SUPPLEMENTAL MATERIAL

Proteinuria as Non-Invasive Marker for Renal Allograft Histology and Failure: an Observational Cohort Study

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Supplemental Figure 1. Overview of the patients and biopsies included. In Cohort 1, renal allograft biopsies were performed for clinical indication only. In Cohort 2, biopsies were performed at pre-specified time points after transplantation (protocol-specified biopsies at 3, 12, and 24 months after transplantation) and for clinical indication. Proteinuria measurements in 24h urine collections were performed at time of a protocol-specified biopsy, or within one week around an indication biopsy.

Supplemental Figure 2. Kaplan Meier survival curves for graft survival according to degree of proteinuria in Cohort 2 at 3 months, 1 and 2 years after Transplantation, and at time of an indication biopsy.

Supplemental Table 1. Hazard Ratio (multivariate models) for Graft Failure According to Renal Function and Proteinuria at 1, 2 and 5 Years after Transplantation in Cohort 1.

Supplemental Table 2. Hazard ratio (univariate models) for post-biopsy graft failure, according to individual clinical and histological parameters in Cox proportional hazards analysis in Cohort 1 and 2.

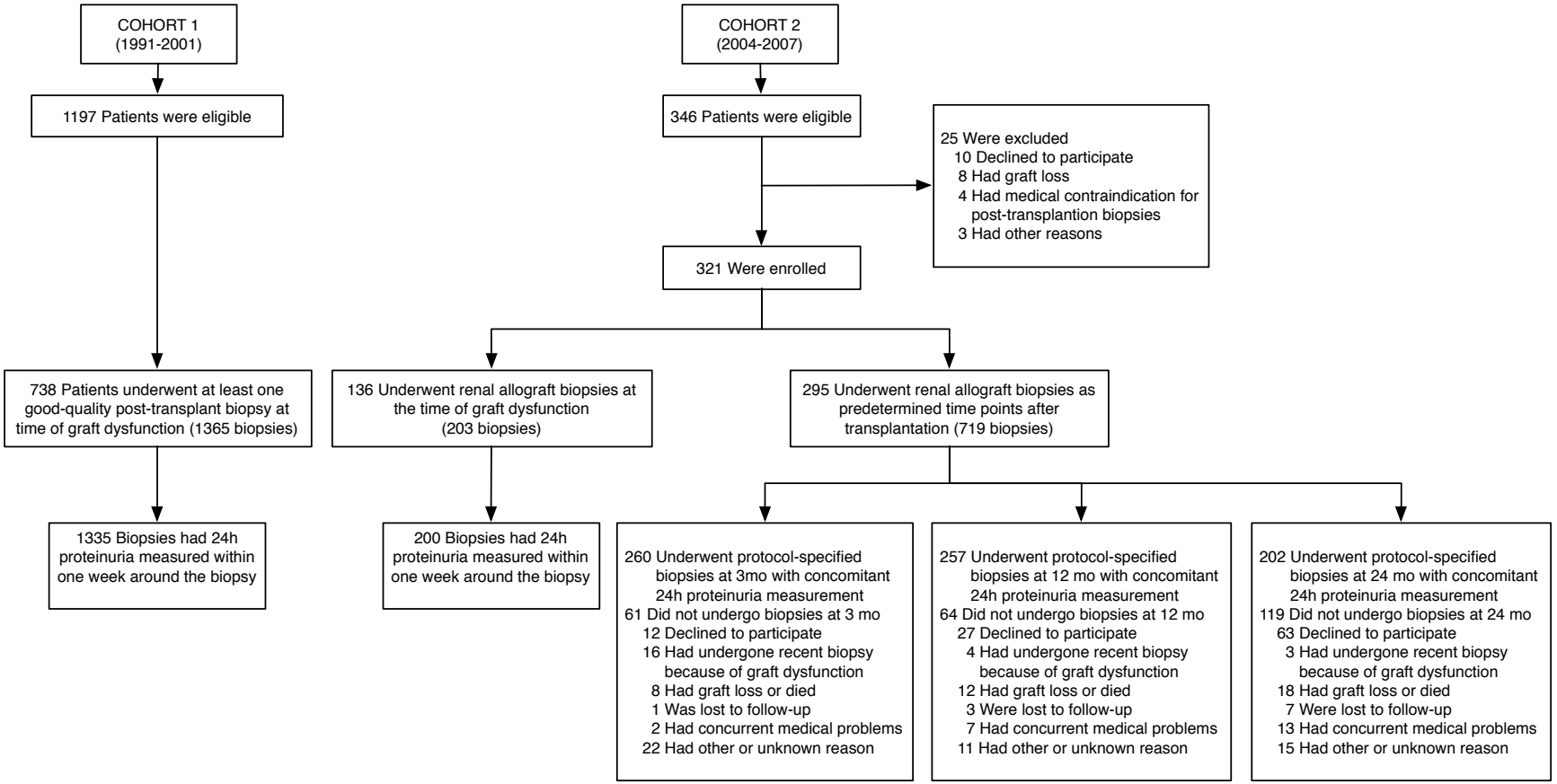
Supplemental Table 3. Hazard ratio (multivariate models) for graft failure according to renal allograft histology, renal function and proteinuria at time of biopsy, adjusted for donor age and time after transplantation in Cox proportional hazards analysis (Cohort 2; N=919 biopsies).

Supplemental Table 4. Univariate analysis of the determinants of 24h proteinuria in Cohort 1 (N=1335 indication biopsies) and in Cohort 2 (N= 919 indication and protocol biopsies).

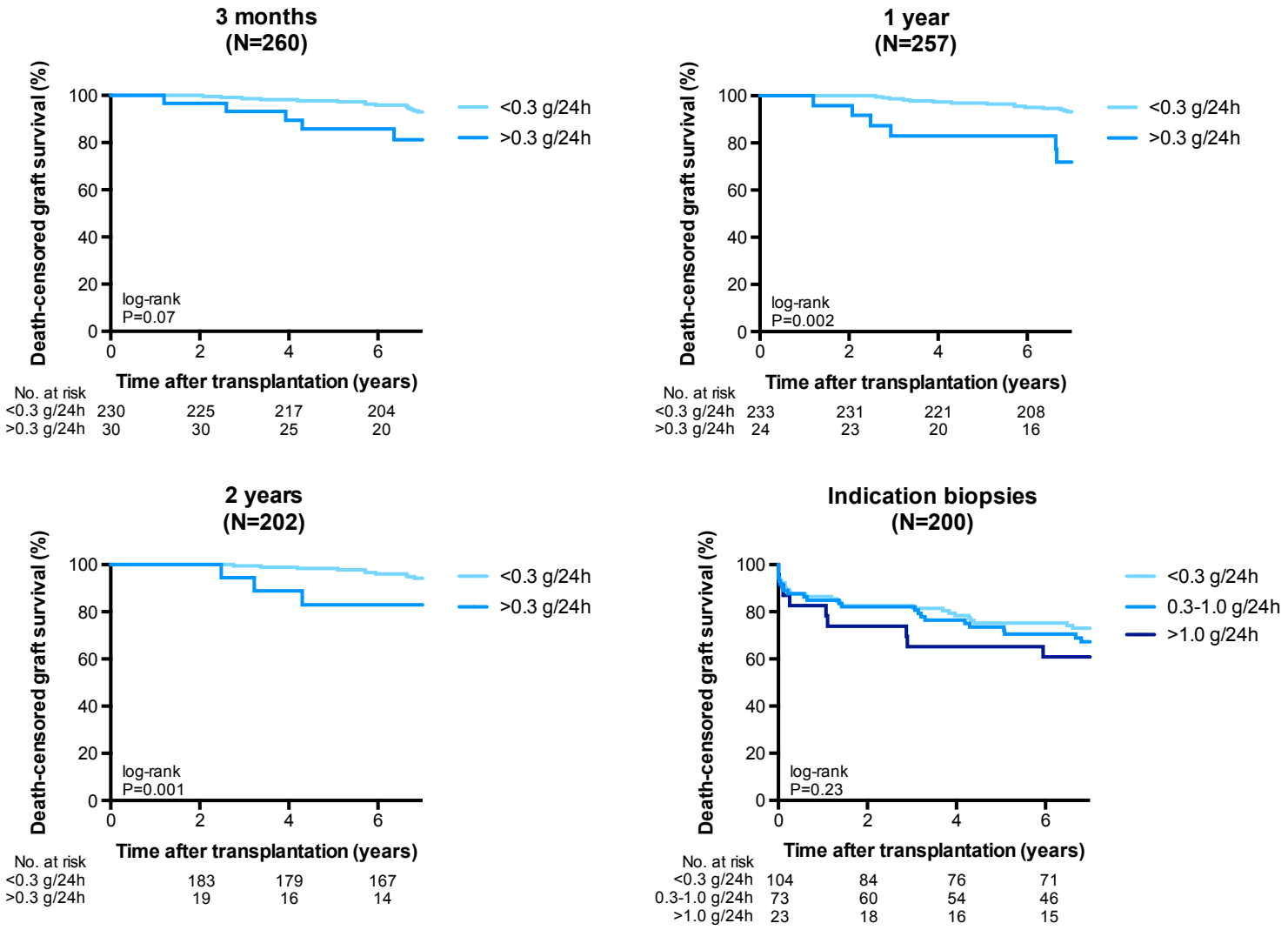
Supplemental Table 5. Association between proteinuria and concomitant renal graft histology after transplantation according to time after transplantation in Cohort 1 (N=1335 indication biopsies).

Supplemental Figure 1. Overview of the patients and biopsies included.

In Cohort 1, renal allograft biopsies were performed for clinical indication only. In Cohort 2, biopsies were performed at pre-specified time points after transplantation (protocol-specified biopsies at 3, 12, and 24 months after transplantation) and for clinical indication. Proteinuria measurements in 24h urine collections were performed at time of a protocol-specified biopsy, or within one week around an indication biopsy. In Cohort 1, renal allograft biopsies were performed for clinical indication only. In Cohort 2, biopsies were performed at pre-specified time points after transplantation (protocol-specified biopsies at 3, 12, and 24 months after transplantation) and for clinical indication. Proteinuria measurements in 24h urine collections were performed at time of a protocol-specified biopsy, or within one week around an indication biopsy.



Supplemental Figure 2. Kaplan Meier Graft Survival Curves according to Degree of Proteinuria in Cohort 2.



Supplemental Table 1. Hazard Ratio (multivariate models) for Graft Failure According to Renal Function and Proteinuria at 1, 2 and 5 Years after Transplantation in Cohort 1.

	1 year after transplantation		2 years after transplantation		5 years after transplantation	
	Adjusted Hazard Ratio (95% CI)	***P* value**	**Adjusted Hazard Ratio (95% CI)**	***P* value**	**Adjusted Hazard Ratio (95% CI)**	***P* value**
COHORT 1[a]	N=778		N=731		N=637	
Proteinuria						
0.3-1.0 vs. <0.3 g/24h	1.96 (1.33-2.89)	<0.001	1.70 (1.12-2.58)	0.01	2.42 (1.56-15.7)	<0.001
1.0-3.0 vs. <0.3 g/24h	2.90 (1.62-5.18)	<0.001	3.91 (2.20-6.94)	<0.001	4.19 (2.27-7.75)	<0.001
>3.0 vs. <0.3 g/24h	8.48 (2.85-25.2)	<0.001	6.52 (1.96-21.7)	0.002	8.94 (2.51-31.9)	<0.001
Estimated GFR						
30-45 vs. >45 mL/min/m2	1.91 (1.28-2.84)	0.001	2.30 (1.50-3.54)	<0.001	2.41 (1.40-4.14)	0.002
15-30 vs. >45 mL/min/m2	3.86 (1.41-6.18)	<0.001	4.52 (2.73-7.49)	<0.001	11.7 (6.76-20.1)	<0.001
<15 vs. >45 mL/min/m2	17.5 (8.33-36.9)	<0.001	44.7 (19.7-102)	<0.001	79.6 (31.8-87.4)	<0.001
COHORT 2	N=257		N=202		--	
Proteinuria						
0.3-1.0 vs. <0.3 g/24h	2.71 (0.79-9.28)	0.11	3.03 (0.83-11.1)	0.09	--	--
>1.0 vs. <0.3 g/24h	13.5 (3.84-16.4)	<0.001	5.55 (1.11-27.8)	0.04	--	--
Estimated GFR						
30-45 vs. >45 mL/min/m2	2.18 (0.96-4.49)	0.06	21.6 (0.71-6.63)	0.18	--	--
<30 vs. >45 mL/min/m2	14.4 (4.59-45.3)	<0.001	15.5 (4.79-50.2)	<0.001	--	--

[a]The data are based on multivariate Cox proportional-hazards models adjusted for donor and recipient age and gender, baseline immunosuppression, repeat transplantation, delayed graft function and T-cell mediated rejection in the first year after transplantation.

Supplemental Table 2. Hazard ratio (univariate models) for post-biopsy graft failure, according to individual clinical and histological parameters in Cox proportional hazards analysis. The number of events was the number of kidney biopsies that preceded graft failure, for each biopsy separately. The number of events indicated in this table therefore does not take into account the fact that multiple biopsies could be performed in one kidney transplant. Hazard ratios and *P* values were calculated using the counting process model in order to account for repeat biopsies within the same patient.

	COHORT 1 (N=1335 Biopsies)				COHORT 2 (N=919 Biopsies)			
Parameter	**Number of biopsies**	**Number of events**	**Hazard ratio (95% CI)**	***P* value**	**Number of biopsies**	**Number of events**	**Hazard ratio (95% CI)**	***P* value**
Donor age								
<40 years	589	216	1		281	35	1	
40-60 years	571	236	1.24 (0.93-1.65)	0.14	453	58	0.57 (0.27-1.21)	0.14
>60 years	175	97	1.62 (1.10-2.37)	0.01	185	46	1.91 (0.90-4.07)	0.09
Recipient age								
<40 years	421	204	1		160	33	1	
40-60 years	660	256	0.97 (0.73-1.31)	0.86	380	57	0.95 (0.40-2.30)	0.92
>60 years	254	89	1.13 (0.77-1.65)	0.53	379	49	1.18 (0.48-2.88)	0.72
Indication vs. protocol biopsy								
Protocol biopsy	--	--	--		719	73	1	
Indication biopsy	--	--	--	--	200	66	8.69 (4.18-18.0)	<0.001
eGFR at time of biopsy								
>45 mL/min/m2	71	12	1		488	36	1	
30-45 mL/min/m2	244	72	1.90 (0.65-5.54)	0.24	244	26	1.60 (0.67-3.80)	0.29
15-30 mL/min/m2	572	242	4.05 (1.49-11.0)	0.006	117	37	6.92 (2.70-17.7)	<0.001
<15 mL/min/m2	439	219	6.71 (2.46-18.3)	<.001	75	40	27.4 (11.1-67.3)	<0.001
Proteinuria at time of biopsy								
<0.3 g/24h	665	228	1		750	87	1	
0.3-1.0 g/24h	401	178	1.32 (0.95-1.84)	0.10	131	37	3.13 (1.44-6.78)	0.004
1.0-3.0 g/24h	217	110	2.42 (1.73-3.39)	<.001	32	12	6.69 (2.22-201)	<0.001
>3.0 g/24h	52	33	3.20 (1.96-5.22)	<.001	6	3	33.7 (7.23-157)	<0.001
Mean arterial pressure at time of biopsy								
<median*	587	212	1		370	57	1	
>median*	587	280	1.59 (1.18-2.14)	0.003	369	64	1.50 (0.72-3.10)	0.28
Use of ACE-inhibitor or ARB								
No	902	371	1		543	94	1	
Yes	278	125	1.23 (0.90-1.68)	0.20	376	45	0.62 (0.32-1.18)	0.14

Supplemental Table 2 (continued)

Parameter	COHORT 1 (N=1335 Biopsies)				COHORT 2 (N=919 Biopsies)			
	Number of biopsies	Number of events	Hazard ratio (95% CI)	*P* value	Number of biopsies	Number of events	Hazard ratio (95% CI)	*P* value
DSA at time of biopsy								
No	--	--	--		853	124	1	
Yes	--	--	--	--	66	15	2.12 (0.93-4.87)	0.08
Tubulitis								
Banff grade 0	676	258	1		673	92	1	
Banff grade 1	368	161	0.94 (0.69-1.29)	0.70	154	24	1.15 (0.27-4.88)	0.85
Banff grade 2-3	291	130	0.79 (0.55-1.14)	0.21	92	23	2.51 (1.11-5.69)	0.03
Interstitial inflammation								
Banff grade 0	639	234	1		727	96	1	
Banff grade 1	202	91	0.98 (0.67-1.42)	0.91	88	13	1.71 (0.59-4.97)	0.32
Banff grade 2-3	494	224	0.89 (0.66-1.20)	0.44	104	30	3.07 (1.15-8.18)	0.02
Intimal arteritis								
Absent	1009	398	1		828	117	1	
Present	317	146	1.03 (0.75-1.42)	0.85	90	22	4.33 (1.67-11.2)	0.003
Glomerulitis								
Banff grade 0-1	1106	435	1		851	121	1	
Banff grade 2-3	225	111	1.61 (1.16-2.25)	0.005	68	18	2.25 (0.87-5.79)	0.09
Peritubular capillaritis								
Absent	1107	434	1		784	105		
Present	215	106	1.43 (1.03-2.01)	0.04	135	34	2.33 (1.10-4.94)	0.03
Microcirculation inflammation								
g+ptc 0-1	1060	416	1		810	112	1	
g+ptc ≥2	275	133	1.51 (1.10-2.06)	0.01	109	27	2.70 (1.27-5.74)	0.01
C4d deposition in peritubular capillaries								
Grade 0-1	917	371	1		832	110	1	
Grade 2-3	342	140	0.83 (0.60-1.16)	0.28	78	28	3.52 (1.56-7.93)	0.002
IF/TA grade								
Banff grade 0	794	279	1		526	60	1	
Banff grade 1	371	166	1.51 (1.09-2.08)	0.01	297	48	0.97 (0.45-2.11)	0.94
Banff grade 2-3	170	104	3.42 (2.44-4.81)	<0.001	95	31	2.76 (1.27-5.99)	0.01

Supplemental Table 2 (continued)

Parameter	COHORT 1 (N=1335 Biopsies)				COHORT 2 (N=919 Biopsies)			
	Number of biopsies	Number of events	Hazard ratio (95% CI)	*P* value	Number of biopsies	Number of events	Hazard ratio (95% CI)	*P* value
Transplant glomerulopathy								
Banff grade 0	1230	491	1		902	133	1	
Banff grade 1	46	22	1.72 (1.00-2.98)	0.05	11	4	3.84 (1.07-13.8)	0.04
Banff grade 2-3	55	33	3.24 (2.10-5.00)	<0.001	6	2	8.14 (1.87-35.5)	0.005
Arteriolar hyalinosis								
Banff grade 0	667	257	1		645	82	1	
Banff grade 1	360	150	0.92 (0.67-1.27)	0.63	193	37	1.09 (0.50-2.35)	0.83
Banff grade 2-3	307	141	1.04 (0.75-1.44)	0.81	81	20	1.59 (0.54-4.66)	0.40
Vascular intimal thickening								
Banff grade 0	1008	386	1		513	69	1	
Banff grade 1	185	87	1.42 (0.99-2.03)	0.05	248	37	0.88 (0.42-1.87)	0.75
Banff grade 2-3	133	72	2.33 (1.65-3.28)	<0.001	157	33	1.49 (0.69-3.20)	0.31
Mesangial matrix increase								
Banff grade 0	1044	402	1		838	123	1	
Banff grade 1	107	49	1.31 (0.85-2.02)	0.23	50	5	1.30 (0.39-4.30)	0.67
Banff grade 2	182	97	1.88 (1.37-2.58)	<0.001	31	11	2.97 (1.03-8.52)	0.04
Glomerulosclerosis								
Banff grade 0	781	302	1		536	70	1	
Banff grade 1	358	156	1.27 (0.92-1.75)	0.15	346	51	1.58 (0.81-3.08)	0.18
Banff grade 2-3	191	88	2.31 (1.67-3.19)	<0.001	35	18	3.98 (1.31-12.1)	0.02
Polyomavirus associated nephropathy								
Absent	1312	529	1		908	135	1	
Present	23	20	4.22 (2.44-7.30)	<0.001	11	4	5.96 (0.74-48.0)	0.09
***De novo*/recurrent glomerular disease**								
Absent	1251	506	1		895	128	1	
Present	84	43	1.71 (1.11-2.64)	0.02	24	11	3.61 (1.24-10.5)	0.02

*Median mean arterial pressure was 103 mm Hg in Cohort 1 and 96 mm Hg in Cohort 2.

Supplemental Table 3. Hazard ratio (multivariate models) for graft failure, according to renal allograft histology, renal function and proteinuria at time of biopsy, adjusted for donor age and time after transplantation in Cox proportional hazards analysis (Cohort 2; N=919 biopsies).

	Parameter	Adjusted Hazard Ratio (95% CI)	*P* value
MODEL 1 WITHOUT PROTEINURIA			
eGFR at time of biopsy	30-45 vs. >45 mL/min/m2	1.18 (0.44-3.15)	0.74
	15-30 vs. >45 mL/min/m2	6.26 (2.22-17.7)	<0.001
	<15 vs. >45 mL/min/m2	21.3 (6.51-69.6)	<0.001
IF/TA grade	Banff grade 1 vs. 0	0.80 (0.34-1.89)	0.61
	Banff grade 2-3 vs. 0	2.42 (0.96-6.24)	0.07
Transplant glomerulopathy	Present vs. absent	10.2 (3.01-34.9)	<0.001
MODEL 2 WITH PROTEINURIA			
Proteinuria at time of biopsy	0.3-1.0 vs. <0.3 g/24h	1.28 (0.52-3.18)	0.59
	1.0-3.0 vs. <0.3 g/24h	1.13 (0.28-4.58)	0.87
	>3.0 vs. <0.3 g/24h	14.9 (2.82-79.2)	0.002
eGFR at time of biopsy	30-45 vs. >45 mL/min/m2	1.65 (0.65-4.23)	0.30
	15-30 vs. >45 mL/min/m2	7.29 (2.60-20.4)	<0.001
	<15 vs. >45 mL/min/m2	22.6 (5.78-88.1)	<0.001

Supplemental Table 4. Univariate analysis of the determinants of 24h proteinuria in Cohort 1 (N=1335 indication biopsies) and in Cohort 2 (N= 919 indication and protocol biopsies).

	Cohort 1 (N=1335)				Cohort 2 (N=919)			
Parameter	**Estimate**	**Standard Error**	**t value**	***P* value**	**Estimate**	**Standard Error**	**t value**	***P* value**
Donor age								
40-60 years vs. <40 years	-0.20	0.08	-2.61	0.01	0.02	0.04	0.51	0.61
>60 years vs. <40 years	-0.29	0.11	-2.60	0.01	0.03	0.05	0.63	0.53
Donor gender								
Female vs. male	0.07	0.07	1.00	0.32	0.06	0.04	1.54	0.12
Recipient age								
40-60 years vs. <40 years	0.06	0.08	0.68	0.50	0.01	0.05	0.10	0.92
>60 years vs. <40 years	-0.21	0.10	-2.06	0.04	-0.07	0.05	-1.36	0.17
Recipient gender								
Female vs. male	-0.11	0.07	-1.51	0.13	-0.08	0.04	-2.00	0.05
Repeat transplantation								
Yes vs. no	0.48	0.10	4.78	<0.001	0.10	0.05	1.91	0.06
Donor-specific antibodies								
Present at time of biopsy vs. absent	--	--	--	--	-0.03	0.07	0.38	0.70
Mean arterial pressure (MAP) at time of biopsy								
>median vs. <median*	0.24	0.06	3.75	<0.001	0.11	0.04	2.86	0.005
Use of ACE-inhibitor or ARB								
Yes vs. no	0.19	0.08	2.39	0.02	-0.04	0.04	1.18	0.24
Use of an mTOR inhibitor								
Yes vs. no	--	--	--	--	0.00	0.10	0.04	0.97
Tubulitis								
Banff grade 1 vs. 0	0.04	0.07	0.55	0.58	0.20	0.04	4.67	<0.001
Banff grade 2-3 vs. 0	0.00	0.08	0.01	0.99	0.14	0.05	2.65	0.008
Interstitial inflammation								
Banff grade 1 vs. 0	0.12	0.09	1.31	0.19	0.19	0.05	3.52	0.005
Banff grade 2-3 vs. 0	0.10	0.07	1.44	0.15	0.27	0.05	5.41	<0.001
Intimal arteritis								
Present vs. absent	0.07	0.07	0.97	0.33	0.25	0.05	4.97	<0.001

Supplemental Table 4 (continued)

Parameter	Cohort 1 (N=1335)				Cohort 2 (N=919)			
	Estimate	Standard Error	t value	*P* value	Estimate	Standard Error	t value	*P* value
Glomerulitis								
Banff grade 1 vs. 0	0.13	0.09	1.47	0.14	0.13	0.07	1.99	0.05
Banff grade 2-3 vs. 0	0.19	0.08	2.32	0.02	0.28	0.06	4.38	<0.001
Peritubular capillaritis								
Banff grade 1 vs. 0	0.15	0.09	1.70	0.09	0.14	0.05	2.59	0.01
Banff grade 2-3 vs. 0	0.66	0.20	3.36	0.0009	0.30	0.08	3.94	0.0001
Microcirculation inflammation								
g+ptc ≥2 vs. 0-1	0.20	0.08	2.59	0.01	0.25	0.05	4.94	<0.001
C4d deposition in peritubular capillaries								
Grade 2-3 vs. 0-1	0.10	0.07	1.39	0.17	0.24	0.06	-4.21	<0.001
IF/TA grade								
Banff grade 1 vs. 0	0.11	0.07	1.49	0.14	-0.05	0.38	-1.31	0.19
Banff grade 2-3 vs. 0	0.29	0.10	2.87	0.005	-0.03	0.06	-0.52	0.60
Transplant glomerulopathy								
Present vs. absent	1.15	0.13	8.71	<0.001	0.92	0.12	7.79	<0.001
Arteriolar hyalinosis								
Banff grade 1 vs. 0	0.14	0.07	1.93	0.05	0.01	0.04	0.31	0.75
Banff grade 2-3 vs. 0	0.01	0.08	0.09	0.93	0.06	0.06	0.98	0.33
Vascular intimal thickening								
Banff grade 1 vs. 0	0.02	0.09	0.19	0.85	0.02	0.04	0.42	0.67
Banff grade 2-3 vs. 0	0.43	0.11	3.89	<0.001	-0.06	0.05	-1.26	0.21
Mesangial matrix increase								
Banff grade 1 vs. 0	-0.21	0.11	-1.895	0.07	0.03	0.08	0.44	0.66
Banff grade 2-3 vs. 0	0.11	0.14	0.77	0.44	0.30	0.09	3.18	0.002
Glomerulosclerosis								
Banff grade 1 vs. 0	-0.03	0.07	-0.41	0.68	-0.01	0.03	-0.25	0.80
Banff grade 2-3 vs. 0	0.21	0.10	2.00	0.046	0.06	0.08	0.71	0.48
De novo*/recurrent glomerular disease								
Present vs. absent	1.09	0.14	7.65	<0.001	0.52	0.11	4.73	<0.001

* Median MAP was 103 mm Hg (range 59-149) in Cohort 1, and 96 mm Hg (range 71-114) in Cohort 2 (*P*<0.001).

Supplemental Table 5. Association between proteinuria and concomitant renal graft histology after transplantation according to time after transplantation in Cohort 1 (N=1335 indication biopsies).

	< 0.3 g/24h	0.3-1 g/24h	1-3 g/24h	> 3 g/24	*P* value
<3 Months Post-Transplant					
N	360	269	108	15	
Any glomerular abnormality*	48% (174/360)	57% (154/269)	48% (52/108)	60% (9/15)	0.31
Glomerulitis grade > 0	36% (129/360)	45% (120/269)	37% (39/107)	33% (5/15)	0.50
Peritubular capillaritis grade > 0	18% (66/357)	22% (60/267)	26% (27/105)	33% (5/15)	0.04
Transplant glomerulopathy grade > 0	0.3% (1/360)	0.0% (0/269)	0.0% (0/107)	0.0% (0/15)	0.37
Mesangial matrix increase > 0	13% (48/360)	12% (33/269)	19% (20/108)	0.0% (0/15)	0.80
C4d deposition in peritubular capillaries grade >1	38% (128/338)	33% (83/251)	44% (45/102)	53% (8/15)	0.32
Global glomerulosclerosis >25%	4.2% (15/360)	5.2% (14/268)	1.9% (2/107)	13% (2/15)	0.18
De novo or recurrent glomerular disease	1.4% (5/360)	1.5% (4/269)	2.8% (3/108)	20% (3/15)	0.005
IF/TA grade > 1	0.6% (2/360)	2.6% (7/269)	2.8% (3/108)	0.0% (0/15)	0.09
Transplant glomerulopathy, microcirculation inflammation and/or de novo/recurrent glomerular disease	26% (92/360)	33% (89/269)	33% (36/108)	40% (6/15)	0.12
> 3 Months Post-Transplant					
N	305	132	109	37	
Any glomerular abnormality*	53% (160/305)	69% (91/132)	78% (85/109)	92% (34/37)	<0.001
Glomerulitis grade > 0	15% (45/305)	29% (37/130)	31% (33/108)	49% (18/37)	<0.001
Peritubular capillaritis grade > 0	7.3% (22/303)	12% (15/130)	12% (13/108)	19% (7/37)	0.01
Transplant glomerulopathy grade > 0	4.3% (13/305)	22% (28/130)	39% (42/108)	46% (17/37)	<0.001
Mesangial matrix increase > 0	25% (76/305)	33% (43/131)	44% (48/108)	57% (21/37)	<0.001
C4d deposition in peritubular capillaries grade >1	11% (32/292)	16% (19/122)	19% (20/103)	19% (7/36)	0.02
Global glomerulosclerosis > 25%	21% (64/304)	31% (40/138)	39% (42/109)	32% (12/37)	0.003
De novo or recurrent glomerular disease	5.5% (20/305)	9.9% (13/132)	22% (24/109)	32% (12/37)	<0.001
IF/TA grade > 1	19% (59/305)	32% (42/132)	40% (44/109)	35% (13/37)	<0.001
Transplant glomerulopathy, microcirculation inflammation and/or de novo/recurrent glomerular disease	16% (50/305)	33% (43/132)	52% (57/109)	76% (28/37)	<0.001

*Any glomerular abnormality was defined as glomerulitis>0, global glomerulosclerosis >25%, mesangial matrix score >0, transplant glomerulopathy and/or *de novo*/recurrent glomerular disease.