enzyme inhibitor, angiotensin receptor blocker, or sodium glucose cotransporter inhibitors, initially reduce the eGFR,[33] which is interpreted as an important renoprotective mechanism of these commonly used drugs.

We observed a significantly greater degree of IFTA in the Black population at most eGFR levels, compared with the non-Black population. Interestingly, the Black population in our study was younger but without significant differences in the diagnosis of DM or HTN. BP at the time of nephrectomy were significantly higher in Black individuals compared with non Black individuals. The difference between Black and non-Black participants was eliminated by removing race from the eGFR equation. Our data are relevant to the controversy surrounding the use of race in estimations of kidney disease.[34,35]

Our study has several limitations. Most importantly, because samples were obtained from nephrectomies, we cannot exclude the role of tumor-associated sclerosis or immune cell infiltration. Even if the cause of IFTA and GS was potentially tumor associated, it would not invalidate our results because our study was not aimed at understanding the cause of IFTA and GS, simply its association with eGFR. The relative percentages of IFTA and GS are internally consistent, but absolute estimations will need external validation. In addition, we relied on electronic medical records to provide information on disease status and laboratory values. Consequently, we were only able to evaluate longitudinal follow-up data in a subset of patients and could not control for differences in AKI incidence or medication use. Another important limitation is the dearth of information on glycemic control and albuminuria, an important diagnostic component of CKD estimation, but often not analyzed in the clinical setting. Our study is also limited by lack of genetic data, including APOL1 genotype information.[36]

In the two-fold definition of CKD, as both a functional and structural entity, this study emphasizes the importance of the presently underappreciated structural component.

Table 5. Clinical and histopathological variables associated with kidney function decline: Variables (fixed effects) associated with eGFR decline in linear mixed model in subjects with eGFR≥60 at time of nephrectomy

Longitudinal eGFR, if Baseline eGFR>60 (n=99)	Coefficient	SE	z	P>|z|	95% Confidence Interval	
Age (per 1 year older)	−0.25	0.09	−2.88	<0.01	−0.41	−0.08
eGFR baseline (per 1 ml/min per 1.73 m^2)	0.75	0.06	12.45	<0.001	0.63	0.87
GS (per 1% difference)	−0.28	0.09	−2.94	<0.01	−0.46	-0.09
Radical nephrectomy (yes/no)	−6.19	1.89	−3.27	<0.01	−9.90	−2.48
Tumor size (per 1 cm)	0.38	0.26	1.47	0.14	−0.13	0.89
Sex, F (yes/no)	−2.25	1.84	−1.22	0.22	−5.85	1.35
Race, Black (yes/no)	−2.88	2.70	−1.07	0.29	−8.17	2.40
BMI (per 1 kg/m^2)	0.05	0.11	0.43	0.67	−0.17	0.26
Diabetes (yes/no)	0.65	1.75	0.37	0.71	−2.78	4.08
HTN (yes/no)	−0.40	1.89	−0.21	0.83	−4.10	3.30
Length of follow up time (per 1 year difference)	−0.04	0.16	−0.25	0.80	−0.36	0.28

F, female.

The study underscores that subjects could have marked histologic damage, despite the preserved eGFR, especially those with DM, HTN, and Black race. Given the important role of fibrosis in predicting kidney function decline, our results indicate the importance of obtaining kidney biopsies on these patients.

In summary, we present a comprehensive analysis defining the relationship between kidney function and structural abnormalities in subjects with and without DM and HTN at different eGFRs, including subjects with preserved kidney function. We observed that the relationship between structural damage and kidney function is weak at earlier stages of CKD but strengthens at later CKD stages. Our study highlights that in the setting of DM, HTN, or Black race, the degree of structural damage is more severe than estimation on the basis of eGFR would indicate. Our results suggest that histologic analysis is an important complementary method for kidney disease evaluation, especially at early disease stages, in patients with DM, HTN, and Black race, because some subjects present with relatively severe structural damage despite preserved eGFR.

SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2021010044/-/DCSupplemental.

Supplemental Table 1. Histopathological variables and definitions.

Supplemental Table 2. Clinical and histopathological characteristics of samples with complete data used in the regression analyses.

Supplemental Table 3. Clinical and select histopathological variables by eGFR sextiles.

Supplemental Tables 4A–C. The degree histopathological damage stratified by presence or absence of HTN and diabetes.

Supplemental Table 5A–C. The degree histopathological damage stratified and by race (Black versus non-Black).

Supplemental Table 6A and B. Clinical and histopathological characteristics of subjects according to race.

Supplemental Table 7A and B. The degree IF and TA stratified by race (Black versus non-Black) without use of race coefficient in estimation of GFR.

Supplemental Table 8A and B. Clinical and histopathological characteristics of samples with estimated glomerular filtration rate ≥ 60 and <60 ml/min per 1.73 m^2.

Supplemental Table 9. Predictors of eGFR decline using linear mixed model of longitudinal eGFR in subjects with eGFR≥ 60 at time of nephrectomy including unbiased histopathology-based clusters.

Supplemental Table 10. Clinical and histopathological characteristics of follow-up samples with complete data used in linear mixed model analyses.

Supplemental Figure 1. Study flow chart and inclusion/exclusion criteria.

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Supplemental Material:

Title: Renal histological analysis provides complementary information to kidney function measurement for patients with early diabetic or hypertensive disease

Running title: Histology-based CKD staging

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Table of Contents

Supplemental Table 1. Histopathologic variables and definitions

Histopathologic Descriptor	Definition
Tubulointerstitial	
Acute Tubular Injury (%)	Tubular epithelial flattening, brush border loss, sloughing, or vacuolar change. Estimated as % of total tubules involved [1]
Tubular atrophy (%)	Small tubules with thick tubular basement membranes lined by small cuboidal or flat cells. Generally accompanied by fibrosis. Includes "thyroidization." Estimated as % of total tubules involved [2]
Tubular Reabsorption droplets	PAS-positive, prominent hyaline droplets in tubular cytoplasm; unifocal (1), multifocal (2), diffuse (3)
Interstitial Fibrosis (%)	Interstitium expanded by collagenous extracellular matrix, scored as estimated % area of sampled cortex
Interstitial Eosinophils	Presence of interstitial eosinophils; unifocal (1), multifocal (2), diffuse (3)
Interstitial Lymphocytes	Presence of interstitial lymphocytes; unifocal (1), multifocal (2), diffuse (3)
Interstitial Plasma cells	Presence of interstitial plasma cells; unifocal (1), multifocal (2), diffuse (3)
Glomerular	
Segmental glomerulosclerosis	Segmental solidification of the glomerular tuft, scored as manually counted % glomeruli involved [2]
Global glomerulosclerosis	Solidification of 100% of the glomerular tuft, scored as manually counted % glomeruli involved [2]
Capillary wall thickening	Judgement of peripheral capillary wall thickening, scored as mild (1), moderate (2), severe (3)
Hypoperfused tuft	global wrinkling and folding of the GBM, also known as ischemic type collapse [3]
Mesangial Matrix Expansion	Expansion of mesangial matrix beyond width of two mesangial nuclei; mild (1), moderate (2), severe (3) [4]
Mesangial Cellularity	Number of mesangial cells in peripheral mesangial areas 4-5 (1), 6-7 (2), >7 (3) [4]
KW Nodules	Round mesangial sclerosing lesions with hypocellular center [5]
Pericapsular Fibrosis	Circumferential expansion of Bowman's capsule by collagenous matrix; focal (1), multifocal (2), diffuse (3)
Vascular	
Arteriolar Hyalinosis	Hyaline eosinophilic material deposition within arteriolar wall; unifocal (1), multifocal (2), diffuse (3)
Intimal Fibrosis	Expansion of vascular wall intima by collagenous material; <50% thickness of media (1), 50-100% thickness of media (2), > 100% thickness of media (3)

KW: Kimmelstiel-Wilson

Supplemental Table 2. Clinical and histopathological characteristics of samples with complete data used in regression analyses.

Clinical and Histopathological Characteristics (n=750)		
eGFR, mean (SD) ml/min/1.73m^2		65 (26.6)
Age, mean (SD), years		61.5 (13.1)
Diabetes mellitus n (%)		267 (35.6)
Hypertension, n (%)		465 (70.4)
Male sex, n (%)		481 (64.1)
Black race, n (%)		160 (21.3)
Body mass index, mean (IQR) kg/m^2		29.5 (25.8-34.4)
Tubulointerstitial		
Acute Tubular Injury, median (IQR) (%)		0 (0-2)
Tubular atrophy, median (IQR) (%)		5 (2-10)
Tubular Reabsorption 0-3 (%)		
	0	80.3
	1	16.9
	2	2.3
	3	0.5
Interstitial Fibrosis, median (IQR) (%)		5 (2-15)
Interstitial Eosinophils 0-3 (%)		
	0	78.4
	1	20.3
	2	1.1
	3	0.3
Interstitial Lymphocytic infiltrates 0-3 (%)		
	0	23.9
	1	49.5
	2	19.2
	3	6.4
Interstitial Plasmocytic infiltrates 0-3 (%)		
	0	65.6
	1	28.1
	2	5.2
	3	1.1

Glomerular		
Segmental Sclerosis, median (IQR) (range) (%)		0 (0)
Glomerulosclerosis, median (IQR) (%)		5.8 (2.8-12.7)
Wall Thickening 0-3 (%)		
	0	91.4
	1	5
	2	3.2
	3	0.4
Hypoperfused 0-3 (%)		
	0	34.3
	1	56.4
	2	8.6
	3	0.7
Mesangial Matrix 0-3 (%)		
	0	77.7
	1	13.3
	2	4.5
	3	4.4
Mesangial Cellularity 0-3 (%)		
	0	82.5
	1	11.8
	2	3.2
	3	2.5
Kimmelstiel-WIlson Nodules present (%)		2.9
Pericapsular Fibrosis 0-3 (%)		
	0	42.1
	1	46.8
	2	11.1
	3	0
Vascular		
Arteriolar Hyalinosis 0-3 (%)		
	0	69.6
	1	24.6
	2	3.9
	3	1.8
Intimal Fibrosis 0-3 (%)		
	0	12.5
	1	45.7
	2	35.7
	3	6.1

eGFR, estimated glomerular filtration rate; IQR, interquartile range

Supplemental Table 3. Clinical and select histological variables by eGFR sextiles

Clinical or Pathologic Variable	Sextile 1: eGFR 3-40 (n=144)	Sextile 2: eGFR 40-56 (n=143)	Sextile 3: eGFR 56-67 (n=143)	Sextile 4: eGFR 67-79 (n=143)	Sextile 5: eGFR 79-91 (n=143)	Sextile 6: eGFR 9-135 (n=143)	p-value
eGFR, median (IQR), ml/min/1.73m^2	23 (10-33)	50 (46-54)	61 (59-65)	73 (70-75)	65 (81-88)	99 (95-108)	<0.001
Age, mean (SD), years	64 (13)	68 (10)	64 (11)	60 (13)	60 (13)	52 (12)	<0.001
Hypertension, n (%)	120 (83.3)	113 (79.0)	104 (72.7)	94 (65.7)	85 (59.4)	79 (55.2)	<0.001
Diabetes, n (%)	71 (49.3)	54 (37.7)	42 (29.4)	51 (35.7)	47 (32.9)	39 (27.3)	0.001
Male sex, n (%)	93 (64.6)	85 (59.4)	92 (64.3)	93 (65.0)	100 (69.9)	85 (59.4)	0.437
Black race, n (%)	40 (27.8)	22 (15.4)	27 (18.9)	30 (20.9)	24 (16.8)	33 (23.1)	0.154
Body mass index, median (IQR), kg/m^2	29.3 (25.5-34.3)	29.6 (26.2-34.8)	29.3 (26.1-33.7)	29.5 (25.6-33.8)	29.3 (26.2-33.8)	29.8 (24.1-35.7)	0.993
Systolic BP, median (IQR), mmHg	139 (125-151)	138 (124-15]	137 (124-148)	134 (122-149)	134 (123-150)	130 (120-143)	0.079
Diastolic BP, median (IQR), mmHg	75 (68-86)	75 (67-85)	77 (69-84)	77 (71-86)	78 (70-82)	78 (71-82)	0.573
Interstitial Fibrosis, median (IQR) (%)	25 (9-75)	8 (5-15)	5 (2-15)	5 (2-10)	5 (2-8)	2 (2-5)	<0.001
Tubular atrophy, median (IQR) (%)	23 (9-70)	7 (5-15)	5 (2-15)	5 (2-8)	5 (2-5)	2 (1-5)	<0.001
Glomerulosclerosis, median (IQR) (%)	22 (10-57)	10 (5-18)	7 (3-16)	4 (2-10)	5 (2-11)	3 (1-8)	<0.001

eGFR, estimated glomerular filtration rate; interquartile range, IQR; BP, blood pressure

Supplemental Table 4A-C. The degree of histopathological damage stratified by presence or absence of hypertension and diabetes.

Adjusted means of **A**, interstitial fibrosis (IF), **B,** tubular atrophy (TA) and **C**, glomerulosclerosis (GS) stratified by presence or absence of hypertension and/or diabetes. Multivariable model adjusted for age, sex, race, body mass index, hypertension, and diabetes. HTN and DM (n=193), HTN alone (n=290), HTN and DM (n=238), DM only (n=29).

A

eGFR	Clinical status	Adjusted Mean IF margin (%)	SE	z	p>|z|	95% CI		p-value vs. No HTN/DM
15	No HTN or DM	20.45	3.04	6.72	<0.001	14.49	26.42	-
	HTN only	25.91	3.35	7.73	<0.001	19.35	32.48	0.0190
	HTN and DM	31.46	3.91	8.04	<0.001	23.79	39.13	0.0001
	DM only	25.06	5.45	4.60	<0.001	14.37	35.74	0.3572
20	No HTN or DM	15.60	2.00	7.79	<0.001	11.68	19.53	
	HTN only	19.86	2.17	9.15	<0.001	15.60	24.12	0.0187
	HTN and DM	24.19	2.56	9.44	<0.001	19.16	29.21	0.0001
	DM only	19.19	4.05	4.74	<0.001	11.25	27.14	0.3580
30	No HTN or DM	9.76	1.11	8.76	<0.001	7.57	11.94	
	HTN only	12.57	1.22	10.29	<0.001	10.17	14.96	0.0193
	HTN and DM	15.42	1.50	10.25	<0.001	12.47	18.37	0.0001
	DM only	12.13	2.61	4.64	<0.001	7.00	17.25	0.3599
45	No HTN or DM	5.65	0.66	8.56	<0.001	4.36	6.94	
	HTN only	7.44	0.73	10.18	<0.001	6.01	8.87	0.0196
	HTN and DM	9.26	0.94	9.81	<0.001	7.41	11.11	0.0001
	DM only	7.16	1.66	4.31	<0.001	3.90	10.42	0.3610
60	No HTN or DM	3.84	0.45	8.58	<0.001	2.96	4.72	
	HTN only	5.18	0.46	11.27	<0.001	4.28	6.09	0.0183
	HTN and DM	6.55	0.62	10.50	<0.001	5.33	7.77	0.0001
	DM only	4.97	1.21	4.11	<0.001	2.60	7.34	0.3600
90	No HTN or DM	2.44	0.35	6.92	<0.001	1.75	3.13	
	HTN only	3.43	0.34	10.00	<0.001	2.76	4.11	0.0173
	HTN and DM	4.44	0.45	9.84	<0.001	3.56	5.33	0.0001
	DM only	3.28	0.87	3.75	<0.001	1.57	4.99	0.3572
120	No HTN or DM	1.46	0.43	3.40	0.001	0.62	2.30	
	HTN only	2.21	0.55	4.05	<0.001	1.14	3.28	0.0285
	HTN and DM	2.97	0.67	4.47	<0.001	1.67	4.28	0.0007
	DM only	2.09	0.79	2.64	0.008	0.54	3.64	0.3620

eGFR, estimated glomerular filtration rate; IF, interstitial fibrosis, HTN, hypertension; DM, diabetes

Supplemental Table 4 cont.

B

eGFR	Clinical status	Adjusted Mean TA (%)	SE	z	p>|z|	95% CI		p-value vs. No HTN/DM
15	No HTN or DM	20.16	3.24	6.23	<0.001	13.82	26.50	-
	HTN only	23.79	3.44	6.91	<0.001	17.04	30.54	0.1166
	HTN and DM	32.00	4.15	7.71	<0.001	23.86	40.14	<0.0001
	DM only	24.40	5.28	4.63	<0.001	14.06	34.74	0.3638
20	No HTN or DM	15.25	2.07	7.36	<0.001	11.19	19.31	
	HTN only	18.06	2.18	8.28	<0.001	13.78	22.33	0.1162
	HTN and DM	24.42	2.62	9.31	<0.001	19.28	29.56	<0.0001
	DM only	18.54	3.82	4.85	<0.001	11.05	26.02	0.3646
30	No HTN or DM	9.42	1.11	8.48	<0.001	7.24	11.59	
	HTN only	11.25	1.18	9.54	<0.001	8.94	13.56	0.1174
	HTN and DM	15.41	1.50	10.26	<0.001	12.47	18.36	0.0001
	DM only	11.56	2.41	4.81	<0.001	6.85	16.28	0.3665
45	No HTN or DM	5.39	0.65	8.26	<0.001	4.11	6.67	
	HTN only	6.56	0.70	9.40	<0.001	5.19	7.92	0.1178
	HTN and DM	9.20	0.96	9.55	<0.001	7.31	11.08	0.0001
	DM only	6.75	1.52	4.43	<0.001	3.77	9.74	0.3678
60	No HTN or DM	3.64	0.44	8.25	<0.001	2.78	4.50	
	HTN only	4.51	0.44	10.28	<0.001	3.65	5.38	0.1154
	HTN and DM	6.49	0.63	10.23	<0.001	5.25	7.74	<0.0001
	DM only	4.66	1.11	4.22	<0.001	2.50	6.83	0.3669
90	No HTN or DM	2.23	0.34	6.45	<0.001	1.55	2.90	
	HTN only	2.86	0.32	8.83	<0.001	2.23	3.50	0.1130
	HTN and DM	4.31	0.44	9.74	<0.001	3.44	5.18	<0.0001
	DM only	2.97	0.78	3.79	<0.002	1.44	4.51	0.3637
120	No HTN or DM	1.06	0.41	2.58	0.010	0.25	1.87	
	HTN only	1.50	0.49	3.06	0.002	0.54	2.47	0.1325
	HTN and DM	2.51	0.65	3.85	<0.001	1.23	3.79	0.0005
	DM only	1.58	0.69	2.30	0.021	0.24	2.92	0.3683

eGFR, estimated glomerular filtration rate; TA, tubular atrophy, HTN, hypertension; DM, diabetes

Supplemental Table 4 cont.

C

eGFR	Clinical status	Mean Adjusted GS (%)	SE	z	p>|z|	95% CI		p-value vs. No HTN/DM
15	No HTN or DM	19.50	3.05	6.39	<0.001	13.52	25.48	
	HTN only	22.98	3.11	7.40	<0.001	16.89	29.07	0.1011
	HTN and DM	29.35	3.55	8.28	<0.001	22.40	36.30	0.0001
	DM only	31.41	6.30	4.99	<0.001	19.07	43.75	0.0310
20	No HTN or DM	15.36	2.09	7.33	<0.001	11.25	19.46	
	HTN only	18.15	2.09	8.70	<0.001	14.06	22.24	0.1012
	HTN and DM	23.27	2.36	9.86	<0.001	18.64	27.90	0.0001
	DM only	24.92	4.73	5.27	<0.001	15.66	34.18	0.0299
30	No HTN or DM	10.19	1.23	8.26	<0.001	7.77	12.60	
	HTN only	12.12	1.23	9.88	<0.001	9.72	14.53	0.1029
	HTN and DM	15.68	1.44	10.86	<0.001	12.85	18.51	0.0001
	DM only	16.82	3.13	5.37	<0.001	10.69	22.96	0.0298
45	No HTN or DM	6.38	0.75	8.47	<0.001	4.91	7.86	
	HTN only	7.70	0.76	10.14	<0.001	6.21	9.18	0.1042
	HTN and DM	10.10	0.97	10.46	<0.001	8.21	11.99	0.0002
	DM only	10.87	2.08	5.23	<0.001	6.80	14.95	0.0300
60	No HTN or DM	4.64	0.52	8.94	<0.001	3.62	5.66	
	HTN only	5.67	0.50	11.31	<0.001	4.68	6.65	0.1036
	HTN and DM	7.54	0.68	11.09	<0.001	6.21	8.88	0.0002
	DM only	8.15	1.57	5.20	<0.001	5.08	11.22	0.0294
90	No HTN or DM	3.21	0.39	8.32	<0.001	2.45	3.96	
	HTN only	4.00	0.40	10.01	<0.001	3.21	4.78	0.1060
	HTN and DM	5.44	0.55	9.87	<0.001	4.36	6.52	0.0003
	DM only	5.90	1.21	4.88	<0.001	3.53	8.27	0.0299
120	No HTN or DM	2.03	0.63	3.20	0.001	0.79	3.27	
	HTN only	2.62	0.79	3.31	0.001	1.07	4.17	0.1360
	HTN and DM	3.71	1.01	1.01	<0.001	1.72	5.69	0.0037
	DM only	4.06	1.34	3.02	0.002	1.43	6.69	0.0471

eGFR, estimated glomerular filtration rate; GS, glomerulosclerosis, HTN, hypertension; DM, diabetes

Supplemental Tables 5 A-C The degree histopathological damage stratified and by race (Black vs. non-Black).

A, Adjusted means for interstitial fibrosis (IF), B, tubular atrophy (TA) and C, glomerulosclerosis (GS) stratified and by race (Black, n=160, vs. non-Black, n=590). Multivariable model adjusted for age, sex, race, body mass index, hypertension, and diabetes.

A

eGFR	Race	Adjusted mean IF (%)	SE	z	p>|z|	95% CI		p-value vs. Non-Black race
15	Non-Black	24.76	3.20	7.73	<0.001	18.48	31.04	
	Black	30.64	3.47	8.84	<0.001	23.84	37.43	0.0224
20	Non-Black	18.96	2.02	9.41	<0.001	15.01	22.91	
	Black	23.55	2.37	9.95	<0.001	18.91	28.19	0.0254
30	Non-Black	11.97	11.97	11.38	<0.001	9.91	14.04	
	Black	15.00	1.57	9.55	<0.001	11.92	18.08	0.0317
45	Non-Black	8.99	1.08	8.30	<0.001	6.87	11.11	
	Black	7.06	0.59	12.01	<0.001	5.91	8.22	0.0376
60	Non-Black	4.90	0.34	14.41	<0.001	4.23	5.57	
	Black	6.35	0.76	8.36	<0.001	4.86	7.83	0.0381
90	Non-Black	3.22	0.28	11.56	<0.001	2.68	3.77	
	Black	4.29	0.51	8.49	<0.001	3.30	5.28	0.0331
120	Non-Black	2.05	0.49	4.15	<0.001	1.08	3.02	
	Black	2.86	0.65	4.39	<0.001	1.58	4.14	0.0370

eGFR, estimated glomerular filtration rate; IF, interstitial fibrosis

B

eGFR	Race	Adjusted Mean TA (%)	SE	z	p>|z|	95% CI		p-value vs. Non-Black race
15	Non-Black	24.01	3.32	7.22	<0.001	17.49	30.52	
	Black	29.51	3.95	7.46	<0.001	21.76	37.27	0.0397
20	Non-Black	18.23	2.03	8.97	<0.001	14.25	22.21	
	Black	22.50	2.59	8.69	<0.001	17.42	27.57	0.0411
30	Non-Black	11.36	1.03	11.04	<0.001	9.35	13.38	
	Black	14.15	1.57	11.04	<0.001	11.07	17.24	0.0457
45	Non-Black	6.63	0.59	11.30	<0.001	5.48	7.78	
	Black	8.40	1.05	8.03	<0.001	6.35	10.45	0.0505
60	Non-Black	4.57	0.34	13.38	<0.001	3.90	5.24	
	Black	5.90	0.73	8.10	<0.001	4.47	7.32	0.0509
90	Non-Black	2.90	0.27	10.93	<0.001	2.38	3.42	
	Black	3.87	0.50	7.80	<0.001	2.90	4.84	0.0470
120	Non-Black	1.53	0.47	3.27	0.001	0.61	2.45	
	Black	2.21	0.61	3.62	<0.001	1.01	3.40	0.0518

eGFR, estimated glomerular filtration rate; TA, tubular atrophy

Supplemental Table 5 cont.

C

eGFR	Race	Adjusted mean GS (%)	SE	z	p>|z|	95% CI		p-value vs. Non-Black race
15	Non-Black	23.58	3.12	7.56	<0.001	17.47	29.69	
	Black	26.21	3.14	8.35	<0.001	20.06	32.36	0.2630
20	Non-Black	18.63	2.03	9.18	<0.001	14.65	22.61	
	Black	20.74	2.20	9.44	<0.001	16.44	25.05	0.2684
30	Non-Black	12.46	1.11	11.18	<0.001	10.27	14.64	
	Black	13.93	1.49	9.36	<0.001	11.01	16.84	0.2774
45	Non-Black	7.92	0.65	12.28	<0.001	6.66	9.19	
	Black	8.91	1.06	8.42	<0.001	6.84	10.99	0.2848
60	Non-Black	5.84	0.39	14.98	<0.001	5.08	6.61	
	Black	6.62	0.77	8.55	<0.001	5.10	8.13	0.2860
90	Non-Black	4.13	0.33	12.53	<0.001	3.48	4.78	
	Black	4.73	0.56	8.50	<0.001	3.64	5.82	0.2811
120	Non-Black	2.72	0.77	3.52	<0.001	1.21	4.24	
	Black	3.17	0.91	3.49	<0.001	1.39	4.95	0.2887

eGFR, estimated glomerular filtration rate; GS, glomerulosclerosis

Supplemental Table 6A-B. A, Clinical and B, histopathologic characteristics of subjects according to race.

A

Clinical Characteristic	Black race (n=176)	Non-Black race (n=631)	p-value (Kruskal-Wallis or chi^2)
eGFR, median (IQR), ml/min/1.73m^2	67 (50-84)	67 (43-86)	0.565
Age, median (IQR), years	59 (49-67)	63 (54-71)	<0.001
Diabetes Mellitus, n (%)	48 (27.3%)	240 (38%)	0.009
Hypertension, n (%)	132 (75%)	430 (68.1%)	0.076
Male sex, n (%)	112 (63.6%)	405 (64.2%)	0.894
Body mass index (kg/m^2), median (IQR)	29.6 (25.5-34.9)	29.4 (25.8-34.3)	0.845
Systolic blood pressure, median (IQR)	141 (125-153)	133 (122-147)	0.004
Diastolic blood pressure, median (IQR)	80 (72-89)	76 (68-82)	<0.001
Dipstick proteinuria (%)			0.036
Negative	45	61	
trace	10	9	
30 mg/dl	17	13	
100 mg/dl	20	8	
300 mg/dl	1	2	
>1000 mg/dl	7	7	

eGFR, estimated glomerular filtration rate; IQR, interquartile range

Supplemental Table 6A-B cont.

B

Histopathologic Characteristic		Black race (n=176)	Non-Black race (n=631)	p-value (Kruskal-Wallis or chi^2)
Acute Tubular Injury, median (IQR) (%)		0 (0)	0 (0-2)	0.692
Tubular atrophy, median (IQR) (%)		5 (2-30)	5 (2-10)	0.002
Tubular Reabsorption 0-3 (%)				0.269
	0	85	80	
	1	12	17	
	2	3	2	
	3	0	1	
Interstitial Fibrosis, median (IQR) (%)		5 (2-30)	5 (2-10)	0.003
Interstitial Eosinophils 0-3 (%)				0.841
	0	78	79	
	1	20	20	
	2	2	1	
	3	0	0	
Interstitial Lymphocytic infiltrates 0-3 (%)				0.002
	0	23	26	
	1	42	51	
	2	21	18	
	3	13	5	
Interstitial Plasmocytic infiltrates 0-3 (%)				0.006
	0	62	67	
	1	26	28	
	2	10	4	
	3	2	1	
Arteriolar Hyalinosis 0-3 (%)				0.980
	0	68	67	
	1	24	24	
	2	6	6	
	3	2	3	
Intimal Fibrosis 0-3 (%)				<0.001
	0	17	13	
	1	36	43	
	2	24	35	
	3	23	9	

Row groups: *Tubulointerstitial* (Acute Tubular Injury through Interstitial Plasmocytic infiltrates); *Vascular* (Arteriolar Hyalinosis and Intimal Fibrosis).

Supplemental Table 6A-B cont.

B cont.

Histopathologic Characteristic		Black race (n=176)	Non-Black race (n=631)	p-value (Kruskal-Wallis or chi^2)
Segmental Sclerosis, median (IQR) (%)		0 (0)	0 (0)	0.135
Glomerulosclerosis, median (IQR) (%)		8.3 (2.9-24.5)	6.5 (2.7-14.9)	0.038
Wall Thickening 0-3 (%)				0.014
	0	84	90	
	1	10	6	
	2	3	3	
	3	3	1	
Hypoperfused 0-3 (%)				<0.0001
	0	33	33	
	1	47	55	
	2	12	10	
	3	8	2	
Mesangial Matrix 0-3 (%)				0.135
	0	74	79	
	1	14	13	
	2	6	4	
	3	6	4	
Mesangial Cellularity 0-3 (%)				0.440
	0	82	83	
	1	8	10	
	2	6	4	
	3	3	3	
Kimmelstiel-Wilson Nodules present (%)		4	3.5	0.757
Pericapsular Fibrosis 0-3 (%)				<0.001
	0	42	44	
	1	33	44	
	2	34	12	
	3	1	0	

Glomerular

Supplemental Table 7A-B. The degree of interstitial fibrosis and tubular atrophy stratified by race (Black vs. non-Black) without use of race coefficient in estimation of GFR.

A, Adjusted means for interstitial fibrosis (IF) and B, tubular atrophy (TA) stratified by race (Black, n=160 vs. non-Black, n=590) without the use of race coefficient in estimation of GFR. Multivariable model adjusted for age, sex, race, body mass index, hypertension, and diabetes.

A

eGFR without Race coefficient	Race	Adjusted mean IF (%)	SE	z	p>|z|	95% CI		p-value vs. Non-Black race
15	Non-Black	25.13	3.20	7.85	<0.001	18.86	31.40	
	Black	27.30	3.02	9.04	<0.001	21.38	33.22	0.3819
20	Non-Black	18.99	2.02	9.40	<0.001	15.03	22.94	
	Black	20.66	2.05	10.06	<0.001	16.63	24.68	0.3831
30	Non-Black	11.78	1.06	11.13	<0.001	9.70	13.85	
	Black	12.86	1.33	9.64	<0.001	10.25	15.48	0.3852
45	Non-Black	6.90	0.57	12.02	<0.001	5.78	8.03	
	Black	7.59	0.89	8.55	<0.001	5.85	9.33	0.3872
60	Non-Black	4.84	0.33	14.46	<0.001	4.19	5.50	
	Black	5.37	0.62	8.63	<0.001	4.15	6.58	0.3882
90	Non-Black	3.29	0.28	11.63	<0.001	2.74	3.85	
	Black	3.69	0.48	7.69	<0.001	2.75	4.63	0.3908
120	Non-Black	1.96	0.58	3.35	0.011	0.81	3.10	
	Black	2.24	0.74	3.03	0.013	0.79	3.70	0.4053

eGFR, estimated glomerular filtration rate; IF, interstitial fibrosis

B

eGFR without Race coefficient	Race	Adjusted Mean TA (%)	SE	z	p>|z|	95% CI		p-value vs. Non-Black race
15	Non-Black	24.46	3.32	7.37	<0.001	17.95	30.96	
	Black	26.13	3.41	7.66	<0.001	19.44	32.82	0.4961
20	Non-Black	18.36	2.03	9.05	<0.001	14.38	22.33	
	Black	19.64	2.21	8.89	<0.001	15.31	23.97	0.497
30	Non-Black	11.26	1.03	10.90	<0.001	9.24	13.29	
	Black	12.09	1.32	9.19	<0.001	9.51	14.67	0.4986
45	Non-Black	6.52	0.58	11.30	<0.001	5.39	7.65	
	Black	7.05	0.85	8.28	<0.001	5.38	8.71	0.5001
60	Non-Black	4.53	0.34	13.42	<0.001	3.87	5.19	
	Black	4.92	0.59	8.29	<0.001	3.76	6.09	0.5008
90	Non-Black	2.94	0.27	10.95	<0.001	2.42	3.47	
	Black	3.24	0.47	6.92	<0.001	2.32	4.15	0.5027
120	Non-Black	1.45	0.54	2.69	0.007	0.39	2.50	
	Black	1.64	0.66	2.48	0.013	0.34	2.94	0.5121

eGFR, estimated glomerular filtration rate; TA, tubular atrophy

Supplemental Table 8A-B. A, Clinical and B, histopathological characteristics of samples with estimated glomerular filtration rate ≥ 60 and < 60 ml/min/1.73m^2.

A

Clinical Characteristic	eGFR ≥ 60 ml/min/1.73m^2 (n=518)	eGFR < 60 ml/min/1.73m^2 (n=341)	p-value (Kruskal-Wallis or chi^2)
eGFR, mean (SD), ml/min/1.73m^2	83 (15)	39 (17)	0.0001
Age, mean (SD), years	58 (13)	66 (12)	0.0001
Hypertension, n (%)	318 (61.4)	277 (81.2)	<0.0001
Diabetes n, (%)	169 (32.6)	135 (39.5)	0.032
Male sex n, (%)	336 (64.9)	212 (62.2)	NS
Black race n, (%)	108 (20.8)	68 (19.9)	NS
Body mass index, median (IQR) kg/m^2	29.5 (25.6-34.2)	29.3 (25.9-34.3)	NS

eGFR, estimated glomerular filtration rate; IQR, interquartile range

B

	Histopathologic Characteristic		eGFR ≥ 60 ml/min/1.73m^2 (n=518)	eGFR < 60 ml/min/1.73m^2 (n=341)	p-value (Kruskal-Wallis or chi^2)
Tubulointerstitial	Acute Tubular Injury, median (IQR) (%)		0 (0-2)	0 (0-2)	NS
	Tubular atrophy, median (IQR) (%)		5 (2-8)	10 (5-30)	0.0001
	Tubular Reabsorption 0-3 (%)		81.1/16.4/2.1/0.4	81.8/15.5/2.1/0.6	NS
		0	81.1	81.8	
		1	16.4	15.5	
		2	2.1	2.1	
		3	0.4	0.6	
	Interstitial Fibrosis, median (IQR) (%)		5 (2-10)	10 (5-30)	0.0001
	Interstitial Eosinophils 0-3 (%)			68/29.3/2.6/0	<0.0001
		0	85.9	68.0	
		1	13.1	29.3	
		2	0.6	2.6	
		3	0.4	0	
	Interstitial Lymphocytic infiltrates 0-3 (%)				<0.0001
		0	35.7	10.6	
		1	49.0	47.2	
		2	12.2	31.1	
		3	3.1	11.1	
	Interstitial Plasmocytic infiltrates 0-3 (%)				<0.0001
		0	77.6	46.9	
		1	19.1	42.2	
		2	2.7	9.4	
		3	0.6	1.5	
Vascular	Arteriolar Hyalinosis 0-2 (%)				<0.0001
		0	73.4	8.0	
		1	21.0	27.6	
		2	5.0	8.2	
		3	0.6	6.2	
	Intimal Fibrosis 0-3 (%)				<0.0001
		0	16.8	9.1	
		1	49.0	29.3	
		2	29.3	37.0	
		3	4.8	24.6	

Supplemental Table 8A-B cont.

B cont.

	Histopathologic Characteristic		eGFR ≥ 60 ml/min/1.73m^2 (n=518)	eGFR < 60 ml/min/1.73m^2 (n=341)	p-value (Kruskal-Wallis or chi^2)
Glomerular	Segmental Sclerosis, median (IQR) (%)		0 (0)	0 (0-0.4)	0.0001
	Glomerulosclerosis, median (IQR) (%)		4.3 (1.8-9.4)	13.5 (6.3-29.2)	0.0001
	Wall Thickening 0-3 (%)				<0.0001
		0	94.2	80.7	
		1	5	10.6	
		2	0.8	6.2	
		3	0	2.6	
	Hypoperfused 0-3 (%)				<0.0001
		0	40.7	23.2	
		1	51.7	56.6	
		2	6.6	14.7	
		3	1.0	5.6	
	Mesangial Matrix 0-3 (%)				<0.0001
		0	84.2	68.4	
		1	11.6	14.8	
		2	2.9	6.7	
		3	1.0	9.1	
	Mesangial Cellularity 0-3 (%)				<0.0001
		0	87.8	76.0	
		1	9.1	10.0	
		2	2.3	7.3	
		3	0.8	6.7	
	Kimmelstiel-Wilson Nodules present (%)		1.4	6.7	<0.0001
	Pericapsular Fibrosis 0/1/2/3 (%)				<0.0001
		0	53.7	28.4	
		1	39.8	44.4	
		2	6.5	26.7	
		3	0	0.3	

Supplemental Table 9. Predictors of eGFR decline in linear mixed model of longitudinal eGFR in subjects with eGFR ≥ 60 at time of nephrectomy including unbiased histopathology-based clusters. Complete fixed effects used in the model include: age, sex, race (as Black or Non-Black), presence of diabetes or hypertension, body mass index, cluster status, nephrectomy status (total or radical vs. partial), tumor size, length of follow up. Variables meeting significance (p <0.05) shown in bold. Coefficients represent fixed effects obtained in linear mixed model using subject-specific eGFR slopes obtained by regressing repeated eGFR values on the time variable (random effects).

Longitudinal eGFR, if baseline eGFR > 60 (n=99)	Coefficient	SE	z	p>|z|	95% CI	
Age (per 1 year older)	**-0.25**	**0.09**	**-2.95**	**0.003**	**-0.42**	**-0.09**
eGFR baseline (per 1 ml/min/1.73m^2 difference)	**0.73**	**0.06**	**11.96**	**<0.001**	**0.61**	**0.85**
Cluster 2	**-6.45**	**2.53**	**-2.55**	**0.011**	**11.42**	**-1.49**
Cluster 3	**-4.20**	**1.79**	**-2.34**	**0.019**	**-7.71**	**-0.68**
Radical Nephrectomy (Yes/No)	**-6.17**	**1.91**	**-3.23**	**0.001**	**-9.91**	**-2.43**
Tumor size (per 1 cm)	0.41	0.26	1.57	0.116	-0.10	0.93
Female Sex (Yes/No)	-2.46	1.85	-1.32	0.185	-6.09	1.18
Black Race (Yes/No)	-2.09	2.74	-0.76	0.445	-7.45	3.27
Body mass index (per 1 kg/m^2)	0.08	0.11	0.74	0.458	-0.13	0.30
Diabetes (Yes/No)	0.90	1.80	0.50	0.616	-2.62	4.43
Hypertension (Yes/No)	-0.63	1.95	-0.32	0.747	-4.44	3.19
Length of Follow up time (per 1 year difference)	-0.14	0.17	-0.83	0.409	-0.47	0.19

eGFR, estimated glomerular filtration rate

Supplemental Table 10. Clinical and histopathological characteristics of follow up samples with complete data used in linear mixed model analyses.

Clinical and Pathologic Characteristics of Follow up data (n=167)		
eGFR, mean (SD), ml/min/1.73m^2		65.6 (23.4)
Follow up eGFR, mean (SD), ml/min/1.73m^2		53.9 (24.8)
Age, mean (SD), years		62.1 (11)
Diabetes mellitus, n (%)		61 (36.5)
Hypertension, n (%)		128 (76.7)
Male sex, n (%)		107 (64.1)
Black race n (%)		21 (12.6)
BMI, mean (SD) kg/m^2		32.2 (7.9)
Follow up time, median (IQR), years		3 (0.8-5.6)
Follow up eGFR slope, median (IQR) ml/min/1.73m^2/year		-5.1 (-12.8 to -1.2)
Percent eGFR slope change, median %/year (IQR)		-6.8 (-20.4 to -2.1)
Partial/Total nephrectomy, n (%)		58/109 (34.7/65.3)
Tumor size, mean (SD) cm		6.2 (3.5)
Tubulointerstitial		
Acute Tubular Injury, median (IQR) (%)		0% (0-2%)
Tubular atrophy, median (IQR) (%)		5% (2-10%)
Tubular Reabsorption 0-3 (%)		
	0	79.0
	1	17.4
	2	2.4
	3	1.2
Interstitial Fibrosis, median (IQR) (%)		5 (2-10%)
Interstitial Eosinophils 0-3 (%)		
	0	79.6
	1	19.8
	2	0.6
	3	0
Interstitial Lymphocytic infiltrates 0-3 (%)		
	0	22.9
	1	58.1
	2	16.8
	3	1.2
Interstitial Plasmocytic infiltrates 0-3 (%)		
	0	70.7
	1	26.9
	2	2.4
	3	0

Glomerular		
Segmental Sclerosis, median (IQR) (range) (%)		0 (0)
Glomerulosclerosis, median (IQR) (%)		5.7 (2.7-12.5)
Wall Thickening 0-3 (%)		
	0	92.2
	1	4.8
	2	3.0
	3	0
Hypoperfused 0-3 (%)		
	0	28.1
	1	61.1
	2	10.2
	3	0.6
Mesangial Matrix 0-3 (%)		
	0	80.8
	1	12
	2	4.2
	3	3.0
Mesangial Cellularity 0-3 (%)		
	0	82.6
	1	12
	2	2.4
	3	3.0
Kimmelstiel-Wilson Nodules present (%)		2.4
Pericapsular Fibrosis 0-3 (%)		
	0	40.1
	1	50.3
	2	9.6
	3	0
Vascular		
Arteriolar Hyalinosis 0-3 (%)		
	0	68.3
	1	26.3
	2	4.2
	3	1.2
Intimal Fibrosis 0-3 (%)		
	0	10.2
	1	44.3
	2	42.5
	3	3

eGFR, estimated glomerular filtration rate; IQR, interquartile range

Supplemental Figure 1. Study flow chart and inclusion/exclusion criteria


```
                    ┌─────────────────────────────┐
                    │         n=1007              │
                    │   Nephrectomy samples       │
                    └─────────────────────────────┘
                              │
                              │      ┌──────────────────────────────────────────────┐
                              ├─────▶│         148 subjects excluded                │
                              │      │  n=132 did not have histology scores         │
                              │      │  n=11 did not meet age criteria >=18 and <100│
                              │      │  n=5 missing serum creatinine               │
                              │      └──────────────────────────────────────────────┘
                              ▼
                    ┌─────────────────────────────┐
                    │          n=859              │
                    │  Complete histopathologic   │
                    │          data               │
                    └─────────────────────────────┘
                              │
                              │      ┌──────────────────────────────────────────────┐
                              ├─────▶│ n=109 subjects excluded due to incomplete age,│
                              │      │ sex, race, BMI, diabetes and hypertension     │
                              │      │ status                                         │
                              │      └──────────────────────────────────────────────┘
                              ▼
                    ┌─────────────────────────────┐
                    │          n=750              │
                    │ Complete clinical/demographic│
                    │          data               │
                    └─────────────────────────────┘
                              │
                              ▼
            ┌─────────────────────────────────────────────┐
            │ n=280 Longitudinal data with > 3 months      │
            │              follow up                       │
            └─────────────────────────────────────────────┘
                              │
                              │      ┌──────────────────────────────────────────────┐
                              ├─────▶│ n=114 subjects excluded due to unknown radical│
                              │      │ vs. partial nephrectomy status or unknown     │
                              │      │ tumor size                                     │
                              │      └──────────────────────────────────────────────┘
                              ▼
                    ┌─────────────────────────────┐
                    │          n=167              │
                    │  Complete follow up cases   │
                    └─────────────────────────────┘
                         ╱                ╲
                        ╱                  ╲
       ┌──────────────────────┐    ┌──────────────────────┐
       │       n=68           │    │        n=99          │
       │ eGFR < 60            │    │ eGFR ≥ 60            │
       │ ml/min/1.73m²        │    │ ml/min/1.73m²        │
       └──────────────────────┘    └──────────────────────┘
```

n=1007
Nephrectomy samples

148 subjects excluded
n=132 did not have histology scores
n=11 did not meet age criteria >=18 and < 100
n=5 missing serum creatinine

n=859
Complete histopathologic data

n=109 subjects excluded due to incomplete age, sex, race, BMI, diabetes and hypertension status

n=750
Complete clinical/demographic data

n=280 Longitudinal data with > 3 months follow up

n=114 subjects excluded due to unknown radical vs. partial nephrectomy status or unknown tumor size

n=167
Complete follow up cases

n=68
eGFR < 60 ml/min/1.73m²

n=99
eGFR ≥ 60 ml/min/1.73m²

Abbreviations: eGFR, estimated glomerular filtration rate; BMI, body mass index

Supplemental References

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