FGF23 is associated with acute kidney injury and death in critically ill patients

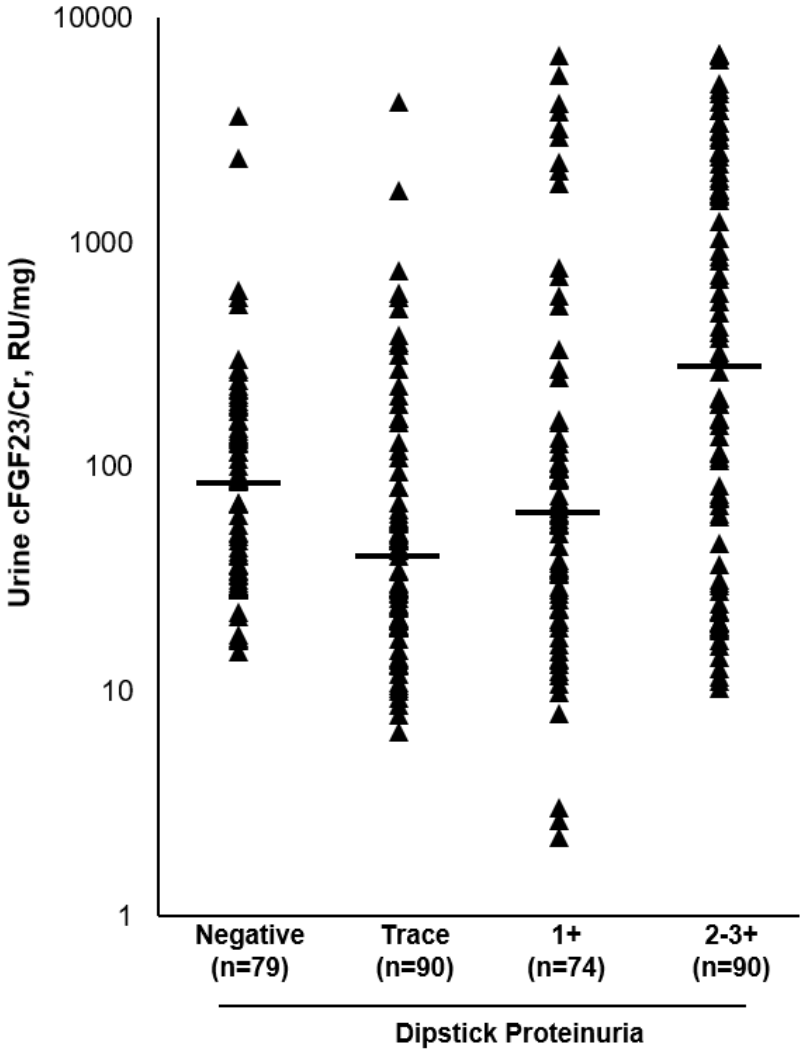
Leaf DE *et al.*

SUPPLEMENTAL MATERIAL

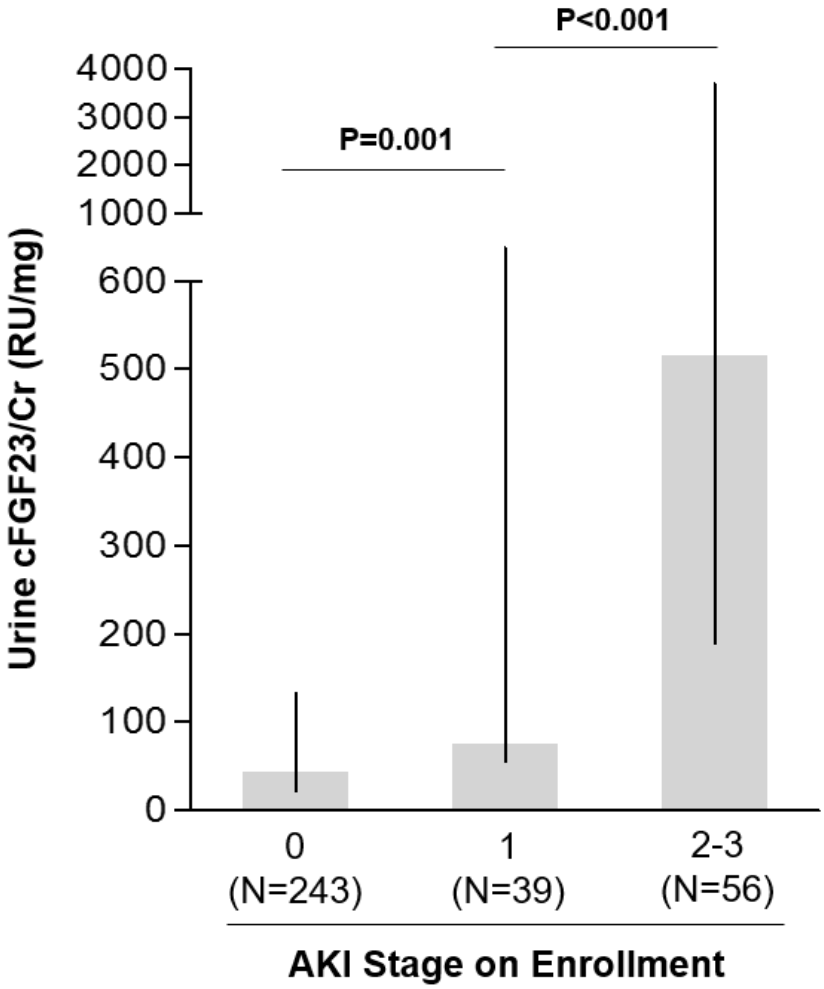
Supplemental Methods

Immunoprecipitation and Western blot characterization of FGF23 in urine.

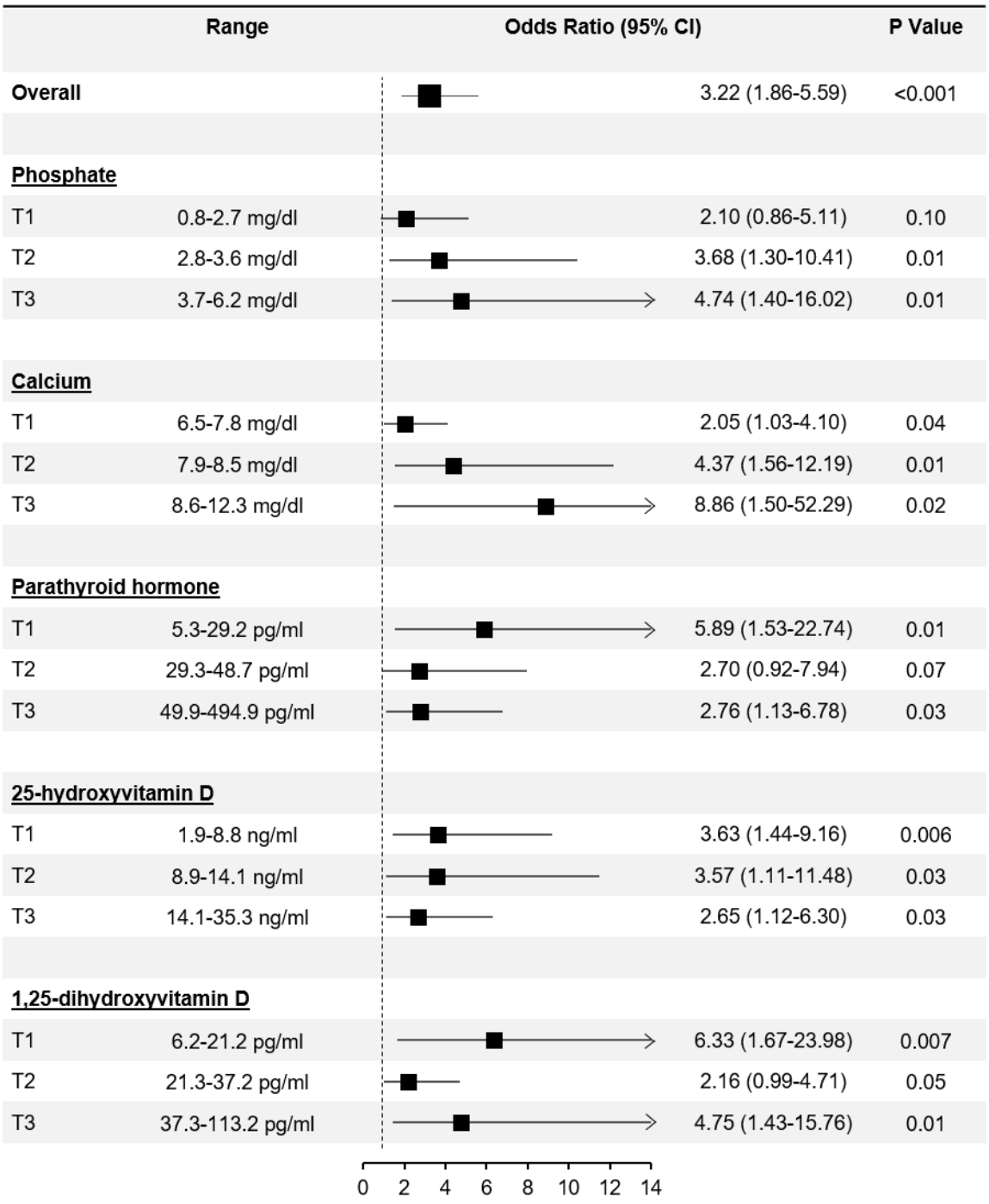
As previously reported,[1] we used the Dynabeads Co-Immunoprecipitation kit (Thermo Fisher Scientific, Grand Island, NY) for immunoprecipitation of FGF23. Affinity-purified goat polyclonal antibody, recognizing epitopes within the human FGF23 C-terminal region (aa 186–206) was covalently coupled to magnetic epoxy Dynabeads (Thermo Fisher Scientific, Grand Island, NY), and 500 µl of each urine sample or recombinant human FGF23 was incubated with the epoxy beads for 1 h at 4ºC with gentle mixing. Bound proteins were eluted from the beads, separated on 4-12% Novex Bis-Tris precast gels (Thermo Fisher Scientific) and transferred onto a polyvinylidene difluoride membrane using the Trans-blot Turbo transfer system (Biorad, Hercules, CA). Membranes were then incubated with an affinity-purified goat polyclonal anti-FGF23 primary antibody recognizing a different C-terminal region (aa 225-244). The signal was visualized using fluorescent secondary anti-goat IgG (Licor, Lincoln, NE) and Odyssey CLx Imaging System (Licor). Both anti-human FGF23 antibodies were a kind gift from Jeffrey Lavigne (Immutopics, San Clemente, CA).



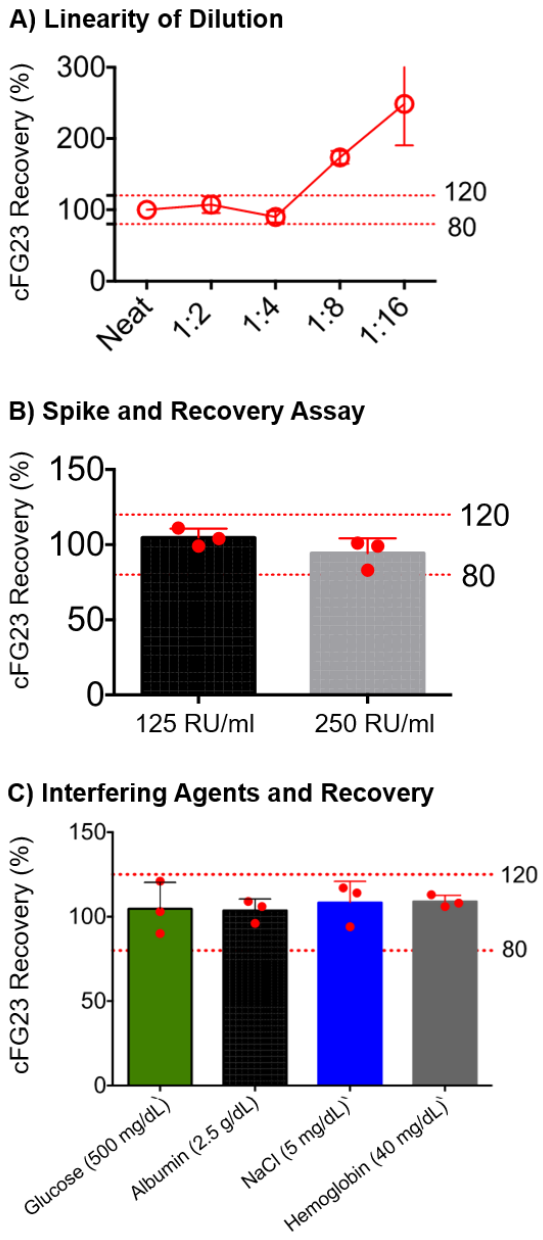
Supplemental Figure 1. Urinary cFGF23/Cr levels by dipstick proteinuria. Bars represent median levels.



Supplemental Figure 2. Urinary cFGF23/Cr levels and AKI stage on enrollment. Bars represent median with interquartile ranges. AKI was staged according to the criteria established by the Kidney Disease Improving Global Outcomes Work Group as follows: stage 1, an increase in serum creatinine ≥ 0.3 mg/dl within 48 hours or ≥ 50% within 7 days; stage 2, an increase in serum creatinine ≥ 100%; stage 3, an increase in serum creatinine ≥ 200%, or an increase ≥ 0.5 mg/dl to a level ≥ 4.0 mg/dl, or need for RRT.[2]



Supplemental Figure 3. Association between plasma cFGF23 levels and AKI/death within tertiles of phosphate, calcium, PTH, 25-hydroxyvitamin D, and 1,25-dihydroxyvitamin D. Plasma cFGF23 levels were natural log-transformed and normalized to one standard deviation. n=121, #events=42.



Supplemental Figure 4. Assay validation of the cFGF23 ELISA assay in urine. A) To assess linearity of dilution, urine samples (n=3) collected from AKI patients were diluted 1:2, 1:4, 1:8 and 1:16 in diluent buffer and the amount of FGF23 recovery was measured. B) Spike and recovery was assessed by spiking neat urine samples (n=3) from AKI patients with 125 RU/ml or 250 RU/ml of recombinant FGF23. C) Urine samples (n=3) from AKI patients were spiked with supraphysiological concentrations of interfering agents, including glucose, albumin, NaCl and hemoglobin, and recovery was calculated. Values are represented as mean ± SD.

End Points	#Events	Unadjusted			Model 1			Model 2		
		Odds Ratio (95% CI)		*P*-value	Odds Ratio (95% CI)		*P*-value	Odds Ratio (95% CI)		*P*-value
Primary End Point										
AKI/Death	105	2.10 (1.43-3.10)		<0.001	1.74 (1.14-2.64)		0.01	1.74 (1.12-2.71)		0.01
Secondary End Points										
AKI	80	2.31 (1.57-3.38)		<0.001	1.94 (1.28-2.94)		0.002	1.81 (1.17-2.79)		0.007
Severe AKI*	37	2.08 (1.40-3.09)		<0.001	2.07 (1.34-3.20)		0.001	1.95 (1.23-3.07)		0.004
RRT/Death	63	1.83 (1.27-2.62)		0.001	1.76 (1.19-2.59)		0.004	1.72 (1.12-2.62)		0.01
Severe Sepsis or Septic Shock	194	1.57 (1.23-2.00)		<0.001	1.56 (1.21-2.02)		<0.001	1.35 (1.01-1.80)		0.04
Hospital Mortality	90	1.46 (1.16-1.84)		0.001	1.45 (1.13-1.87)		0.003	1.31 (0.99-1.75)		0.06
90-day Mortality	118	1.42 (1.14-1.77)		0.002	1.34 (1.06-1.70)		0.01	1.23 (0.94-1.60)		0.13
1-year Mortality	161	1.35 (1.09-1.68)		0.007	1.37 (1.08-1.74)		0.01	1.27 (0.97-1.66)		0.09
		ß	**S.E.**	***P*-value**	**ß**	**S.E.**	***P*-value**	**ß**	**S.E.**	***P*-value**
Ventilator-Free Days	N/A	-2.22	0.61	<0.001	-2.28	0.66	<0.001	-1.61	0.67	0.02
Hospital-Free Days	N/A	-2.07	0.44	<0.001	-2.13	0.48	<0.001	-1.80	0.50	<0.001

Supplemental Table 1. Urinary cFGF23 levels (without normalization to the urinary creatinine concentration) and adverse outcomes. Urinary cFGF23 levels were natural log-transformed, given their skewed distribution, and normalized to one standard deviation. Model 1 is adjusted for age, gender, baseline eGFR, hypertension, and diabetes mellitus. Model 2 is further adjusted for ICU type (surgical versus medical), mechanical ventilation, and APACHE II score. Analyses that include AKI or RRT as an end point are restricted to patients who did not have AKI on enrollment (n=243). All other analyses include the entire cohort (n=350). *Severe AKI was defined as doubling of serum creatinine or need for RRT.[2] Abbreviations: RRT/Death, renal replacement therapy or in-hospital mortality.

Exposure	Unadjusted		Adjusted*	
	Odds Ratio (95% CI)	*P*-value	Odds Ratio (95% CI)	*P*-value
Plasma cFGF23	3.06 (1.71-5.47)	<0.001	2.66 (1.42-5.00)	0.002
Urinary cFGF23/Cr	2.60 (1.51-4.49)	<0.001	2.04 (1.17-3.56)	0.01

Supplemental Table 2. Plasma and urinary cFGF23 levels and risk of AKI/death. *In the adjusted models, plasma and urinary cFGF23 are adjusted for each other.

Supplemental References

1. Smith ER, Cai MM, McMahon LP, Holt SG. Biological variability of plasma intact and C-terminal FGF23 measurements. *J Clin Endocrinol Metab* 97**:** 3357-3365, 2012.

2. Kidney Disease; Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO clinical practice guideline for acute kidney injury. *Kidney Int Suppl 2***:** 1-138, 2012.