APPENDICES

Appendix 1/Table S1: Relative risk of donor acute kidney injury associated with urinary creatinine and creatinine-indexed urinary biomarker concentrations

				Relative Risk (95% CI) for Severe AKI	
Urine Biomarker	**Quantile**	**Range**	**Event Rate (%)**	**Unadjusted**	**Adjusted [1]**
Creatinine	Q1	n_1=434 [0.02, 20.63]	28 (6%)	1.0 (referent)	1.0 (referent)
	Q2	n_2=435 [20.66, 54.29]	50 (11%)	1.59 (1.01, 2.50)	1.64 (1.04, 2.58)
	Q3	n_3=434 [54.52, 381.73]	34 (8%)	1.09 (0.67, 1.78)	1.03 (0.62, 1.69)
Microalbumin/Creatinine	Q1	n_1=402 [0.00, 0.04]	13 (3%)	1.0 (referent)	1.0 (referent)
	Q2	n_2=403 [0.04, 0.11]	23 (6%)	1.77 (0.91, 3.44)	1.88 (0.97, 3.67)
	Q3	n_3=402 [0.11, 6.39]	73 (18%)	5.62 (3.16, 9.97)	6.18 (3.47, 11.00)
NGAL/Creatinine	Q1	n_1=434 [0.00, 0.56]	15 (3%)	1.0 (referent)	1.0 (referent)
	Q2	n_2=434 [0.56, 2.78]	22 (5%)	1.46 (0.77, 2.78)	1.50 (0.78, 2.87)
	Q3	n_3=434 [2.81, 23254.29]	75 (17%)	5.57 (3.25, 9.54)	6.21 (3.63, 10.62)
KIM-1/Creatinine	Q1	n_1=434 [0.64, 30.24]	31 (7%)	1.0 (referent)	1.0 (referent)
	Q2	n_2=434 [30.37, 69.15]	31 (7%)	1.01 (0.63, 1.63)	1.11 (0.67, 1.82)
	Q3	n_3=434 [69.16, 25343.75]	49 (11%)	1.81 (1.18, 2.79)	2.00 (1.26, 3.16)
IL-18/Creatinine	Q1	n_1=434 [0.02, 0.85]	16 (4%)	1.0 (referent)	1.0 (referent)
	Q2	n_2=434 [0.86, 2.23]	30 (7%)	1.89 (1.05, 3.42)	1.99 (1.10, 3.60)
	Q3	n_3=434 [2.24, 1695.43]	66 (15%)	4.69 (2.76, 7.96)	5.14 (3.02, 8.75)
L-FABP/Creatinine	Q1	n_1=433 [0.00, 0.20]	14 (3%)	1.0 (referent)	1.0 (referent)
	Q2	n_2=433 [0.20, 0.99]	25 (6%)	1.77 (0.93, 3.36)	1.82 (0.95, 3.49)
	Q3	n_3=433 [0.99, 2252.25]	72 (17%)	5.80 (3.33, 10.12)	6.31 (3.62, 11.02)

				Relative Risk (95% CI) for Severe AKI	
Urine Biomarker	**Quantile**	**Range**	**Event Rate (%)**	**Unadjusted**	**Adjusted [1]**

Acute kidney injury (AKI) was defined as at least a 2-fold increase in donor serum creatinine from the admission to terminal value.

[1] Adjusted for the donor variables that comprise the KDRI, except terminal serum creatinine. These are: age (years), height (cm), weight (kg), Black race, history of hypertension, history of diabetes, stroke as cause of death, HCV status, and DCD status.

Appendix 2/Table S2: Donor urinary biomarker concentrations, by recipient delayed graft function status

Urine Biomarker	ALL (N=2441)	No DGF (N=1685)	DGF (N=756)	P*
Microalbumin, mg/dL	1.97 [0.66, 5.47]	1.76 [0.61, 4.95]	2.32 [0.8, 6.48]	<0.001
NGAL, ng/mL	39.6 [12.3, 135.8]	34 [10.5, 106.1]	53.7 [17.8, 214.5]	<0.001
KIM-1, pg/mL	1360.95 [624.23, 3201.52]	1314.87 [591.64, 3077.98]	1584.56 [717.37, 3356.05]	0.001
IL-18, pg/mL	43.4 [19.97, 106.98]	40.5 [17.94, 99.69]	52.2 [24.98, 126.69]	<0.001
L-FABP, pg/mL	12.4 [3.6, 54.4]	10.8 [3.2, 43.2]	16 [4.4, 72.4]	<0.001
Creatinine, mg/mL	36.18 [14.45, 67.3]	34.76 [13.53, 66.17]	38.19 [17.2, 70.31]	0.042
Creatinine-Indexed Urine Biomarkers				
Microalbumin/Creatinine	0.06 [0.03, 0.14]	0.06 [0.03, 0.14]	0.07 [0.03, 0.14]	0.186
NGAL/Creatinine	1.07 [0.37, 5.24]	0.96 [0.33, 4]	1.49 [0.48, 10.17]	<0.001
KIM-1/Creatinine	43.18 [22.76, 89.15]	41.36 [22.14, 85.49]	46.46 [24.55, 96.67]	0.037
IL-18/Creatinine	1.29 [0.65, 3.17]	1.26 [0.64, 2.83]	1.49 [0.69, 4.42]	0.004
L-FABP/Creatinine	0.43 [0.14, 1.54]	0.4 [0.13, 1.37]	0.54 [0.17, 1.97]	<0.001

Values reported are median [interquartile range].
* Wilcoxon rank sum test.

Appendix 3/Table S3: Statistics related to improvement in prediction of delayed graft function with the addition of donor urinary biomarkers to statistical models

Urine Biomarker	Stat	Estimate	SE	P
log Microalbumin	ΔAUC	0.000		0.890
	IDI	0.000	0.000	0.460
	NRI	0.004	0.002	0.074
	NRI_{ne}	0.006		
	NRI_{e}	-0.001		
log NGAL	ΔAUC	0.003		0.076
	IDI	0.002	0.001	0.031
	NRI	0.008	0.005	0.124
	NRI_{ne}	0.011		
	NRI_{e}	-0.003		
log KIM-1	ΔAUC	0.001		0.350
	IDI	0.001	0.001	0.235
	NRI	0.003	0.004	0.339
	NRI_{ne}	0.006		
	NRI_{e}	-0.003		
log IL-18	ΔAUC	0.001		0.258
	IDI	0.000	0.000	0.380
	NRI	0.002	0.003	0.370
	NRI_{ne}	0.004		
	NRI_{e}	-0.001		
log L-FABP	ΔAUC	0.000		0.380
	IDI	0.000	0.000	0.499
	NRI	-0.001	0.002	0.559
	NRI_{ne}	0.000		
	NRI_{e}	-0.001		

Area under the curve (AUC) of clinical model is 0.714. Predictors used for clinical model are: donor age (years), height (cm), weight (kg), black race, history of hypertension, history of diabetes, stroke as cause of death, hepatitis C serostatus, donation after circulatory determination of death, and terminal serum creatinine (mg/dL).
Casewise deletion was applied to missing values of all biomarkers, resulting in 2258 observations. Pre-specified risk categories for the NRI were defined as low (<5%), medium (5-10%), or high (>10%) risk.

ΔAUC = change in AUC after adding the biomarker to the clinical model

IDI=integrated discrimination improvement

NRI =Overall Net Reclassification Improvement calculated as NRI_{ne} + NRI_{e}

NRI_{ne} = Non-Event Net Reclassification Improvement.

NRI_{e} = Event Net Reclassification Improvement.

Appendix 4/Table S4: Relative risk of delayed graft function after kidney transplantation associated with donor urinary creatinine and creatinine-indexed urinary biomarker concentrations

				Relative Risk (95% CI) for DGF			
Urine Biomarker	**Quantile**	**Range**	**Event Rate (%)**	**Unadjusted**	**Adjusted for donor variables only [1]**	**Adjusted for donor variables & SCr [2]**	**Adjusted for donor, transport, & recipient variables [3]**
Microalbumin/ Creatinine	Q1	n_1=753 [0.00, 0.04]	233 (31%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
	Q2	n_2=753 [0.04, 0.11]	216 (29%)	0.93 (0.79, 1.11)	0.94 (0.80, 1.10)	0.92 (0.78, 1.07)	0.96 (0.82, 1.13)
	Q3	n_3=754 [0.11, 6.39]	241 (32%)	1.04 (0.88, 1.22)	1.03 (0.88, 1.20)	0.87 (0.74, 1.02)	0.91 (0.78, 1.07)
NGAL/ Creatinine	Q1	n_1=813 [0.00, 0.54]	213 (26%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
	Q2	n_2=811 [0.55, 2.70]	243 (30%)	1.17 (0.98, 1.39)	1.12 (0.95, 1.32)	1.08 (0.92, 1.28)	1.05 (0.90, 1.24)
	Q3	n_3=813 [2.73, 23254.29]	300 (37%)	1.42 (1.20, 1.69)	1.42 (1.21, 1.67)	1.14 (0.96, 1.36)	1.17 (0.99, 1.39)
KIM-1/ Creatinine	Q1	n_1=813 [0.64, 29.98]	232 (29%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
	Q2	n_2=812 [30.01, 68.36]	255 (31%)	1.10 (0.93, 1.30)	1.14 (0.98, 1.34)	1.09 (0.94, 1.28)	1.08 (0.93, 1.26)
	Q3	n_3=812 [68.52, 25343.75]	269 (33%)	1.11 (0.94, 1.32)	1.19 (1.01, 1.41)	1.09 (0.92, 1.28)	1.07 (0.91, 1.26)
IL-18/ Creatinine	Q1	n_1=812 [0.02, 0.84]	237 (29%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
	Q2	n_2=813 [0.84, 2.21]	226 (28%)	0.96 (0.81, 1.15)	1.01 (0.86, 1.19)	0.98 (0.83, 1.15)	1.00 (0.86, 1.17)
	Q3	n_3=812 [2.21, 1695.43]	293 (36%)	1.24 (1.06, 1.47)	1.23 (1.05, 1.44)	1.05 (0.89, 1.24)	1.06 (0.90, 1.25)
L-FABP/ Creatinine	Q1	n_1=810 [0.00, 0.20]	217 (27%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
	Q2	n_2=811 [0.20, 0.98]	251 (31%)	1.18 (0.99, 1.39)	1.20 (1.03, 1.40)	1.15 (0.98, 1.34)	1.14 (0.97, 1.32)
	Q3	n_3=811 [0.99, 2252.25]	287 (35%)	1.31 (1.10, 1.56)	1.29 (1.09, 1.52)	1.08 (0.91, 1.29)	1.07 (0.90, 1.26)

[1] Donor variables used for adjustment: age (years), height (cm), weight (kg), Black race, history of hypertension, history of diabetes, stroke as cause of death, HCV status, and DCD status.
[2] Includes donor variables listed above plus terminal serum creatinine.
[3] Includes all variables listed above plus cold ischemia time and the following recipient variables: age (years), black race, gender, previous kidney transplant, diabetes as the cause of end stage renal disease, need for pre-transplant blood transfusion, number of human leukocyte antigen mismatches, panel reactive antibody (%), body mass index, and pre-transplant dialysis.

Appendix 5/ Table S5: Regression of 6-month eGFR (ml/min/1.73 m^2)* on delayed graft function

	Linear Regression Coefficient (95% CI)			
	Unadjusted	**Adjusted for donor variables only[1]**	**Adjusted for donor variables & SCr[2]**	**Adjusted for donor, transport, & recipient variables[3]**
DGF (vs. no DGF)	-11.53 (-13.53, -9.54)	-8.95 (-10.85, -7.04)	-8.64 (-10.58, -6.71)	-7.53 (-9.51, -5.55)

* eGFR calculated using Chronic Kidney Disease Epidemiology Collaboration equation.

[1] Donor variables used for adjustment: age (years), height (cm), weight (kg), Black race, history of hypertension, history of diabetes, stroke as cause of death, HCV status, and DCD status.

[2] Includes donor variables listed above plus terminal serum creatinine.

[3] Includes all variables listed above plus cold ischemia time and the following recipient variables: age (years), black race, gender, previous kidney transplant, diabetes as the cause of end stage renal disease, need for pre-transplant blood transfusion, number of human leukocyte antigen mismatches, body mass index, and pre-transplant dialysis.

Appendix 6/Table S6: Regression of 6-month eGFR on urine biomarker tertiles: Recipients without delayed graft function (n=1685)

			Linear Regression Coefficient (95% CI)			
Biomarker	**Quantile**	**Range**	**Unadjusted**	**Adjusted for donor variables only[1]**	**Adjusted for donor variables & SCr[2]**	**Adjusted for donor, transport, & recipient variables[3]**
Microalbumin	Q1	n_1=563 [0.50, 0.89]	(Referent)	(Referent)	(Referent)	(Referent)
	Q2	n_2=504 [0.90, 3.84]	-1.99 (-5.14, 1.17)	0.40 (-2.32, 3.13)	0.70 (-2.02, 3.42)	0.73 (-1.96, 3.43)
	Q3	n_3=503 [3.86, 44.86]	-4.88 (-7.87, -1.90)	-2.44 (-5.10, 0.22)	-1.59 (-4.30, 1.11)	-1.81 (-4.49, 0.86)
NGAL	Q1	n_1=620 [0.00, 18.10]	(Referent)	(Referent)	(Referent)	(Referent)
	Q2	n_2=565 [18.20, 83.40]	-3.60 (-6.67, -0.52)	-1.76 (-4.48, 0.97)	-1.49 (-4.21, 1.23)	-1.27 (-3.99, 1.44)
	Q3	n_3=496 [83.60, 6000.00]	-4.40 (-7.47, -1.33)	-3.15 (-5.81, -0.50)	-1.84 (-4.67, 1.00)	-1.40 (-4.23, 1.43)
KIM-1	Q1	n_1=600 [58.96, 843.30]	(Referent)	(Referent)	(Referent)	(Referent)
	Q2	n_2=548 [844.28, 2430.76]	-0.95 (-4.13, 2.22)	-0.83 (-3.56, 1.90)	-0.63 (-3.34, 2.08)	-0.66 (-3.35, 2.03)
	Q3	n_3=535 [2432.46, 37759.01]	-2.40 (-5.37, 0.57)	-1.06 (-3.68, 1.57)	-0.75 (-3.37, 1.87)	-0.10 (-2.68, 2.48)
IL-18	Q1	n_1=605 [2.58, 26.59]	(Referent)	(Referent)	(Referent)	(Referent)
	Q2	n_2=558 [26.63, 76.69]	-1.20 (-4.35, 1.95)	-1.34 (-4.03, 1.35)	-1.25 (-3.93, 1.43)	-1.22 (-3.85, 1.41)
	Q3	n_3=518 [77.41, 1448.69]	-3.61 (-6.62, -0.60)	-2.54 (-5.19, 0.11)	-1.63 (-4.36, 1.09)	-1.13 (-3.87, 1.61)
L-FABP	Q1	n_1=601 [1.00, 5.60]	(Referent)	(Referent)	(Referent)	(Referent)
	Q2	n_2=570 [6.00, 30.00]	-2.70 (-5.76, 0.36)	-1.55 (-4.22, 1.11)	-1.23 (-3.88, 1.42)	-1.52 (-4.13, 1.10)
	Q3	n_3=508 [30.40, 250.00]	-5.01 (-8.16, -1.86)	-3.56 (-6.30, -0.83)	-2.66 (-5.47, 0.14)	-2.32 (-5.10, 0.47)
Creatinine	Q1	n_1=594 [0.04, 20.69]	(Referent)	(Referent)	(Referent)	(Referent)
	Q2	n_2=545 [20.78, 54.75]	0.90 (-2.15, 3.94)	-0.74 (-3.44, 1.95)	-0.55 (-3.22, 2.13)	-0.19 (-2.83, 2.46)
	Q3	n_3=544 [54.76, 381.73]	-0.89 (-4.04, 2.25)	-2.01 (-4.81, 0.79)	-1.92 (-4.70, 0.86)	-1.07 (-3.86, 1.71)

[1] Donor variables used for adjustment: age (years), height (cm), weight (kg), Black race, history of hypertension, history of diabetes, stroke as cause of death, HCV status, and DCD status.
[2] Includes donor variables listed above plus terminal serum creatinine.
[3] Includes all variables listed above plus cold ischemia time and the following recipient variables: age (years), black race, gender, previous kidney transplant, diabetes as the cause of end stage renal disease, need for pre-transplant blood transfusion, number of human leukocyte antigen mismatches, panel reactive antibody (%), body mass index, and pre-transplant dialysis.

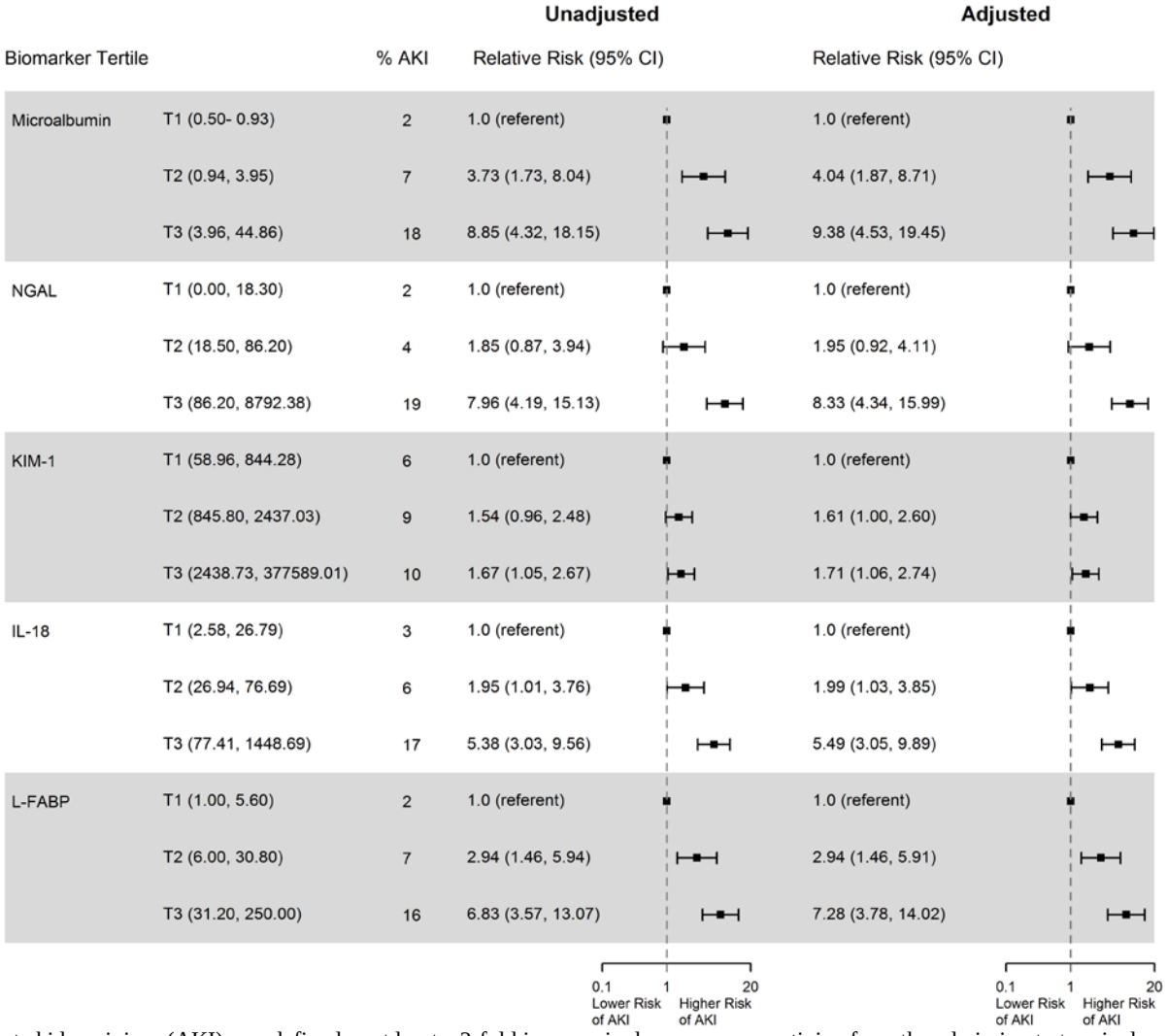
P-values for biomarker x DGF interaction term (separate models) are: microalbumin, $p = 0.015$; NGAL, $p < 0.001$; KIM-1, $p = 0.290$; IL-18, $p = 0.014$; L-FABP, $p = 0.006$

Appendix 7/Table S7: Regression of 6-month eGFR on Biomarker Tertiles, Recipients with Delayed Graft Function (n=756)

			Linear Regression Coefficient (95% CI)			
Urine Biomarker	**Quantile**	**Range**	**Unadjusted**	**Adjusted for donor variables only [1]**	**Adjusted for donor variables & SCr [2]**	**Adjusted for donor, transport, & recipient variables [3]**
Microalbumin	Q1	n_1=192 [0.50, 0.89]	(Referent)	(Referent)	(Referent)	(Referent)
	Q2	n_2=247 [0.91, 3.80]	-0.06 (-4.83, 4.70)	1.32 (-2.92, 5.55)	1.40 (-2.84, 5.64)	1.35 (-2.94, 5.63)
	Q3	n_3=251 [3.86, 44.86]	2.10 (-2.76, 6.96)	2.38 (-1.99, 6.75)	2.63 (-1.96, 7.23)	3.00 (-1.63, 7.62)
NGAL	Q1	n_1=193 [0.00, 18.10]	(Referent)	(Referent)	(Referent)	(Referent)
	Q2	n_2=246 [18.30, 83.40]	-1.71 (-6.70, 3.28)	-0.33 (-4.86, 4.21)	-0.22 (-4.78, 4.34)	-0.16 (-4.86, 4.54)
	Q3	n_3=317 [84.50, 8792.38]	2.62 (-2.04, 7.27)	1.63 (-2.55, 5.81)	2.04 (-2.38, 6.46)	2.28 (-2.18, 6.74)
KIM-1	Q1	n_1=213 [58.96, 831.84]	(Referent)	(Referent)	(Referent)	(Referent)
	Q2	n_2=266 [844.28, 2430.76]	-3.96 (-8.64, 0.72)	-2.99 (-7.18, 1.20)	-2.99 (-7.18, 1.20)	-2.94 (-7.18, 1.31)
	Q3	n_3=277 [2432.46, 37759.01]	-1.53 (-6.32, 3.25)	-0.26 (-4.43, 3.90)	-0.25 (-4.43, 3.93)	0.31 (-4.03, 4.65)
IL-18	Q1	n_1=207 [2.58, 26.56]	(Referent)	(Referent)	(Referent)	(Referent)
	Q2	n_2=255 [26.68, 74.44]	1.68 (-3.26, 6.62)	2.37 (-2.03, 6.77)	2.42 (-1.96, 6.80)	2.98 (-1.40, 7.37)
	Q3	n_3=294 [77.41, 1448.69]	4.16 (-0.47, 8.80)	5.17 (0.95, 9.39)	5.47 (1.18, 9.77)	5.53 (1.20, 9.85)
L-FABP	Q1	n_1=214 [1.00, 5.60]	(Referent)	(Referent)	(Referent)	(Referent)
	Q2	n_2=237 [6.00, 30.00]	-0.22 (-4.97, 4.52)	-0.53 (-4.87, 3.82)	-0.44 (-4.81, 3.94)	-0.36 (-4.85, 4.13)
	Q3	n_3=304 [30.40, 250.00]	3.39 (-1.30, 8.08)	2.50 (-1.85, 6.84)	2.79 (-1.78, 7.36)	3.47 (-1.10, 8.05)

[1] Donor variables used for adjustment: age (years), height (cm), weight (kg), Black race, history of hypertension, history of diabetes, stroke as cause of death, HCV status, and DCD status.
[2] Includes donor variables listed above plus terminal serum creatinine.
[3] Includes all variables listed above plus cold ischemia time and the following recipient variables: age (years), black race, gender, previous kidney transplant, diabetes as the cause of end stage renal disease, need for pre-transplant blood transfusion, number of human leukocyte antigen mismatches, panel reactive antibody (%), body mass index, and pre-transplant dialysis.

<u>Appendix 8/Figure S1</u>: Relative risk of donor acute kidney injury associated with donor urinary biomarkers



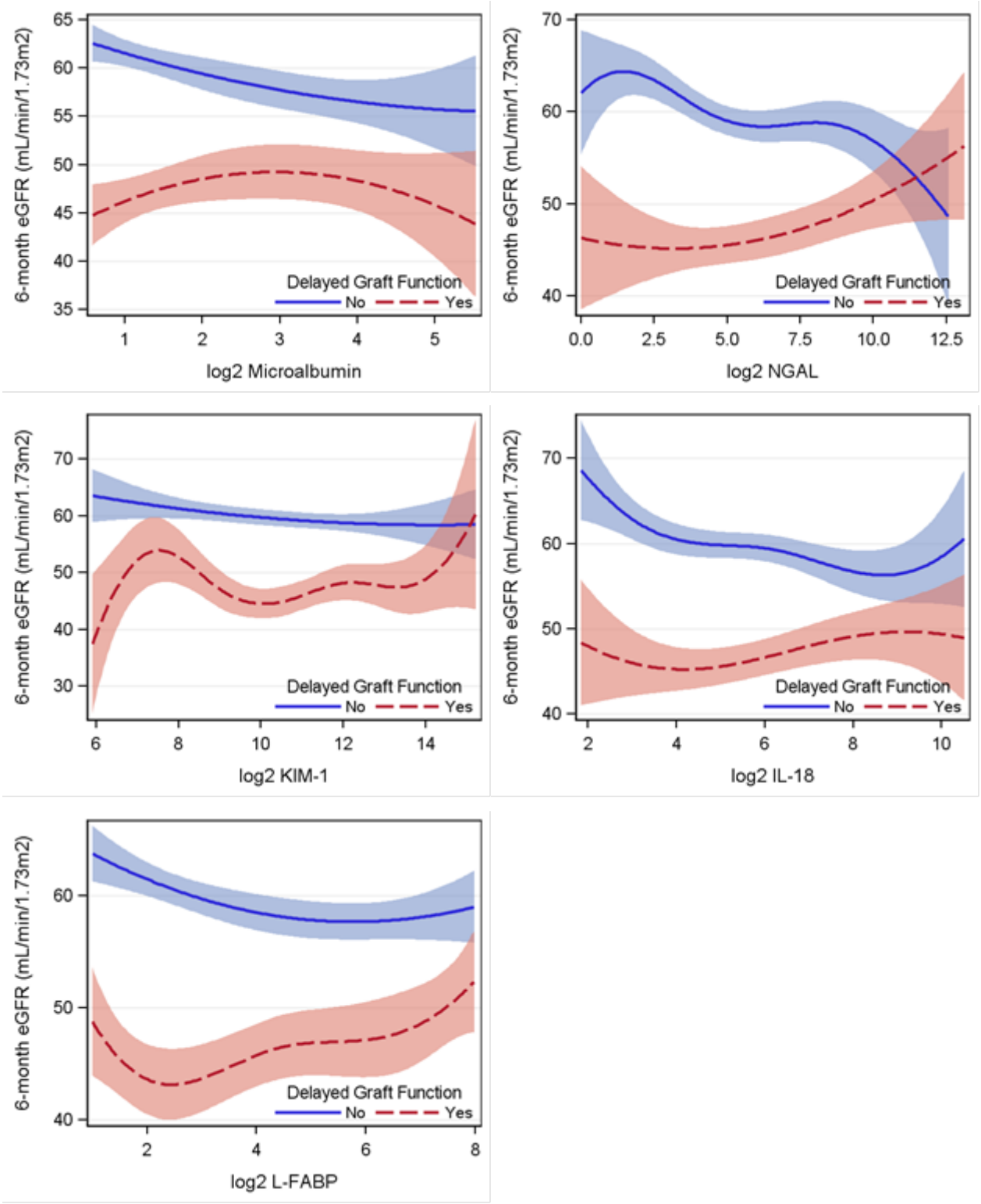
Acute kidney injury (AKI) was defined as at least a 2-fold increase in donor serum creatinine from the admission to terminal value.

NGAL, neutrophil gelatinase associated lipocalin; KIM-1, kidney injury molecule-1, IL-18, interleukin-18; L-FABP, liver-type fatty acid binding protein.

Biomarkers were log (base 2) transformed. Original urinary concentrations are microalbumin, mg/dL; NGAL, ng/mL; KIM-1, pg/mL; IL-18, pg/mL; L-FABP, ng/mL; creatinine, mg/dL.

[1] Adjusted models were adjusted for the donor variables that comprise the Kidney Donor Profile Index, except terminal serum creatinine because it is part of the AKI outcome definition. These are: age (years), height (cm), weight (kg), Black race, history of hypertension, history of diabetes, stroke as cause of death, Hepatitis C serostatus status, and Donation after Circulatory Determination of Death status.

Appendix 9/Figure S2: Spline Plots showing the association of donor urinary biomarkers and recipient eGFR, stratified by recipient delayed graft function



Cubic penalized B-spline curves depicting the unadjusted, bivariate relationship between 6-month eGFR and the indicated log (base 2) transformed biomarkers, stratified by delayed graft function. Splines are based on 5 internal knots. Shaded regions are 95% confidence limits.

Appendix 10: Methods related to measurement of urinary biomarkers

Urine albumin was measured using the immunoturbidimetry assay from Randox on RxDaytona, as per the manufacturer's instructions. Microalbumin calibrators and the urine level 2 and level 3 controls were also obtained from Randox Laboratories (Randox Laboratories, Boston, MA). The minimum detectable concentration of microalbumin is 5.11 mg/L. The inter-assay CV for Level 2 and level 3 urine controls was 3.96 and 5.96% respectively.

Urine NGAL measurement was performed with the Architect platform that is clinically approved for use in Europe (Abbott Diagnostics). The concentration of KIM-1 and IL-18 was measured using the Meso Scale Discovery platform (Meso Scale diagnostics, Gaithersburg, Maryland, USA), which employs proprietary electrochemiluminescence detection methods combined with patterned arrays. The intra-assay coefficient of variation (CV) was <10% and the inter-assay CV for the calibrators was 5.2 – 9.2% and 7.2 – 10.66% for KIM-1 and IL-18 respectively. The average lower limit of detection obtained from 24 runs was 0.43 pg/mL for KIM-1 and 0.169 pg/mL for IL-18. Urine L-FABP was measured using a new method with clinical chemistry analyzers, based on latex-enhanced immunoturbidimetry that employs anti-human L-FABP mouse monoclonal antibodies. The concentration is determined by measuring the change in absorbance that results from agglutination of latex particles. The interassay CV was between 1 – 3.5%. The lower and upper limits of detection were 0.5 ng/mL and 250 ng/mL respectively.