Supplemental Online Content

Leaf DE *et al.* Fibroblast Growth Factor 23 and Death in Critically Ill Patients

Tables

Figures

Characteristic	All Patients in the ATN Study (n=1124)	Biorepository Subcohort (n=817)	P Value
Demographics			
Age, yr, median (IQR)	61 (50–71)	62 (51–72)	0.30
Male sex – no. (%)	793 (71)	568 (70)	0.60
White race – no. (%)	835 (74)	628 (77)	0.19
Comorbidities – no. (%)			
Diabetes mellitus	275 (25)	199 (25)	0.90
Congestive heart failure	260 (24)	198 (25)	0.63
Chronic liver disease	131 (12)	95 (12)	0.95
Chronic lung disease†	135 (12)	103 (13)	0.74
Chronic kidney disease‡	390 (37)	293 (38)	0.67
Malignancy	224 (20)	158 (19)	0.74
Baseline kidney function			
Creatinine, mg/dl, median (IQR)§	1.1 (0.9–1.4)	1.1 (0.9–1.4)	0.94
eGFR, median (IQR)‖	71 (52–94)	71 (50–94)	0.62
Medical ICU – no. (%)	531 (47)	361 (44)	0.18
Severity of illness			
Sepsis – no. (%)	708 (63)	527 (65)	0.49
APACHE II score, median (IQR)**	26 (21–32)	26 (21–31)	0.64
Mechanical ventilation – no. (%)	905 (81)	661 (81)	0.86
Hypotension – no. (%)††	695 (62)	515 (63)	0.59
Enrollment labs, median (IQR)			
White cell count, per mm^3	13 (8–19)	13 (9–19)	0.68
Hemoglobin, g/dl	10 (9–11)	10 (9–11)	0.83
Creatinine, mg/dl	3.9 (2.9–5.1)	3.9 (2.9–5.1)	0.67
Albumin, g/dl	2.3 (1.9–2.9)	2.3 (1.9–2.8)	0.70
60-day mortality – no. (%)	591 (53)	415 (51)	0.41

Supplemental Table 1. Enrollment characteristics in the ATN study, all patients versus biorepository subcohort. Percentages are based on the number of patients without missing data.

†Defined as chronic hypoxemia, hypercapnea, pulmonary hypertension, or ventilator dependence.
‡Defined as baseline eGFR<60 ml/min/1.73m^2.
§Baseline serum creatinine (SCr) was defined as the pre-morbid SCr at the time of screening or, if unavailable, the lowest SCr within 4 days prior to screening.
‖eGFR is reported in ml/min per 1.73m^2 and was determined using the CKD-EPI equation.
**APACHE II score is an ICU severity of illness scoring system ranging from 0 to 71, with higher scores indicating more severe disease.
††Defined as requirement for vasopressor support for greater than 1 hour.

Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; IQR, interquartile range; SCr, serum creatinine.

Biomarker	Measured in Singulate or Duplicate	Assay Type	Manufacturer	Lower Limit of detection	Interassay CV (%)
ATN Study					
cFGF23	Duplicate	ELISA	Immutopics	1.5 RU/ml	9.4
iFGF23	Duplicate	ELISA	Immutopics	1 pg/ml	5.1
25D	Singulate	LCMS	N/A	1.0 ng/ml	6.7
1,25D	Singulate	LCMS	N/A	3.4 pg/ml	12.0
$24,25D_3$	Singulate	LCMS	N/A	0.06 ng/ml	9.1
PTH	Singulate	ELISA	LifeSpan Biosciences	1.27 pg/ml	4.8
VALID					
cFGF23	Singulate	ELISA	Immutopics	1.5 RU/ml	4.2
iFGF23	Singulate	ELISA	Immutopics	1 pg/ml	3.3

Supplemental Table 2. Assay performance characteristics. Abbreviations: 25D, 25-hydroxyvitamin D; 1,25D, 1,25-dihydroxyvitamin D; $24,25D_3$, 24,25-dihydroxyvitamin D_3; cFGF23, C-terminal fibroblast growth factor 23; CV, coefficient of variation (calculated from blinded replicate samples); ELISA, enzyme-linked immunosorbent assay; iFGF23, intact fibroblast growth factor 23; LCMS, liquid chromatography-tandem mass spectrometry; PTH, parathyroid hormone.

Characteristic	cFGF23 quartiles in the ATN Study					cFGF23 quartiles in VALID				
cFGF23, RU/ml – range	Q1 (n=204) <1072	Q2 (n=204) 1078–3587	Q3 (n=204) 3608–12,951	Q4 (n=205) >12,983	P_{trend}	Q1 (n=177) <211	Q2 (n=177) 212–595)	Q3 (n=178) 602–1868	Q4 (n=178) >1886	P_{trend}
Demographics										
Age, yr, median (IQR)	62 (52–74)	63 (53–74)	62 (52–73)	60 (49–70)	0.20	53 (35–63)	53 (42–67)	61 (47–70)	59 (50–71)	<0.001
Male sex – no. (%)	146 (72)	147 (72)	144 (71)	131 (64)	0.24	123 (69)	109 (62)	111 (62)	96 (54)	0.03
White race – no. (%)	151 (74)	156 (76)	152 (75)	169 (82)	0.16	145 (82)	154 (87)	164 (92)	162 (91)	0.01
Comorbidities – no. (%)										
Diabetes mellitus	44 (22)	52 (27)	53 (27)	50 (25)	0.74	24 (14)	38 (21)	53 (30)	51 (29)	<0.001
Congestive heart failure	39 (20)	40 (21)	44 (22)	75 (38)	<0.001	8 (5)	20 (11)	26 (15)	36 (20)	<0.001
Chronic liver disease	10 (5)	13 (7)	38 (19)	34 (17)	<0.001	8 (5)	8 (5)	17 (10)	44 (25)	<0.001
Chronic lung disease/COPD[†]	17 (9)	36 (18)	23 (12)	27 (14)	0.04	22 (12)	33 (19)	24 (13)	28 (16)	0.37
Chronic kidney disease[‡]	74 (40)	82 (41)	66 (34)	71 (38)	0.49	12 (7)	41 (23)	56 (31)	74 (42)	<0.001
Malignancy	35 (17)	49 (24)	33 (16)	41 (20)	0.18	21 (12)	38 (21)	22 (12)	28 (16)	0.05
Baseline kidney function										
Creatinine, mg/dl, median (IQR)[§]	1.1 (0.9–1.4)	1.1 (0.9–1.4)	1.0 (0.8–1.4)	1.0 (0.8–1.3)	0.16	0.7 (0.6–0.9)	0.9 (0.7–1.1)	0.9 (0.7–1.3)	1.0 (0.8–1.5)	<0.001
eGFR, median (IQR)[‖]	71 (51–94)	67 (47–92)	72 (52–95)	70 (50–94)	0.41	107 (88–128)	93 (62–113)	84 (55–106)	68 (43–94)	<0.001
Medical ICU – no. (%)	105 (51)	89 (44)	84 (41)	83 (40)	0.10	62 (35)	82 (46)	74 (42)	111 (62)	<0.001
Severity of illness										
Sepsis – no. (%)	116 (57)	131 (64)	132 (65)	148 (72)	0.01	55 (31)	69 (40)	84 (47)	89 (50)	0.001
APACHE II score, median (IQR)[¶]	23 (18–29)	25 (20–30)	27 (22–32)	28 (25–33)	<0.001	21 (17–25)	24 (20–29)	27 (22–33)	29 (25–35)	<0.001
Mechanical ventilation – no. (%)	140 (69)	161 (79)	180 (88)	180 (88)	<0.001	132 (75)	124 (70)	133 (75)	123 (69)	0.51
Hypotension – no. (%)[††]	96 (47)	103 (50)	143 (70)	173 (84)	<0.001	82 (46)	100 (57)	127 (71)	141 (79)	<0.001
AKI and RRT data on enrollment										
AKI – no. (%)[‡‡]	204 (100)	204 (100)	204 (100)	205 (100)	0.99	58 (33)	71 (40)	96 (54)	123 (69)	<0.001
AKI severity					0.99					<0.001
Stage 1 – no. (%)	0 (0)	0 (0)	0 (0)	0 (0)		49 (28)	50 (28)	66 (37)	73 (41)	
Stage 2 – no. (%)	0 (0)	0 (0)	0 (0)	0 (0)		6 (3)	19 (11)	25 (14)	31 (17)	
Stage 3 – no. (%)	204 (100)	204 (100)	204 (100)	205 (100)		3 (2)	2 (1)	5 (3)	19 (11)	
Oliguria – no. (%)[§§]	139 (68)	153 (75)	174 (85)	188 (92)	<0.001	–	–	–	–	–
RRT before enrollment – no. (%)	135 (66)	132 (65)	152 (75)	142 (69)	0.15	–	–	–	–	–
Enrollment labs, median (IQR)										
White cell count, per mm^3	13 (8–17)	13 (8–18)	13 (9–19)	16 (10–23)	0.001	13 (9–17)	14 (10–19)	13 (8–19)	15 (9–21)	0.04
Hemoglobin, g/dl	9.9 (9.1–10.9)	9.9 (9.1–10.8)	9.7 (8.8–10.8)	9.7 (8.8–10.5)	0.13	10.5 (9.3–11.8)	9.9 (9.1–11.2)	9.7 (8.6–10.8)	9.4 (8.5–10.5)	<0.001
Creatinine, mg/dl	4.4 (3.1–5.9)	4.1 (3.2–5.3)	3.8 (2.8–4.9)	3.3 (2.7–4.4)	<0.001	0.9 (0.7–1.1)	1.0 (0.8–1.3)	1.4 (0.9–1.9)	1.8 (1.2–2.7)	<0.001
Albumin, g/dl	2.3 (1.9–2.7)	2.2 (1.7–2.7)	2.4 (2.0–2.8)	2.5 (2.0–3.0)	0.004	–	–	–	–	

Supplemental Table 3. Enrollment characteristics across cFGF23 and iFGF23 quartiles

Characteristic	iFGF23 quartiles in the ATN Study					iFGF23 quartiles in VALID				
iFGF23, pg/ml – range	Q1 (n=100) <30	Q2 (n=100) 30–88	Q3 (n=100) 88–240	Q4 (n=100) >241	P_{trend}	Q1 (n=177) <24	Q2 (n=177) 24–44	Q3 (n=178) 44–87	Q4 (n=178) >87	P_{trend}
Demographics										
Age, yr, median (IQR)	61 (44–72)	63 (54–76)	62 (53–75)	62 (54–72)	0.20	55 (37–65)	55 (44–67)	59 (46–68)	58 (50–70)	0.007
Male sex – no. (%)	58 (58)	72 (72)	64 (64)	77 (77)	0.02	122 (69)	111 (63)	104 (58)	102 (57)	0.10
White race – no. (%)	76 (76)	77 (77)	80 (80)	77 (77)	0.92	158 (89)	156 (88)	158 (89)	153 (86)	0.78
Comorbidities – no. (%)										
Diabetes mellitus	19 (21)	30 (30)	20 (21)	30 (30)	0.19	36 (20)	41 (23)	47 (26)	42 (24)	0.61
Congestive heart failure	15 (16)	18 (19)	31 (32)	43 (45)	<0.001	12 (7)	18 (10)	24 (13)	36 (20)	0.001
Chronic liver disease	10 (11)	12 (13)	10 (10)	13 (13)	0.91	12 (7)	13 (7)	20 (11)	32 (18)	0.002
Chronic lung disease/COPD[†]	6 (6)	10 (10)	14 (14)	14 (14)	0.22	21 (12)	24 (14)	37 (21)	25 (14)	0.09
Chronic kidney disease[‡]	30 (31)	41 (44)	33 (36)	49 (51)	0.03	20 (11)	29 (16)	53 (30)	81 (46)	<0.001
Malignancy	18 (18)	22 (22)	20 (20)	23 (23)	0.83	31 (18)	26 (15)	25 (14)	27 (15)	0.82
Baseline kidney function										
Creatinine, mg/dl, median (IQR)[§]	1.0 (0.8–1.2)	1.1 (0.9–1.4)	1.0 (0.8–1.3)	1.3 (1.0–1.5)	<0.001	0.7 (0.6–0.9)	0.8 (0.6–1.0)	0.9 (0.7–1.2)	1.1 (0.9–1.5)	<0.001
eGFR, median (IQR)[‖]	71 (55–98)	67 (51–85)	73 (49–95)	59 (44–83)	0.01	103 (87–125)	96 (72–116)	84 (54–107)	63 (42–94)	<0.001
Medical ICU – no. (%)	47 (47)	51 (51)	38 (38)	38 (38)	0.15	70 (40)	67 (38)	75 (42)	117 (66)	<0.001
Severity of illness										
Sepsis– no. (%)	64 (64)	62 (62)	69 (69)	65 (65)	0.77	82 (46)	58 (33)	64 (36)	93 (52)	<0.001
APACHE II score, median (IQR)[¶]	27 (23–32)	27 (21–33)	27 (21–33)	26 (22–30)	0.85	24 (19–30)	24 (19–29)	26 (20–31)	28 (22–33)	<0.001
Mechanical ventilation – no. (%)	88 (88)	84 (84)	82 (82)	65 (65)	<0.001	135 (76)	135 (76)	127 (71)	115 (65)	0.04
Hypotension – no. (%)[††]	65 (65)	58 (58)	65 (65)	55 (55)	0.36	112 (63)	111 (63)	109 (61)	118 (66)	0.79
AKI and RRT data on enrollment										
AKI – no. (%)[‡‡]	100 (100)	100 (100)	100 (100)	100 (100)	0.99	83 (47)	67 (38)	78 (44)	120 (67)	<0.001
AKI severity					0.99					<0.001
Stage 1 – no. (%)	0 (0)	0 (0)	0 (0)	0 (0)		60 (34)	54 (31)	53 (30)	71 (40)	
Stage 2 – no. (%)	0 (0)	0 (0)	0 (0)	0 (0)		20 (11)	11 (6)	17 (10)	33 (19)	
Stage 3 – no. (%)	100 (100)	100 (100)	100 (100)	100 (100)		3 (2)	2 (1)	8 (4)	16 (9)	
Oliguria – no. (%)[§§]	88 (88)	82 (82)	74 (74)	77 (77)	0.07	–	–	–	–	–
RRT before enrollment – no. (%)	71 (71)	70 (70)	72 (72)	55 (55)	0.03	–	–	–	–	–
Enrollment labs, median (IQR)										
White cell count, per mm^3	13 (8–20)	14 (8–19)	14 (10–20)	14 (10–23)	0.56	14 (9–19)	14 (10–19)	14 (9–19)	14 (9–19)	0.97
Hemoglobin, g/dl	9.8 (9.1–11.0)	9.9 (8.9–10.7)	9.7 (9.0–10.5)	9.8 (8.9–11.1)	0.58	9.9 (8.8–10.9)	10.0 (9.1–11.4)	10.3 (8.9–11.2)	9.4 (8.7–10.5)	0.003
Creatinine, mg/dl	3.6 (2.6–4.6)	3.7 (2.9–5.4)	3.8 (2.9–5.0)	4.4 (3.5–5.4)	0.003	1.0 (0.8–1.2)	1.0 (0.8–1.3)	1.3 (0.9–1.7)	1.7 (1.2–2.9)	<0.001
Albumin, g/dl	2.3 (1.9–2.8)	2.1 (1.7–2.6)	2.3 (1.9–2.9)	2.6 (2.1–3.0)	<0.001	–	–	–	–	

Supplemental Table 3 (continued)

Supplemental Table 3. Enrollment characteristics across cFGF23 and iFGF23 quartiles. Percentages are based on the number of patients without missing data. P values were calculated using Kruskal Wallis and chi-squared tests for continuous and categorical variables, respectively, across the four quartiles of cFGF23 and iFGF23.

[†]Defined in the ATN study as chronic hypoxemia, hypercapnea, pulmonary hypertension, or ventilator dependence; defined in VALID as COPD.
[‡]Defined as baseline eGFR<60 ml/min/1.73m^2.
[§]Defined in the ATN study as the pre-morbid serum creatinine (SCr) at the time of screening or, if unavailable, the lowest SCr within 4 days prior to screening; defined in VALID as the lowest SCr within 365 days prior to hospitalization. If no SCr values were available prior to hospitalization, the lowest SCr during hospitalization (excluding values obtained during renal replacement therapy) was used as the baseline.
[‖]Reported in ml/min per 1.73m^2 and determined using the CKD-EPI equation.
[**]An ICU severity of illness scoring system ranging from 0 to 71, with higher scores indicating more severe disease.
[††]Defined in the ATN study as requirement for vasopressor support for greater than 1 hour; defined in VALID as systolic blood pressure ≤90 mm Hg or need for vasopressor support.
[‡‡]All patients in the ATN study had AKI requiring renal replacement therapy on enrollment; in VALID, AKI on enrollment was defined as an increase in SCr ≥ 0.3 mg/dl within 48 hours or ≥ 50% within 7 days prior to enrollment.
[§§]Defined in the ATN study as an average urine output <20ml/h for >24h prior to enrollment.

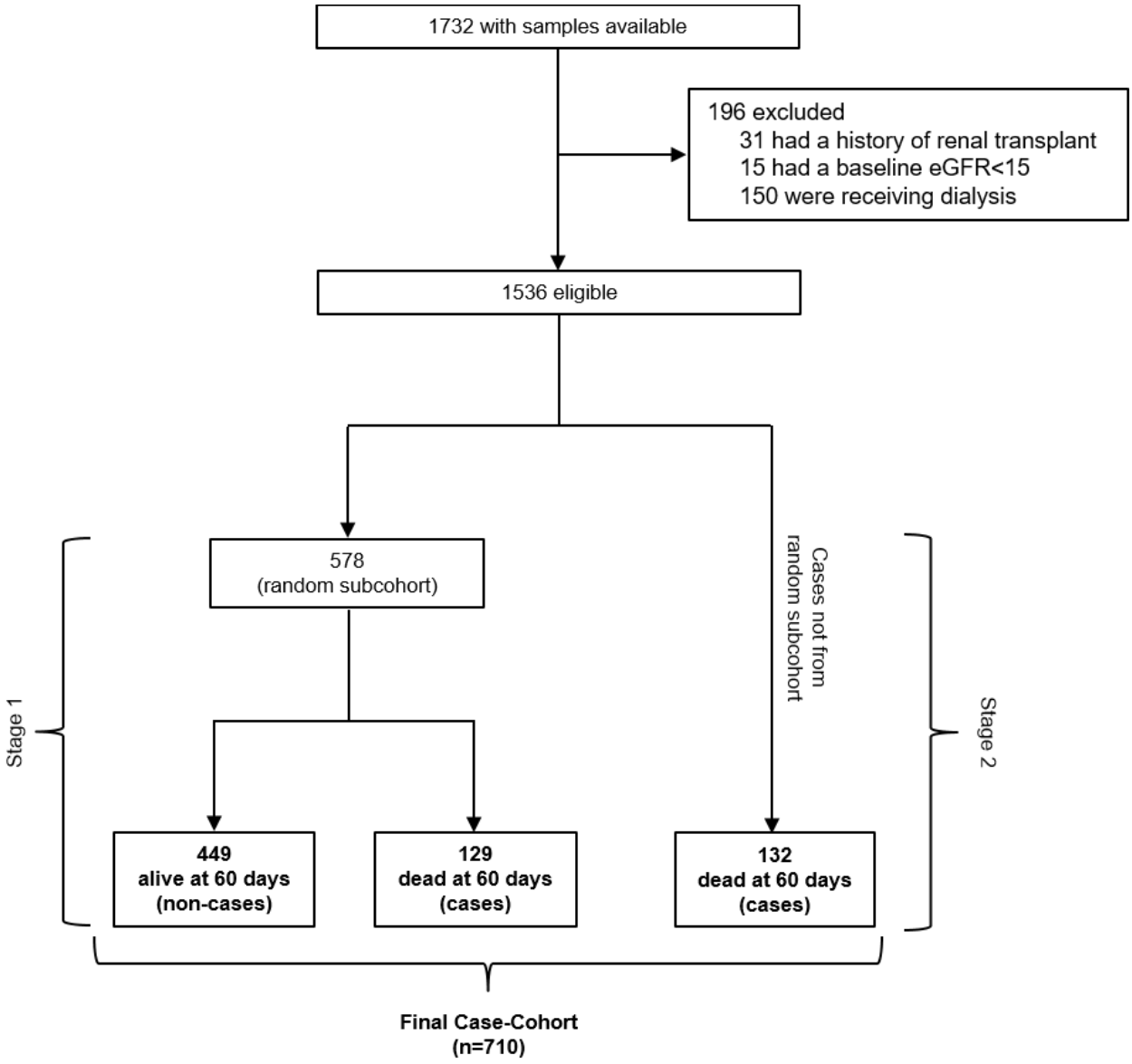
Abbreviations: AKI, Acute Kidney Injury; APACHE II, Acute Physiology and Chronic Health Evaluation II; COPD, chronic obstructive pulmonary disease; CRRT, continuous renal replacement therapy; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; IQR, interquartile range.

	Odds Ratios (and 95% CIs) for Death				
	Model 3	**Model 3 + 25D**	**Model 3 + 1,25D**	**Model 3 + 25D and 1,25D**	**Model 3 + 25D, 1,25D, and PO_4**
cFGF23 (RU/ml)	1.57 (1.17–2.12)	1.61 (1.19–2.17)	1.56 (1.16–2.11)	1.59 (1.18–2.16)	1.60 (1.18–2.17)
iFGF23 (pg/ml)	1.40 (1.08–1.84)	1.47 (1.12–1.92)	1.38 (1.05–1.81)	1.47 (1.11–1.94)	1.52 (1.13–2.05)

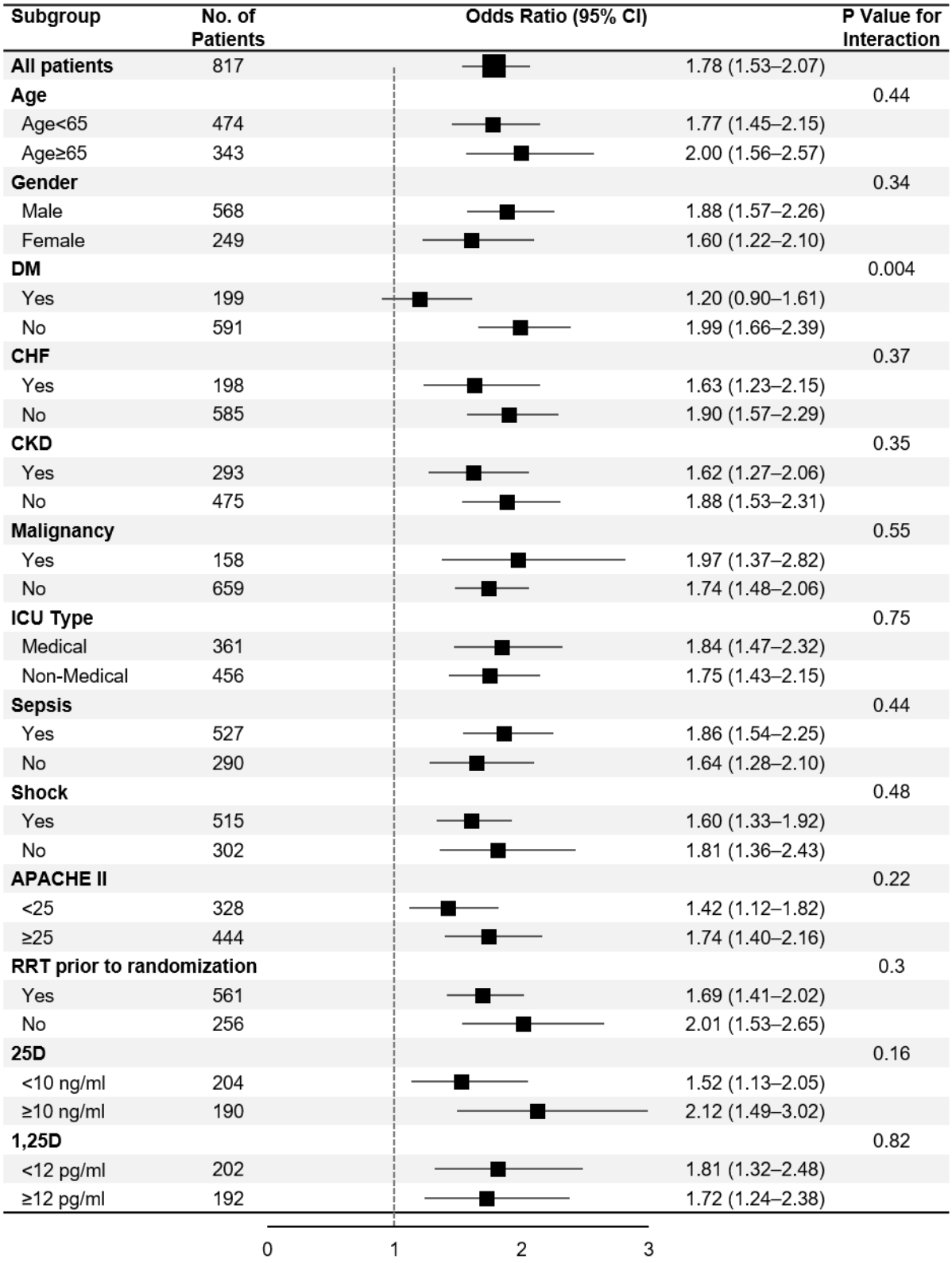
Supplemental Table 4. Odds Ratios (and 95% CIs) for Death According to FGF23 Levels in the ATN Study – Exploratory Analysis further adjusted for Vitamin D Metabolite and Phosphate Levels. Model 3 is adjusted for demographics and comorbidities (age, gender, race, baseline eGFR, diabetes, cancer, congestive heart failure, and chronic liver disease) as well as severity of illness (ICU type, mechanical ventilation, APACHE II score, RRT before randomization, treatment group, type of RRT, oliguria, sepsis, hypotension, WBC count, hemoglobin, and serum/plasma levels of albumin, creatinine, and interleukin-6). "Model 3 + 25D" is adjusted for the covariates in model 3, as well as 25-hydroxyvitamin D (25D) levels. "Model 3 +1,25D" is adjusted for the covariates in model 3, as well as 1,25-dihydroxyvitamin D (1,25D) levels. "Model 3 + 25D and 1,25D" is adjusted for the covariates in model 3, as well as both 25D and 1,25D levels. "Model 3 + 25D, 1,25D, and PO4" is adjusted for the covariates in model 3, as well as 25D, 1,25D, and phosphate (PO4) levels. In all models, FGF23 levels are natural log-transformed and standardized to one standard deviation.

Exposure	Unadjusted		Adjusted*	
	Odds Ratio (95% CI)	*P*-value	Odds Ratio (95% CI)	*P*-value
cFGF23	1.76 (1.41–2.20)	<0.001	1.81 (1.43–2.28)	<0.001
iFGF23	1.09 (0.89–1.32)	0.42	0.92 (0.74–1.13)	0.43

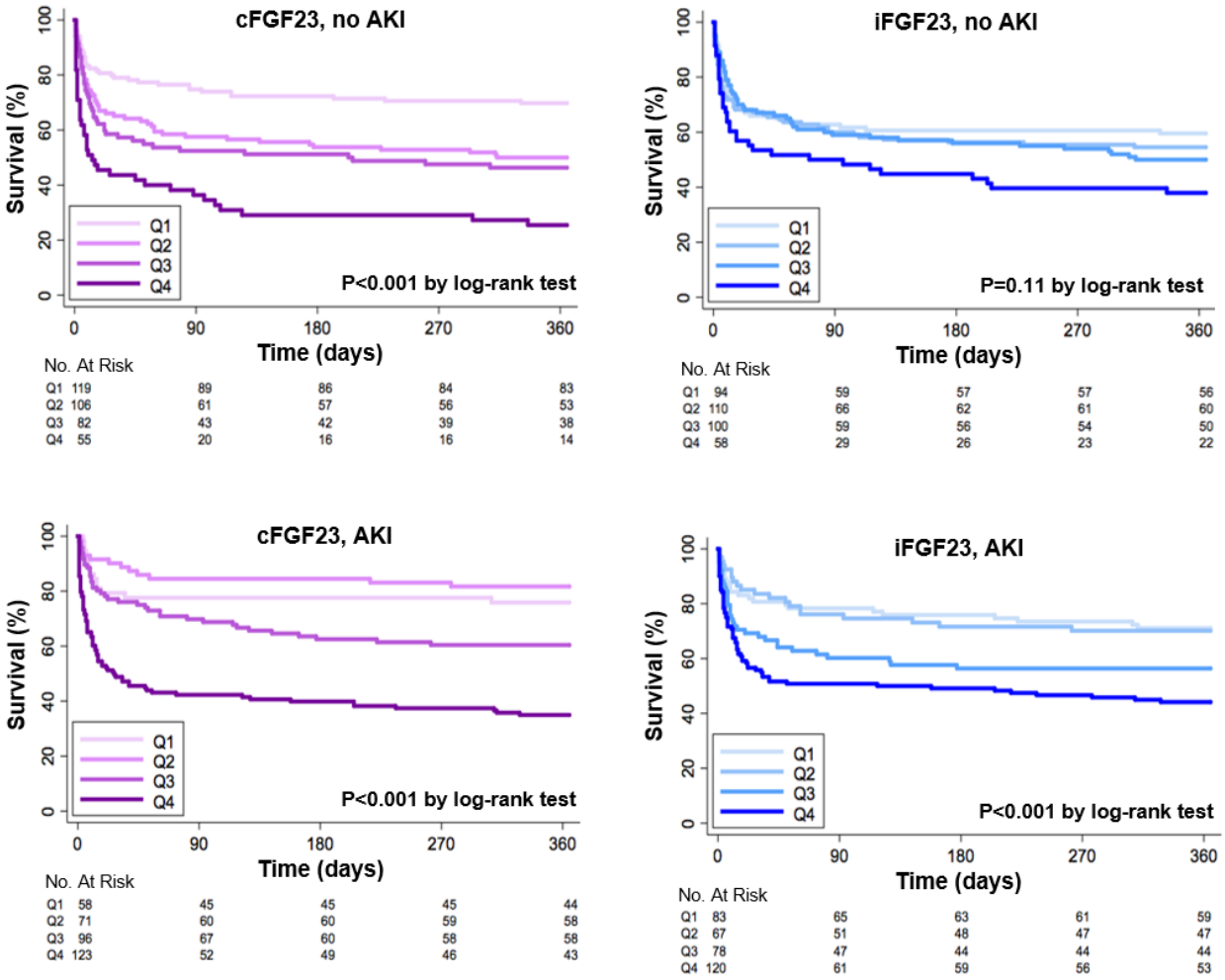
Supplemental Table 5. Odds Ratios (and 95% CIs) for Death According to FGF23 Levels in the ATN Study – Exploratory Analysis in which cFGF23 and iFGF23 are adjusted for each other. *In the adjusted models, cFGF23 and iFGF23 are adjusted for each other. These analyses are limited to patients who had both cFGF23 and iFGF23 levels available (n=400). FGF23 levels are natural log-transformed and standardized to one standard deviation.



Supplemental Figure 1. Patient flow diagram for the case-cohort design in VALID. The case-cohort in VALID was constructed using a conventional two-stage process. In stage 1, a random subcohort (n=578) was selected out of the 1536 eligible patients. Among the patients in this random subcohort, 449 were alive at 60 days (non-cases), and 129 were dead at 60 days (cases). In stage 2, 132 additional cases (patients who were not included in the random subcohort from stage one and who died by day 60) were added to create the final case-cohort of n=710. Thus, the total number of cases includes cases from both the random subcohort (n=129) as well as oversampled cases from outside the random subcohort (n=132).



Supplemental Figure 2. Subgroup analyses. The forest plot shows risk of 60-day mortality based on plasma cFGF23 levels in subgroups from the ATN study. cFGF23 levels were natural log-transformed and standardized to one standard deviation.



Supplemental Figure 3. Risk of 1-Year Mortality According to cFGF23 and iFGF23 quartiles in VALID, stratified by AKI status on enrollment. Kaplan-Meier curves showing survival during the first year following enrollment by cFGF23 and iFGF23 quartiles, stratified according to AKI status on enrollment. AKI was defined as an increase in serum creatinine ≥ 0.3 mg/dl within 48 hours or ≥ 50% within 7 days prior to enrollment.