PLOS ONE

RESEARCH ARTICLE

Plasma total fibroblast growth factor 23 levels are associated with acute kidney injury and mortality in children with acute respiratory distress syndrome

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Citation: Hanudel MR, Zinter MS, Chen L, Gala K, Lim M, Guglielmo M, et al. (2019) Plasma total fibroblast growth factor 23 levels are associated with acute kidney injury and mortality in children with acute respiratory distress syndrome. PLoS ONE 14(9): e0222065. https://doi.org/10.1371/journal.pone.0222065

Editor: Yu Ru Kou, National Yang-Ming University, TAIWAN

Received: July 15, 2019

Accepted: August 21, 2019

Published: September 5, 2019

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: The work in this manuscript has been performed with the support of Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) research grants UG1-HD083166 (AS) and R01-HD092471 (AS).

Competing interests: The authors have declared that no competing interests exist.

Abstract

Acute respiratory distress syndrome (ARDS) has high rates of mortality and multisystem morbidity. Pre-clinical data suggest that fibroblast growth factor 23 (FGF23) may contribute to pulmonary pathology, and FGF23 is associated with mortality and morbidity, including acute kidney injury (AKI), in non-ARDS cohorts. Here, we assess whether FGF23 is associated with AKI and/or mortality in a cohort of 161 pediatric ARDS patients. Plasma total (intact + C-terminal) FGF23 and intact FGF23 concentrations were measured within 24 hours of ARDS diagnosis (Day 1), and associations with Day 3 AKI and 60-day mortality were evaluated. 35 patients (22%) developed AKI by 3 days post-ARDS diagnosis, and 25 (16%) died by 60 days post-ARDS diagnosis. In unadjusted models, higher Day 1 total FGF23 was associated with Day 3 AKI (odds ratio (OR) 2.22 [95% confidence interval (CI) 1.62, 3.03], p<0.001), but Day 1 intact FGF23 was not. In a model adjusted for demographics and disease severity, total FGF23 remained associated with AKI (OR 1.52 [95% CI 1.02, 2.26], p = 0.039). In unadjusted models, both higher Day 1 total and intact FGF23 were associated with 60-day mortality (OR 1.43 [95% CI 1.07, 1.91], p = 0.014; and OR 1.44 [95% CI 1.02, 2.05], p = 0.039, respectively). In the adjusted model, only total FGF23 remained associated with 60-day mortality (OR 1.62 [95% CI 1.07, 2.45], p = 0.023). In a subgroup analysis of patients with Day 1 plasma IL-6 concentrations available, inflammation partially mediated the association between total FGF23 and AKI. Our data suggest both inflammation-dependent and inflammation-independent associations between total FGF23 and clinical outcomes in pediatric ARDS patients.

Introduction

Acute respiratory distress syndrome (ARDS) is defined as the presence of hypoxia in the context of a new lung infiltrate occurring within seven days of a known insult [1]. In children, ARDS is accompanied by high mortality rates—with an estimated overall mortality rate of 24% [2]—and extra-pulmonary comorbidities, including renal dysfunction, which occurs commonly and contributes substantially to morbidity and mortality [3–5]. ARDS may be precipitated by a pulmonary insult, such as pneumonia or aspiration (direct ARDS), or by a non-pulmonary insult, such as sepsis or transfusion reaction (indirect ARDS), resulting in non-cardiogenic pulmonary edema and massive pulmonary inflammation [1, 6, 7].

One factor that may contribute to pulmonary inflammation is fibroblast growth factor 23 (FGF23). FGF23 is a predominantly bone-derived hormone that acts on the kidney and physiologically functions to maintain phosphate homeostasis; however, FGF23 can also have pathologic, "off-target" effects. Specifically, FGF23 can induce cardiomyocyte hypertrophy [8], impair neutrophil function [9], and stimulate hepatic secretion of the inflammatory cytokines interleukin-6 (IL-6) and C-reactive protein [10], as has been demonstrated in *in vitro* and murine studies. Recently, it has also been shown that FGF23 can stimulate IL-6 release from cultured bronchial epithelial cells [11], suggesting a possible pro-inflammatory role of FGF23 in pathologic pulmonary conditions such as ARDS.

Therefore, FGF23 may induce inflammation, but interestingly, inflammation also affects FGF23. Inflammation promotes FGF23 proteolysis, resulting in increased levels of FGF23 fragments [12]. In the circulation, concentrations of both total FGF23 (intact FGF23 + C-terminal FGF23) and intact FGF23 alone can be measured. Whereas intact FGF23 is known to be biologically active, the effects of FGF23 fragments remain unclear.

In many human cohorts, higher circulating concentrations of total FGF23 and/or intact FGF23 have been associated with adverse clinical outcomes, including all-cause mortality [13–16], cardiovascular morbidity [8, 17–20], progression of chronic kidney disease [14, 21, 22], development of acute kidney injury (AKI) [23–28], and infection-related hospitalization [29]. However, whether FGF23 levels are associated with poor clinical outcomes in pediatric ARDS is unknown, and characterization of the FGF23 profile in this population may improve risk stratification and better define the pathophysiology of this heterogeneous clinical condition [30]. Therefore, in the current study, we assessed whether circulating total and intact FGF23 levels are associated with the development of AKI and/or mortality in a multicenter cohort of pediatric ARDS patients.

Methods

Study subjects

Data were collected from a multicenter observational study of pediatric intensive care unit patients with ARDS admitted between 2008 and 2016. Subjects were enrolled in five academic pediatric intensive care units: Children's Hospital Los Angeles; Children's Hospital Central California; American Family Children's Hospital, University of Wisconsin-Madison; and the University of California San Francisco (UCSF) Benioff Children's Hospitals in Oakland and San Francisco. The study was approved by the individual Institutional Review Boards at participating centers.

Pediatric patients with bilateral chest X-ray infiltrates, receiving respiratory support in the form of continuous positive airway pressure (CPAP), bilevel positive airway pressure (BiPAP), or invasive positive pressure ventilation, were screened for eligibility. Guardians were approached for informed written consent if the patients met the American-European

Consensus Conference definition of Acute Lung Injury/Acute Respiratory Distress Syndrome [31]. Chest x-ray results used for diagnosing ARDS were based on interpretations performed by site investigators. Exclusion criteria included patients <1 month of age, <36 weeks corrected gestational age, >18 years of age, and/or with a documented Do Not Resuscitate or Do Not Intubate order at the time of screening.

Data collection

Demographic data, anthropometric data, and causes of lung injury were obtained from the medical record. On the day of ARDS diagnosis (Day 1), plasma was collected for measurement of total (intact + C-terminal) FGF23 and intact FGF23, assessed with ELISA kits (Quidel, San Diego, CA). FGF23 can be proteolytically cleaved into fragments. The total FGF23 assay uses a C-terminal capture antibody and a C-terminal detection antibody, both of which recognize epitopes distal to the FGF23 cleavage site. Thus, the total FGF23 assay detects both full-length, intact FGF23 protein and C-terminal FGF23 proteolytic fragments. Conversely, the intact FGF23 assay uses an N-terminal capture antibody and a C-terminal detection antibody, thus detecting only full-length FGF23 (**Fig 1**) [32]. As the human total FGF23 assay measures concentrations in RU/ml, but the human intact FGF23 assay measures concentrations in pg/ml, direct calculation of C-terminal FGF23 fragment concentrations is not possible with these assays.

Serum creatinine was assessed daily, and glomerular filtration rate (GFR) was estimated using the revised Schwartz equation [33]. Consistent with the pediatric Risk, Injury, Failure,

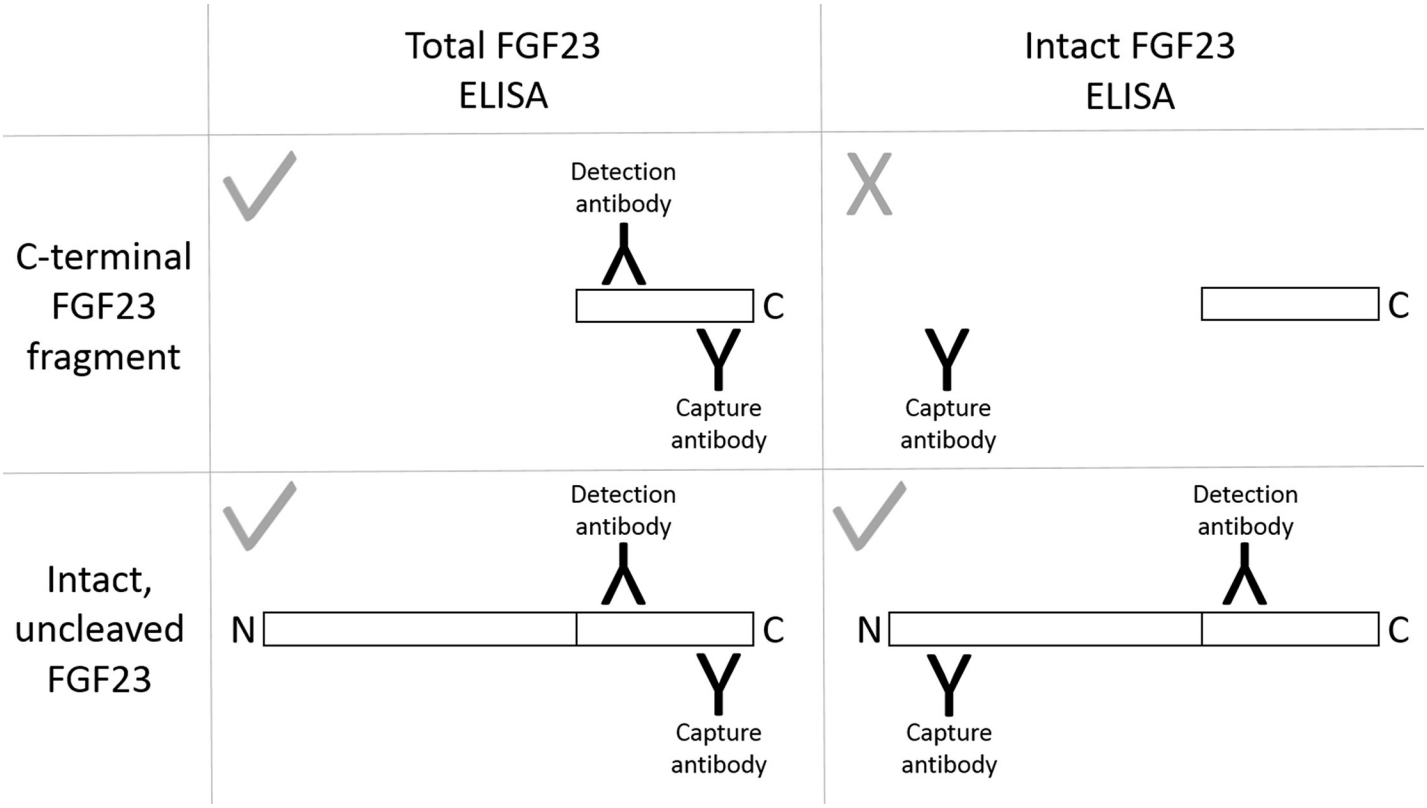

Fig 1. The total FGF23 and intact FGF23 enzyme-linked immunosorbent assays (ELISA). Whereas the total fibroblast growth factor 23 (FGF23) ELISA detects both the full-length, intact protein and its C-terminal proteolytic fragments, the intact FGF23 assay detects only the full-length form of the hormone.

https://doi.org/10.1371/journal.pone.0222065.g001

Loss, End-Stage (pRIFLE) criteria [34], AKI was defined as a decrease in estimated GFR (eGFR) of ≥50%. As baseline (prior to Day 1) serum creatinine measurements were not available, a baseline eGFR of 120 mL/min/1.73 m^2 was assumed, as has been done in previous analyses of AKI in this cohort [35]. As a measurement of disease severity, Pediatric Risk of Mortality (PRISM) 3 scores were calculated [36]. PRISM 3 is a score developed specifically for mortality prediction, utilizing the worst values of 17 laboratory and vital sign parameters from the first 24 hours of intensive care [36]. PaO_2/FiO_2 (P/F) ratios were also calculated. For those subjects without recorded arterial blood gas data, with pulse oximetry saturations between 80 and 97%, and for whom FiO_2 was available, predicted P/F was calculated using the saturation/FiO_2 ratio [37]. A subgroup of patients had Day 1 plasma IL-6 concentrations available, which were measured with a Luminex multiplex ELISA (Myriad RBM, Austin, TX).

Statistical analysis

All analyses were performed using STATA statistical software, version 13.1 (StataCorp, College Station, TX). Continuous data are presented as medians and interquartile ranges (IQR), and categorical data are presented as frequencies (percentage). Statistical tests used to compare data between groups were the Wilcoxon rank-sum test for continuous variables and the chi-squared test for categorical variables. Logistic regression modeling was performed to assess whether FGF23 levels at the time of diagnosis were associated with AKI at 3 days post-diagnosis or with mortality at 60 days post-diagnosis. Given skewed data distributions, natural log transformed FGF23 levels were used in the regression models. In the Day 3 AKI models, covariates included demographics (age, sex); measures of disease severity (P/F ratio, PRISM score); and the presence or absence of AKI at the time of diagnosis. In the 60-day mortality models, covariates included demographics (age, sex); measures of disease severity (P/F ratio, PRISM score); and the presence or absence of AKI. These models were fitted using a complete cases approach, and numbers of included patients are reported for each regression model. Separate analyses were performed including plasma IL-6 as a covariate, due to only a subset of patients having non-missing data. Given skewed data distributions, natural log transformed IL-6 was used in the regression models. Correlations between IL-6 and FGF23 concentrations were assessed with Spearman's rank correlation coefficients. Also, in this subset, we performed mediation analysis [38, 39] to quantify the degree to which variation in IL-6 concentrations mediated associations between FGF23 levels and clinical outcomes. In all analyses, a two-sided p-value <0.05 was considered significant.

Results

Cohort characteristics

The cohort included 161 pediatric ARDS patients, 57% male, 70% Caucasian, with a median age of 4.4 [IQR 1.1, 11.7] years (**Table 1**). The major risk factors for ARDS were pneumonia (60%), sepsis (19%), and aspiration (6%). At the time of ARDS diagnosis (Day 1), the median plasma total FGF23 level in this cohort was markedly elevated at 223 [IQR 114, 774] RU/ml. (In healthy children, median total FGF23 concentrations range from 50 to 105 RU/ml, depending on age [40].) Contrastingly, the Day 1 median intact FGF23 level was not elevated (23 [IQR 11, 60] pg/ml). (In healthy children, the median intact FGF23 concentration is 35 [range 9–120] pg/ml [41].) Of the 161 subjects, 35 (22%) had AKI on Day 3 post-ARDS diagnosis (**S1 Table**), and 25 (16%) died by 60 days post-ARDS diagnosis.

Table 1. Cohort characteristics at the time of ARDS diagnosis, stratified by the presence/absence of Day 3 AKI and stratified by 60-day mortality.

Variable	All (n = 161)	No Day 3 AKI (n = 126, 78%)	Day 3 AKI (n = 35, 22%)	p value	Survived (n = 136, 84%)	Deceased (n = 25, 16%)	p value
Age (years)	4.4 (1.1, 11.7)	5.9 (1.5, 11.8)	2.2 (0.4, 11.3)	0.21	3.4 (1.1, 11.3)	10.4 (2.9, 13.9)	0.11
Sex (% male)	92 (57%)	71 (56%)	21 (60%)	0.70	73 (54%)	19 (76%)	0.038
Race:				0.21			1.00
Caucasian/white	112 (70%)	91 (72%)	21 (60%)		95 (70%)	17 (68%)	
African-American/black	14 (9%)	9 (7%)	5 (14%)		12 (9%)	2 (8%)	
Asian/Pacific Islander	13 (8%)	8 (6%)	5 (14%)		11 (8%)	2 (8%)	
Other	22 (14%)	18 (14%)	4 (11%)		18 (13%)	4 (16%)	
ARDS etiology:				0.40			0.49
Pneumonia	96 (60%)	78 (62%)	18 (51%)		80 (58%)	16 (64%)	
Sepsis	30 (19%)	21 (17%)	9 (26%)		25 (18%)	5 (20%)	
Aspiration	9 (6%)	6 (5%)	3 (9%)		7 (5%)	2 (8%)	
Trauma	6 (4%)	5 (4%)	1 (3%)		5 (4%)	1 (4%)	
Transfusion	1 (1%)	1 (1%)	0 (0%)		1 (1%)	0 (0%)	
Other	18 (11%)	15 (12%)	3 (9%)		18 (13%)	0 (0%)	
Missing	1 (1%)	0 (0%)	1 (3%)		0 (0%)	1 (4%)	
Primary insult (% direct ARDS)	107 (66%)	86 (68%)	21 (62%)	0.48	89 (65%)	18 (75%)	0.36
Type of respiratory support:				0.67			0.65
Conventional mechanical ventilation	144 (89%)	112 (89%)	32 (91%)		121 (89%)	23 (92%)	
High frequency oscillatory ventilation	7 (4%)	6 (5%)	1 (3%)		6 (4%)	1 (4%)	
CPAP or BiPAP	10 (6%)	8 (6%)	2 (6%)		9 (7%)	1 (4%)	
Kidney function (% AKI)	35 (22%)	n/a	n/a	n/a	28 (21%)	7 (28%)	0.41
Day 1 PaO_2/FiO_2 ratio	153 (91, 228)	162 (93, 223)	129 (89, 243)	0.50	163 (92, 246)	135 (84, 172)	0.06
Day 1 oxygenation index	9 (5, 18)	8 (5, 16)	12 (6, 19)	0.25	8 (5, 16)	10 (8, 24)	0.07
Day 1 PRISM score	11 (6, 18)	10 (5, 17)	16 (10, 22)	0.001	11 (5, 17)	15 (9, 19)	0.04
Day 1 serum IL-6 (pg/ml) (n = 135)	75 (26, 227)	70 (18, 185)	138 (41, 1540)	0.004	72 (24, 222)	107 (28, 689)	0.21
Day 1 plasma total FGF23 (RU/ml)	223 (114, 774)	166 (89, 466)	944 (357, 6556)	<0.001	202 (100, 669)	529 (172, 1490)	0.017
Day 1 plasma intact FGF23 (pg/ml)	23 (11, 60)	21 (11, 41)	28 (13, 105)	0.09	21 (10, 43)	38 (13, 128)	0.037

Data presented as numbers (percentages) or medians (interquartile range).

https://doi.org/10.1371/journal.pone.0222065.t001

Day 1 FGF23 levels and Day 3 AKI

Cohort characteristics at the time of ARDS diagnosis, stratified by the presence or absence of Day 3 AKI, are shown in Table 1. Day 1 total FGF23 concentrations were significantly higher in subjects with Day 3 AKI than in those without Day 3 AKI (median 944 [IQR 357, 6556] RU/ml vs. median 166 [IQR 89, 466] RU/ml, p<0.001) (Fig 2A). However, Day 1 intact FGF23 levels did not significantly differ between the two groups (median 28 [IQR 13, 105] pg/ml in the AKI group vs. median 21 [IQR 11, 41] pg/ml in the non-AKI group, p = 0.09) (Fig 2B).

Higher Day 1 total FGF23 levels were significantly associated with the presence of Day 3 AKI (odds ratio (OR) 2.21 [95% confidence interval (CI) 1.61, 3.03], p<0.001) (Table 2, **Model 1**). The association persisted after adjustment for the presence of Day 1 AKI (OR 1.51 [95% CI 1.05, 2.17], p = 0.027) (Table 2, **Model 2**), and after further adjustment for Day 1 covariates (age, sex, P/F ratio, and PRISM score) (OR 1.52 [95% CI 1.02, 2.26], p = 0.039) (Table 2, **Model 3**). On the contrary, Day 1 intact FGF23 concentrations were not significantly associated with Day 3 AKI in any of the models.

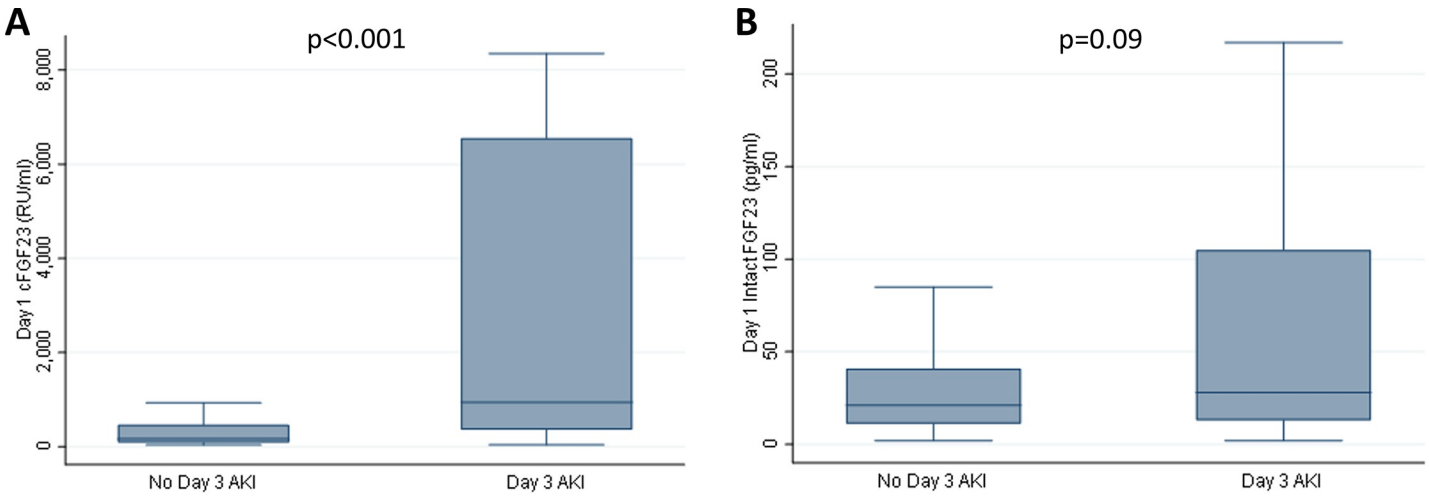

Fig 2. Day 1 plasma FGF23 concentrations stratified by the presence or absence of Day 3 AKI. Day 1 plasma total fibroblast growth factor 23 (cFGF23) concentrations were significantly higher in subjects who had acute kidney injury (AKI) on Day 3 than in those that did not (**Fig 2A**). Day 1 plasma intact FGF23 concentrations were not significantly different between the two groups (**Fig 2B**). Data are presented as medians and interquartile ranges, and the Wilcoxon rank-sum test was used to compare groups.

https://doi.org/10.1371/journal.pone.0222065.g002

Day 1 FGF23 levels and 60-day mortality

Cohort characteristics at the time of ARDS diagnosis, stratified by mortality, are shown in Table 1. Day 1 total FGF23 concentrations were significantly higher in subjects that died than in those that survived (median 529 [IQR 172, 1490] RU/ml vs. median 202 [IQR 100, 669] RU/ml, p = 0.017) (Fig 3A). Day 1 intact FGF23 concentrations were also significantly higher in subjects that died than in those that survived (median 38 [IQR 13, 128] pg/ml vs. median 21 [IQR 10, 43] pg/ml, p = 0.037) (Fig 3B).

Both higher Day 1 total FGF23 levels and higher Day 1 intact FGF23 levels were significantly associated with mortality at 60 days (OR 1.43 [95% CI 1.07, 1.91], p = 0.014; and OR 1.44 [95% CI 1.02, 2.05], p = 0.039, respectively) (Table 3, **Model 1**). After adjustment for Day 1 covariates (age, sex, presence/absence of AKI, P/F ratio, and PRISM score), Day 1 total FGF23 levels remained significantly associated with 60-day mortality (OR 1.62 [95% CI 1.07, 2.45], p = 0.023), but Day 1 intact FGF23 levels did not (OR 1.30 [95% CI 0.89, 1.90], p = 0.17) (Table 3, **Model 2**).

Subgroup of patients with Day 1 IL-6 concentrations

Given that stronger associations were observed with total FGF23 than with intact FGF23, and that inflammation is known to increase total FGF23 out of proportion to intact FGF23 [12], we assessed how the addition of an inflammatory marker to our models evaluating total

Table 2. Multivariable logistic regression modeling, with a dependent variable of acute kidney injury (AKI) at three days post-ARDS diagnosis.

Dependent Variable	Independent Variable	Model 1	Model 2	Model 3
Day 3 AKI	Total FGF23	2.22 (1.62, 3.03), p<0.001	1.51 (1.05, 2.17), p = 0.027	1.52 (1.02, 2.26), p = 0.039
Day 3 AKI	Intact FGF23	1.28 (0.95, 1.72), p = 0.11	0.99 (0.69, 1.42), p = 0.95	1.02 (0.69, 1.52), p = 0.92

In these models, the independent variable is Day 1 plasma total FGF23 or intact FGF23, both of which were log-transformed to correct for skewness. Model 1 is unadjusted. Model 2 is adjusted for the presence or absence of Day 1 AKI. Model 3 is adjusted for Day 1 AKI, age, sex, P/F ratio, and PRISM score. Data shown are odds ratios and 95% confidence intervals. As only subjects with complete covariate data are included in the regression analysis, n = 150 for all models.

https://doi.org/10.1371/journal.pone.0222065.t002

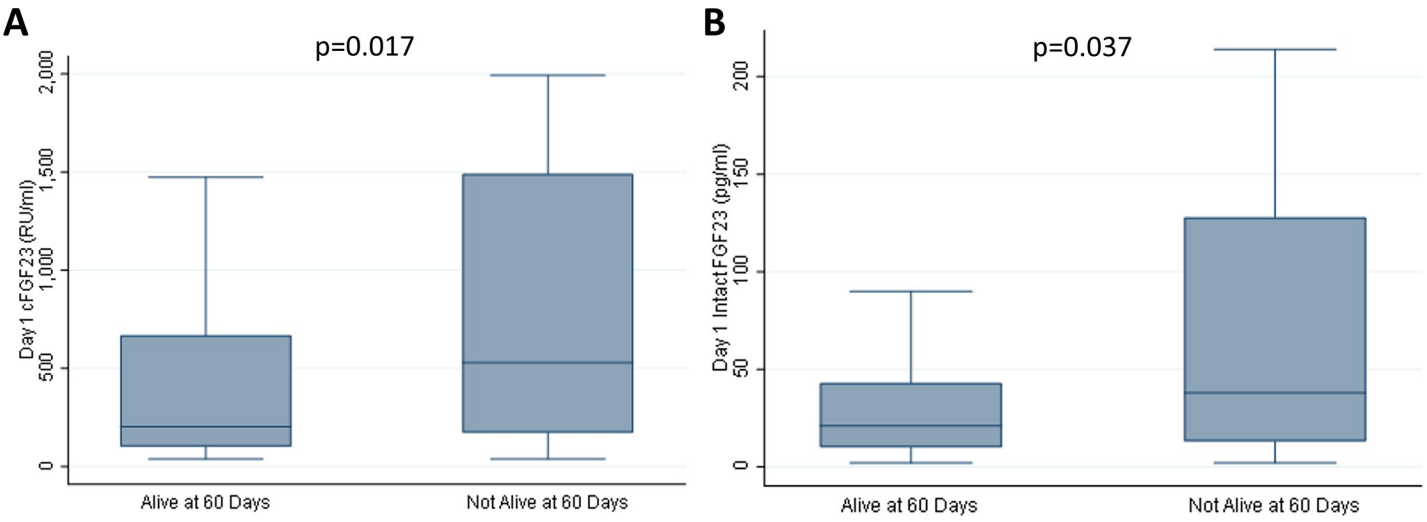

Fig 3. Day 1 plasma FGF23 concentrations stratified by 60-day mortality. Day 1 plasma total fibroblast growth factor 23 (cFGF23) concentrations (**Fig 3A**) and intact FGF23 concentrations (**Fig 3B**) were significantly higher in subjects who did not survive. Data are presented as medians and interquartile ranges, and the Wilcoxon rank-sum test was used to compare groups.

https://doi.org/10.1371/journal.pone.0222065.g003

FGF23 affected the results. In a subgroup of patients (n = 135), Day 1 plasma IL-6 concentrations were available. In these patients, plasma IL-6 levels were markedly elevated compared to what is observed in healthy subjects [42]. Total FGF23 levels positively correlated with plasma IL-6 concentrations (Spearman's rank correlation coefficient = 0.35, $p<0.001$), but intact FGF23 levels did not (Spearman's rank correlation coefficient = 0.06, p = 0.45).

Regarding the outcome of AKI, the addition of IL-6 as a covariate to the subgroup adjusted model decreased the Day 1 total FGF23 OR from 1.42 (95% CI 0.95, 2.14; p = 0.09) to 1.26 (95% CI 0.81, 1.96; p = 0.31) (**Table 4**). In this adjusted model, plasma IL-6 concentrations were independently associated with Day 3 AKI (OR 1.59 [95% CI 1.13, 2.22], p = 0.007), similar to what has been observed in other analyses of this cohort [35]. Given the association between IL-6 and AKI, we performed mediation analysis to quantify the degree to which variation in IL-6 concentrations mediated the FGF23-AKI association. In this analysis, although the p-value for the indirect effect did not reach statistical significance (p = 0.12), likely contributed to by decreased sample size, it was estimated that 36.4% of the association between total FGF23 and AKI was explained by IL-6 (**S2 Table**), suggesting partial mediation.

Regarding the outcome of mortality, the addition of IL-6 as a covariate to the subgroup adjusted model did not alter the Day 1 total FGF23 OR (1.38 [95% CI 0.90, 2.11], p = 0.14 without IL-6 vs. 1.35 [95% CI 0.88, 2.09], p = 0.17 with IL-6) (**Table 4**).

Table 3. Multivariable logistic regression modeling, with a dependent variable of mortality by 60 days post-ARDS diagnosis.

Dependent Variable	Independent Variable	Model 1	Model 2
Day 60 mortality	Total FGF23	1.43 (1.07, 1.91), p = 0.014	1.62 (1.07, 2.45), p = 0.023
Day 60 mortality	Intact FGF23	1.44 (1.02, 2.05), p = 0.039	1.30 (0.89, 1.90), p = 0.17

In these models, the independent variable is Day 1 plasma total FGF23 or intact FGF23, both of which were log-transformed to correct for skewness. Model 1 is unadjusted. Model 2 is adjusted for age, sex, the presence or absence of Day 1 AKI, P/F ratio, and PRISM score. Data shown are odds ratios and 95% confidence intervals. As only subjects with complete covariate data are included in the regression analysis, n = 150 for both models.

https://doi.org/10.1371/journal.pone.0222065.t003

Table 4. Multivariable logistic regression modeling in the subgroup of patients with Day 1 plasma IL-6 concentrations available.

Dependent Variable	Independent Variable	Model 1	Model 2	Model 3
Day 3 AKI	Total FGF23	1.99 (1.44, 2.75), p<0.001	1.42 (0.95, 2.14), p = 0.09	1.26 (0.81, 1.96), p = 0.31
Day 60 mortality	Total FGF23	1.27 (0.92, 1.75), p = 0.14	1.38 (0.90, 2.11), p = 0.14	1.35 (0.88, 2.09), p = 0.17

This subgroup includes 135 subjects. In these models, the dependent variable is acute kidney injury (AKI) at three days post-ARDS diagnosis or mortality by 60 days post-ARDS diagnosis. The independent variable is Day 1 plasma total FGF23, which was log-transformed to correct for skewness. Model 1 is unadjusted. Model 2 is adjusted for age, sex, the presence or absence of Day 1 AKI, P/F ratio, and PRISM score. Model 3 is further adjusted for log-transformed Day 1 plasma IL-6. Data shown are odds ratios and 95% confidence intervals.

https://doi.org/10.1371/journal.pone.0222065.t004

Discussion

In this study of a multicenter cohort of pediatric ARDS patients, we found that higher levels of plasma total FGF23, but not intact FGF23, measured at the time of ARDS diagnosis (Day 1) are associated with Day 3 AKI in unadjusted and adjusted models. We also found that higher levels of both Day 1 total and intact FGF23 are associated with 60-day mortality in unadjusted models, but in adjusted models, this association with mortality persists for only total FGF23.

FGF23 is a hormone that is primary secreted by osteocytes. Intracellularly, FGF23 production is regulated at both the transcriptional and post-translational stages. Prior to secretion, translated, full-length FGF23 protein can be cleaved by furin into N-terminal and C-terminal fragments, the biological functions of which are unclear. Therefore, what is secreted into the circulation is a mix of intact FGF23 protein and FGF23 proteolytic fragments, and post-translational cleavage mechanisms determine how much of the total FGF23 secreted from the cell is intact and how much is fragmented.

As the total FGF23 ELISA detects both intact and fragmented FGF23 in the circulation, it functions as a surrogate marker of total FGF23 translated and secreted. A profile of circulating FGF23 concentrations characterized by increased total FGF23 but normal intact FGF23 is consistent with complete coupling of increased FGF23 transcription with increased post-translational cleavage, resulting in increased circulating levels of FGF23 fragments. Notably, several stimuli couple increased FGF23 transcription with increased post-translational cleavage, including inflammation [12], iron deficiency [12, 43–45], erythropoietin [46–51], and parathyroid hormone [52], resulting in elevated concentrations of circulating total FGF23 but not intact FGF23. Indeed, in murine models of acute and chronic inflammation, bone *Fgf23* mRNA expression and circulating total FGF23 are increased, but circulating levels of intact FGF23 remain normal or near-normal [12]. As ARDS is an inflammatory condition, inflammation may have contributed to the differing results we observed for total FGF23 vs. intact FGF23.

In our cohort, circulating levels of total FGF23 were elevated, but not intact FGF23, consistent with what is observed in the presence of inflammation [12]. A subgroup of patients had plasma IL-6 concentrations—an inflammatory cytokine—available for analysis. Plasma IL-6 concentrations were elevated, and positively correlated with total FGF23 but not intact FGF23. In this subgroup, plasma IL-6 was independently associated with AKI, and the addition of IL-6 as a covariate to the adjusted regression model partially attenuated the association between total FGF23 and AKI, with mediation analysis demonstrating partial mediation by IL-6. Given the presence of partial mediation, these data suggest both inflammation-dependent and inflammation-independent associations between total FGF23 and AKI. Regarding possible inflammation-dependent effects, previous studies have demonstrated that higher circulating IL-6 levels are independently associated with AKI [35, 53–55], and that IL-6 may play a role in AKI pathophysiology [56]. Regarding possible inflammation-independent effects, the

pathophysiologic mechanisms related to high concentrations of FGF23 fragments remain to be elucidated. Although several conditions result in high FGF23 fragment concentrations, studies assessing the direct effects of these fragments are limited. However, one recent study provides some data demonstrating a direct adverse effect of the C-terminal FGF23 fragment on cardiomyocytes [57].

In the limited subgroup of patients with both IL-6 and FGF23 levels, the addition of IL-6 as a covariate to the adjusted regression model examining the association of total FGF23 with mortality did not alter the odds ratio, suggesting inflammation-independent effects. However, it should be noted that the p-value associated with this odds ratio did not reach statistical significance, possibly contributed to by decreased power associated with the smaller subgroup sample size.

In a similar study, Leaf et al assessed FGF23-mortality associations in the Validating Acute Lung Injury biomarkers for Diagnosis (VALID) cohort, which comprised 710 adult ARDS patients recruited from a single ICU [58]. In that study, the highest vs. lowest quartile of FGF23 concentrations at enrollment was independently associated with a significantly increased risk of both 60-day and 1-year mortality after adjustment for demographics, comorbidities, and measures of illness severity [58]. Although both total FGF23 and intact FGF23 levels were independently associated with mortality, the association effect sizes were stronger for total FGF23 than intact FGF23, similar to what we observed in our pediatric ARDS cohort. Of note, in the VALID cohort, circulating IL-6 concentrations were not available for analysis, so this marker of inflammation could not be added to the models. However, in another critically ill cohort, without ARDS, but in which plasma IL-6 levels were available, Leaf et al observed that both total FGF23 and intact FGF23 levels remained associated with mortality after adjustment for IL-6, suggesting inflammation-independent effects of FGF23 [58].

Although our study is novel in its assessment of FGF23 in the pediatric ARDS population, it has some limitations, including a lack of IL-6 concentrations in all patients and possible contributions from unmeasured ARDS-relevant factors. Another limitation is that we measured FGF23 concentrations only at the time of ARDS diagnosis; it is unknown whether changes in FGF23 over time are associated with clinical outcomes. Lastly, as this was an observational study, only associative data was generated, without providing any evidence of causality between predictor variables and clinical outcomes.

In conclusion, based on pre-clinical data suggesting that FGF23 may contribute to pulmonary pathology [11, 59, 60], we assessed whether circulating FGF23 levels are associated with clinical outcomes in a multicenter cohort of pediatric ARDS patients. Total (intact + C-terminal) FGF23, but not intact FGF23 alone, was associated with AKI in unadjusted and adjusted models. Total and intact FGF23 were associated with mortality in unadjusted models, but only total FGF23 was associated with mortality in an adjusted model. In a subset of patients, plasma IL-6 concentrations correlated with total FGF23 but not intact FGF23, and partially mediated the association between total FGF23 and AKI, suggesting both inflammation-dependent and inflammation-independent effects. Building on the findings of previous studies, this study further demonstrates the robustness and utility of total FGF23 as a biomarker of adverse clinical outcomes in critically ill patient populations, potentially helping to identify those at highest risk. Further mechanistic studies are required to assess the possible direct pathophysiologic effects of FGF23 fragments.

Supporting information

S1 Table. Numbers of subjects with and without acute kidney injury (AKI).
(DOCX)

S2 Table. Mediation analysis of interleukin-6 in the association between total FGF23 and acute kidney injury. All coefficients are adjusted for age, sex, the presence or absence of Day 1 AKI, P/F ratio, and PRISM score. The 95% confidence intervals for the indirect effects were determined after running 10,000 bootstrap samples. The estimated size of the mediated effect (proportion mediated) was calculated as the indirect effect divided by the total effect, multiplied by 100.
(DOCX)

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