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Higher Plasma Cystatin C is associated with Mortality after Acute Respiratory Distress Syndrome: Findings from a Fluid and Catheter Treatment Trial (FACTT) Substudy

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Abstract

Background: Cystatin C is a well validated marker of glomerular filtration rate in chronic kidney disease. Higher plasma concentrations of cystatin C are associated with worse clinical outcomes in heterogenous populations of critically-ill patients and may be superior to creatinine in identifying kidney injury in critically-ill patients. We hypothesized that elevated levels of plasma cystatin C in patients with Acute Respiratory Distress Syndrome (ARDS) would be associated with mortality risk.

Methods: In a retrospective study, cystatin C was measured by nephelometry on plasma obtained at enrollment from 919 patients in the Fluid and Catheter Treatment Trial. Multivariable logistic regression was performed testing the association between quartiles of cystatin C and 60-day mortality. Analyses were stratified by Acute kidney injury (AKI) status identified in the first 7 days after enrollment by Kidney Disease: Improving Global Outcomes (KDIGO) criteria.

Results: Cystatin C was significantly higher among those patients who died compared to those who survived to 60 days [1.2 (0.9 – 1.9) mg/L *vs.* 0.8 (0.6 – 1.2) mg/L, p <0.001]. Compared to the lower three quartiles, subjects in the highest quartile of cystatin C had a significantly higher odds of death at 60 days [OR 1.8 (1.2-2.6), p=0.003 in adjusted analyses]; the odds of death incrementally rose in higher cystatin C quartiles compared to the lowest quartile (OR 1.1, 1.8, and 2.5). In adjusted analyses stratified by AKI status, compared to subjects in the lower three quartiles, subjects in the highest quartile of cystatin C with AKI had a significantly higher odds of death at 60 days both in

participants with AKI [OR 1.6 (1.0-2.4), *p*=0.048] and those without AKI [OR 2.4 (1.2-5.0), *p*=0.017]. In adjusted analyses, there was no significant association between sex-stratified baseline creatinine quartiles and mortality.

Conclusions: Higher plasma levels of cystatin C on enrollment were strongly associated with mortality at 60-days in patients with ARDS with and without AKI identified by creatinine-based definitions**.** Compared to creatinine, cystatin C may be a better biomarker of kidney function in patients with ARDS and therefore identify patients with multiple organ failure at higher risk of death.

Word count (344/350)

Key Words: Cystatin C, Acute Respiratory Distress Syndrome (ARDS), Acute Kidney Injury (AKI)

Background

Identifying patients who have the highest risk of death after the Acute Respiratory Distress Syndrome (ARDS) and understanding the biology driving this risk is important to both clinicians and researchers. Studying the predictive value of biomarkers measured early in the course of ARDS may help with risk stratification for important clinical outcomes including death and multiple organ dysfunction after ARDS. Acute kidney injury (AKI) has been associated with increased mortality among critically ill patients, and the development of AKI after ARDS by definition marks the development of multiple organ dysfunction(1-4). Cystatin C is a 13kDa inhibitor of cysteine proteases and a housekeeping gene expressed in all nucleated cells at a steady rate. Because of its small size and basic pH, this molecule is freely filtered at the glomerulus, then reabsorbed and fully catabolized, but not secreted by the proximal renal tubule. These properties make cystatin C an ideal marker for glomerular filtration rate (GFR). Cystatin C is a well validated marker of kidney function in chronic kidney disease and may be a superior marker of acute kidney injury leading to impaired GFR compared to serum creatinine among critically ill patients(5, 6). Furthermore, plasma cystatin C measurements are clinically available in many institutions. Elevated cystatin C is associated with higher mortality in heterogenous cohorts of critically-ill patients(5, 7, 8), but this finding has not been studied in a large cohort of patients with ARDS.

In this retrospective cohort study, we measured cystatin C in plasma samples obtained from 919 subjects with ARDS on enrollment in the Fluid and Catheter Treatment Trial (FACTT)(9). Using adjusted logistic regression models, we tested the association between plasma cystatin C and 60-day mortality. We hypothesized that plasma cystatin C measured early in the course of ARDS would identify a subset of the most severely-ill patients and that this biomarker would add predictive and biological

104 information to mortality prediction models in ARDS above and beyond identification of
105 AKI cases using creatinine-based definitions.
106
107 **Methods**
108 The ARDS Network FACTT trial is a large randomized controlled trial with a factorial
109 design comparing a fluid conservative to a fluid liberal management strategy and
110 comparing the use of pulmonary artery catheters to central venous catheters in the
111 management of 1,000 patients with ARDS(9). Subjects were enrolled within 48 hours of
112 developing ARDS and patients with end stage renal disease or requiring renal
113 replacement therapy were excluded from the study. For this retrospective cohort study,
114 919 plasma samples were available for cystatin C measurement, which were made on a
115 Dade-Behring BNII nephelometer. Estimated glomerular filtration (eGFR) rate was not
116 reported in this cohort because these estimates would be unreliable given the available
117 plasma creatinine and cystatin C measurements were not made at steady state. Sex
118 stratified multivariate models adjusted for baseline creatinine were performed separately
119 from the analyses stratified by AKI, which are the main focus of our analysis. AKI was
120 identified by applying Kidney Disease: Improving Global Outcomes (KDIGO) criteria to
121 all available creatinine (Cr) measurements in the first 7 days after study enrollment. AKI
122 cases were identified as an increase in Cr ≥ 0.3 mg/dl over 48 hours, to levels greater
123 than or equal to 1.5 times baseline Cr or dialysis initiation within 7 days. Baseline Cr was
124 defined by serum Cr at study enrollment. We repeated adjusted and stratified analyses
125 after reclassifying AKI cases by accounting for the effect of fluid balance on the volume
126 of distribution of creatinine(10). Previous work using latent class analysis (LCA) has
127 identified subphenotypes with different mortality rates and differential response to
128 therapy within large randomized controlled trials of patients with ARDS, including
129 FACTT. We adjusted for these subphenotypes which have also been characterized as

hypoinflammatory or subphenotype 1, and a hyperinflammatory or subphenotype 2. (11, 12) All variables considered for inclusion in the multivariable logistic regression models were examined for distribution and missingness and appropriate model checking was performed. Multiple imputation was used to address 4% missing data for the APACHE III variable. Logistic regression models were used to test the association between cystatin C and mortality adjusted for important confounders. Because the linearity assumption of the logistic regression models was violated when considering cystatin C as a continuous variable, even when testing several options for transformation of the independent variable, including the commonly used inverse function, cystatin C was analyzed by quartiles defined using data from the full cohort. Cystatin C was divided into quartiles at the following cut points: Quartile 1 (0.2-0.67 mg/L), Quartile 2 (0.68-0.90 mg/L), Quartile 3 (0.91-1.37 mg/L), Quartile 4 (1.38-5.2 mg/L). The same quartile cut-offs from Cystatin C measurements in the full cohort were used in all analyses including stratified analyses. Results from a postestimation linear test for trend are reported to describe the association between cystatin C quartiles and mortality in adjusted analyses. Sensitivity analyses were performed excluding subjects with clinical and demographic characteristics that are believed to influence plasma cystatin C levels and possibly act as confounders of the association between cystatin C and mortality, including cancer, trauma, and recent surgery. Furthermore, likelihood ratio testing was used to eliminate variables from models that were adjusted for a variety of other factors known to affect cystatin C levels. When compared to the parsimonious final models presented here, the models with additional variables did not improve the model fit. The following variables were tested and eliminated from the final model: body mass index (BMI), diabetes, baseline white blood cell count (WBC), serum albumin, and a history of cardiovascular disease. All analyses were performed using STATA version 15 (Statacorp, College Station, TX).

Results

The demographic and clinical characteristic of the 919 subjects included in this analysis of the FACTT study are displayed in **Table 1**. The median age of subjects was 49 years and 53% of subjects were female. The mortality rate was 28%. The incidence of AKI was 53% and 61% after adjusting for fluid balance. The median APACHE III score was 91 (IQR 70-117). The most common primary risk factor for ARDS was pneumonia (426 subjects, 46%) followed by sepsis (218 subjects, 24%). A total of 394 subjects (43%) had sepsis listed as either a primary or secondary risk factor for ARDS. The median baseline creatinine value measured in the full cohort was 1.0 mg/dL (IQR 0.7-1.5mg/dL). The median cystatin C level at enrollment was 0.9 mg/L, and the interquartile range was 0.7-1.4 mg/L. Plasma cystatin C levels were higher among those in the hyperinflammatory subphenotype compared with those in the hypoinflammatory subphenotype [1.3 (0.9-2.1) mg/L *vs.* 0.8 (0.6-1.1) mg/L, *p*-value<0.0001]. Cystatin C was higher among those who died compared to those who survived [1.2 (0.9-1.9) mg/L *vs.* 0.8 (0.6-1.2) mg/L, p <0.001] (**Table 1**). This difference remained statistically significant when the cohort was stratified by AKI status (**Figure 1**). In subjects with AKI, cystatin C was higher among those who died compared to those who survived [1.3(1.0-1.9) mg/L *vs.* 0.9(0.7-1.4)mg/L, p <0.0001]; and the same pattern was observed in subjects without AKI [1.1(0.7-1.7) mg/L *vs.* 0.8(0.6-1.0) mg/L, p <0.0001].

The crude 60-day mortality rate by cystatin C quartile is shown in **Figure 2**. The highest quartile of cystatin in the full cohort had an elevated mortality rate that was observed in both AKI strata. Even among subjects without AKI by creatinine-based definitions, the 60-day mortality rate in the highest quartile of cystatin C was 40%.

In the full cohort, a multivariate logistic regression model adjusted for sepsis, age, sex, APACHE III score, AKI status, treatment arm and LCA subphenotype, showed

that higher quartiles of cystatin C were strongly associated with 60-day mortality. In contrast, in adjusted analyses there was no significant association between sex-stratified baseline creatinine quartiles and mortality (**Table 2**). In the full cohort, subjects in the highest quartile of cystatin C had a more than the two-fold odds of death compared to those in the lowest quartile [OR 2.5 (1.5-4.2); linear test for trend *p*=0.002](**Table 4)** and compared to subjects in the lower three quartiles combined [OR 1.8 (1.2-2.6, *p*=0.003)], (**Table 3**). The relationship between cystatin C and 60-day mortality in adjusted models was not significantly different between subjects with and without AKI. Post estimation tests for linear trend for the association between cystatin C quartiles and mortality were significant in the full cohort and among subjects with AKI, but not among subjects without AKI (**Table 4**). However, there was no statistically significant interaction between AKI status and cystatin C in adjusted models. Additionally, there was no significant interaction between LCA subphenotype and cystatin C in the full cohort or in the analyses stratified by AKI status.

The subjects in the highest quartile of cystatin C who did not meet the creatinine-based definition of AKI (n=65) had a substantially elevated risk of death compared to subjects without AKI in the lower three quartiles of cystatin C [OR 2.4 (1.2-5.0, *p*=0.017)], (**Table 3**). These subjects were of particular interest in this analysis. We examined the clinical and demographic characteristics of this group in an effort to understand the drivers of the observed statistical association (**Table 5**). The median age of these subjects was 58 and 31% were female. The creatinine trends over the first 8 study days among subjects with the highest quartile of cystatin C but no AKI are plotted in **Figure 3**. These data show that the daily creatinine recorded among these individuals was either down-trending or stable over time. The median value of baseline creatinine on study enrollment among these 65 patients was 1.5 mg/dL (IQR 1.1-2.2 mg/dL). Only

10 of these 65 subjects died before study day 9 and the median survival time among those who died was 16 days.

Discussion

Here we have found for the first time that higher plasma cystatin C concentrations measured early in the course of ARDS are associated with higher mortality and that this association persists after adjustment for AKI defined by creatinine-based criteria. Earlier studies reported that elevated cystatin C is associated with higher mortality in heterogenous cohorts of critically-ill patients(5, 7, 8), but prior to this study, this finding had not been validated in a large cohort of patients with ARDS. Additionally, to the best of our knowledge, this is the first study to test the association of cystatin C and mortality among critically ill patients cared for in North America.

The association between cystatin C and death among patients with ARDS may work through several pathways. First, and in our opinion, the most likely explanation among patients with ARDS, the association between elevated plasma cystatin C and death after ARDS is driven by the glomerular filtration rate pathway, capturing kidney dysfunction in a way not captured by other measures of illness severity. This dysfunction could be either acute, chronic, or both. Plasma cystatin C may identify additional patients with AKI and therefore multiorgan failure who do not meet creatinine-based definitions of AKI. In this cohort of patient with ARDS, the highest quartile of cystatin C measurements identified 65 individuals, 7% of the full cohort, with likely kidney dysfunction that was not detected by current creatinine-based definitions of AKI (**Table 5**, **Figure 3**). This finding is consistent with data from other populations showing that cystatin C may be a superior marker of glomerular filtration rate than creatinine in critically-ill patients(5, 6, 13). Thus, if cystatin C is a more sensitive marker of AKI than creatinine among those with ARDS, it can accurately identify subjects with multiorgan

failure, a well-established risk factor for death among critically ill patients (14-19). Additionally, cystatin C is a more sensitive marker of chronic kidney disease (CKD) than creatinine in many populations and CKD is a known risk factor for death after critical-illness.(20, 21) Second, in theory, it is possible that the association between cystatin C and mortality after ARDS is working through a non-glomerular filtration rate pathway. This alternative explanation is speculative, and we are unable to test the possibility of a biological process to distinguish cystatin C's role as a GFR marker from other hypothesized pathways, such as increased cystatin C production from inflammation. In sensitivity analyses excluding subjects with available data on conditions known to increase cystatin C production, we did not find any evidence supporting this hypothesis. Although we are unable to test the mechanism of association between elevated cystatin C and mortality after ARDS, it seems most plausible that this biomarker is detecting reduced glomerular filtration rates (either acute or chronic) and thus identifying patients with ARDS who have kidney dysfunction and multiple organ failure. Regardless of the mechanisms driving this association, multivariable models adjusted for important potential confounders or mediators including APACHE III score and LCA subphenotype showed a robust association between cystatin C and mortality among patients with ARDS, suggesting that this biomarker provides valuable prognostic information not otherwise captured by established markers of critical illness severity.

The 65 subjects in the highest quartile of cystatin C but without AKI by creatinine based definitions were of particular interest. Studying basic demographic assessments of age, sex, and BMI, gave no indication that these individuals may have had lower muscle mass that would make creatinine a less-reliable marker of GFR. The data presented in **Table 5** and **Figure 2** do not clearly suggest that these 65 patients were simply patients with previously undiagnosed chronic kidney disease, but with the available information we cannot rule out that possibility. Only 10 of these 65 subjects

died before study day 9 and the median survival time among those who died was 16 days. These data suggest that survival bias censoring the trajectory of creatinine values does not explain why this group of subjects with high cystatin C did not have AKI by a creatinine-based definition. Taken to together these data suggest that this group of study subjects was not classified as having AKI because they were most likely on the downward trajectory or plateau phase of their creatinine measurements, and the AKI occurred earlier in their course of illness before study enrollment in FACTT.

Because our findings suggest that a single measurement of plasma cystatin C early in the course of ARDS provides prognostic information about mortality beyond creatinine and creatinine-based definitions of AKI, this may be an appealing biomarker to measure for both research and clinical care purposes. Plasma cystatin C measurement is widely available in many clinical settings and may allow clinicians to identify patients with ARDS at highest risk of death, early in their course of illness. Interestingly, cystatin C differed between LCA subphenotypes. Furthermore, it appears to provide additional information beyond both APACHE III score and LCA subphenotype and therefore may be of interest in future clinical research studies focused on enhancing enrollment of subjects at highest risk of death or studies using risk stratification to assign or evaluate treatment interventions. Specifically, more work is needed to understand the relationship between cystatin C and LCA subphenotypes in ARDS.

This study has several strengths. The FACTT study enrolled a large number of patients with well-adjudicated ARDS. Detailed data collection of laboratory values including creatinine allowed for the rigorous adjudication of AKI by KDIGO criteria. Detailed documentation of clinical data allowed us to adjust for APACHE III score and sepsis in multivariable analyses. Prior work using clinical data and biomarkers measured in patients enrolled in the FACTT trial identified subphenotype latent classes with differential response to therapy. We included adjustment for subphenotypes in our

models to strengthen the importance of this novel finding of the association between cystatin C and mortality after ARDS. Our study has some limitations. The retrospective design of this study does not allow us to test the mechanisms driving the association between cystatin C and mortality after ARDS. As these plasma biomarkers are not in steady state during critical illness, timing of creatinine and cystatin C measurements in this cohort do not allow for meaningful estimation of glomerular filtration rate or accurate classification of chronic kidney disease prior to study enrollment.

Conclusions

The strong association between mortality and elevated plasma cystatin C measured early in the course of ARDS was robust to adjustment for many important confounders or potential mediators and this association persists after adjustment for AKI defined by creatinine-based criteria. Among patients with ARDS, cystatin C may identify kidney dysfunction and multiple organ failures that increase the risk of death and are not captured by other commonly measured assessments of severity of illness. These findings are likely to be of interest to a broad audience of both clinicians and investigators who are designing clinical trials.

List of Abbreviations

ARDS Acute Respiratory Distress Syndrome

AKI Acute Kidney Injury

FACTT Fluid and Catheter Treatment Trial

KDIGO Kidney Disease: Improving Global Outcomes

LCA Latent Class Analysis

Declarations

- Ethics approval and consent to participate: This study is a secondary data analysis of deidentified data from a previously published clinical trial. The authors did not have access to the key linking file between the data set and protected health information and therefore the study did not meet criteria for human subjects research according to the University of California San Francisco Institutional Review Board.
- Consent for publication: Not applicable
- Availability of data and material: The parent FACTT data is available from the National Heart, Lung and Blood Institute. If requested, the investigators will work the National Heart, Lung and Blood Institute and the requestors to make the cystatin C measurements available.
- Competing interests: None
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- Authors' contributions:

CMH created the data analysis plan, analyzed and interpreted the data, and was a major contributor in writing the manuscript and presenting the results. **KDL** designed the study, verified the data analysis methods, supervised the interpretation of the findings of

the results, and contributed to the writing of the manuscript. **MAM** helped to design the study, supervised the findings of this work, contributed to the interpretation of results. **MGS** supervised the measurements of cystatin C, contributed to the data analysis plan and to the interpretation of results. **YDK** contributed to the data analysis plan, helped to check and analyze the data to identify cases of acute kidney injury by creatinine-based definitions, and contributed to the interpretation of results and writing of the manuscript. **AGB** contributed to the interpretation and presentation of results and writing the manuscript. **All authors provided critical feedback, read, and approved the final manuscript.**

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Authors' information (optional): Not applicable.

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TABLE 1. Demographics, Clinical Characteristics, and Biomarkers by 60 Day Mortality

	Full Cohort n=919	Alive at 60 day n=658	Dead by 60 days n=261	*p* –value
Age	49 (38 – 61)	47 (37 – 57)	57 (41 – 70)	**<0.001**
Female Sex	489 (53)	338 (52)	151 (58)	0.076
BMI	27 (23 – 32)	28 (24 – 33)	26 (22 – 31)	**0.005**
Race/Ethnicity				**<0.001**
White	602 (66)	459 (70)	143 (55)	
Black	193 (21)	121 (18)	72 (28)	
Hispanic	124 (13)	78 (12)	46 (18)	
Fluid Conservative Arm	467 (51)	342 (52)	125 (48)	
Baseline WBC (10^3/μL)	11.8 (7.2-17.1)	12.0 (7.8-17.1)	10.6 (5.8-17.3)	**0.046**
Baseline Creatinine (mg/dL)	1.0 (0.7 – 1.5)	0.8 (0.7 – 1.4)	1.2 (0.9 – 1.8)	**<0.001**
Cystatin C (mg/L)	0.9 (0.7 – 1.4)	0.8 (0.6 – 1.2)	1.2 (0.9 – 1.9)	**<0.001**
AKI [#]				
Actual	486(53)	306 (47)	180(69)	**<0.001**
Adjusted for Fluid Balance	559(61)	348 (53)	211(81)	**<0.001**
APACHE III Score	91 (70 – 117)	85 (65 – 104)	114 (91 – 133)	**<0.001**
Primary ARDS Risk Factor				**0.002**
Pneumonia	426 (46)	303 (46)	123(47)	
Sepsis	218 (24)	138 (21)	80 (31)	
Aspiration	138 (15)	104 (16)	34 (13)	
Trauma	71 (8)	62 (9)	9 (3)	
Multiple Transfusion	9 (1)	7 (1)	2 (1)	
Other	57 (6)	44 (7)	13 (5)	
Hyperinflammatory LCA Subphenotype[α]	252 (27)	137 (54)	115 (46)	**<0.001**
Comorbidities				
Solid Tumor	14 (2)	8 (1)	6 (2)	0.25
Lymphoma	13 (1)	4 (1)	9 (4)	**0.001**
Leukemia	20 (3)	9 (1)	11 (4)	**0.009**
Recent Surgery	46 (5)	37 (6)	9 (3)	0.17

BMI = body mass index, WBC= white blood cell count, AKI = acute kidney injury by KDIGO criteria, IQR = interquartile range
[#] AKI by Kidney Disease: Improving Global Outcomes (KDIGO) criteria
α LCA=Latent Class Analysis, Famous *et al.* AJRCCM 2017
data presented as n(%) or median (IQR)
p value refers to a comparison of those survivors to those who died using rank-sum, Pearson chi^2, or Fisher Exact tests as appropriate

TABLE 2. No Association between Sex-Stratified Baseline Creatine Quartiles and 60-day Mortality in Adjusted Logistic Regression Models			
		OR (95%CI)	*p*-value
Female Sex (n=489)			
Covariates: Sepsis, Age, Sex, APACHE III*, Treatment Arm, LCA subphenotype$^{\alpha}$			
Baseline Creatinine			0.67
	Q2	0.6 (0.3-1.3)	
	Q3	0.8 (0.4-1.5)	
	Q4	1.1 (0.6-2.2)	
Male Sex (n=430)			
Covariates: Sepsis, Age, Sex, APACHE III*, Treatment Arm, LCA subphenotype$^{\alpha}$			
Baseline Creatinine			0.26
	Q2	1.4 (0.7-2.8)	
	Q3	1.9 (0.9-3.8)	
	Q4	2.1 (1.0-4.4)	
All analyses compare stated Quartile to first quartile (Q1). *APACHE III scores exclude renal variables in these models with creatinine as predictor *p*-value refers to post estimation global test for the null hypothesis that creatine quartiles are not associated with death α LCA=Latent Class Analysis, Famous *et al.* AJRCCM 2017			

TABLE 3. Higher Cystatin C is Associated with 60-Day Mortality in Subjects With and Without AKI		
	OR (95%CI)	*p*-value
Full Cohort (n=919)		
Cystatin C Q4	1.8 (1.2-2.6)	0.003
With Acute Kidney Injury (n=486)		
Cystatin C Q4	1.6 (1.0-2.4)	0.048
Without Acute Kidney Injury (n=433)		
Cystatin C Q4	2.4 (1.2-5.0)	0.017
All analyses compare the highest quartile to the lower three quartiles. Q4- highest quartile of cystatin C with quartiles determined by ranges of cystatin C in full cohort. All models adjusted for sepsis, age, sex, APACHE III, treatment arm, and LCA subphenotype. Full Cohort model also adjusted for Acute Kidney Injury (AKI) by Kidney Disease: Improving Global Outcomes (KDIGO) creatine-based definition		

TABLE 4. The Adjusted Association between 60-day Mortality and Cystatin C Overall and Stratified by AKI Status			
		OR (95%CI)	*p*-value
Full Cohort (n=919)			
Covariates: Sepsis, Age, Sex, APACHE III, AKI, Treatment Arm, LCA subphenotype[α]			
Cystatin C			0.0002
	Q2	1.1 (0.6-1.8)	
	Q3	1.8 (1.1-3.1)	
	Q4	2.5 (1.5-4.2)	
With Acute Kidney Injury (n=486)			
Covariates: Sepsis, Age, Sex, APACHE III, Treatment Arm, LCA subphenotype[α]			
Cystatin C			0.0032
	Q2	1.2 (0.6-2.6)	
	Q3	2.6 (1.3-4.9)	
	Q4	2.7 (1.4-5.3)	
Without Acute Kidney Injury (n=433)			
Covariates: Sepsis, Age, Sex, APACHE III, Treatment Arm, LCA subphenotype[α]			
Cystatin C			0.094
	Q2	0.7 (0.3-1.6)	
	Q3	0.9 (0.4-2.2)	
	Q4	2.0 (0.8-5.2)	
*All analyses compare stated Quartile to first quartile (Q1). *p*-value refers to post estimation linear test for trend across cystatin C quartiles AKI- Acute Kidney Injury by Kidney Disease: Improving Global Outcomes (KDIGO) creatine-based definition α LCA=Latent Class Analysis, Famous *et al.* AJRCCM 2017			

TABLE 5. Characteristics of 65 Subjects Without Acute Kidney Injury In the Highest Quartile of Cystatin C	
Age (years)	58 (46-69)
Female Sex	20 (31%)
BMI	28.1 (24.2-37.1)
Baseline Serum Creatinine (mg/dL)	1.5 (1.1-2.2)
Baseline Serum Creatinine >1.5mg/dL	32 (49%)
Cystatin C (mg/L)	1.8 (1.6-2.3)
Mortality at 60 days	26 (40%)
Survival Time to 60 days (days)	60 (23-60)
Died Before Study Day 8	10 (15%)
Survival Time Among Those Who Died (days)	16 (1-33)
Acute Kidney Injury defined by Kidney Disease: Improving Global Outcomes (KDIGO) creatinine-based definition BMI = body mass index data presented as n(%) or median (IQR)	

Figures

Figure 1. Cystatin C and Mortality by Acute Kidney Injury Status.

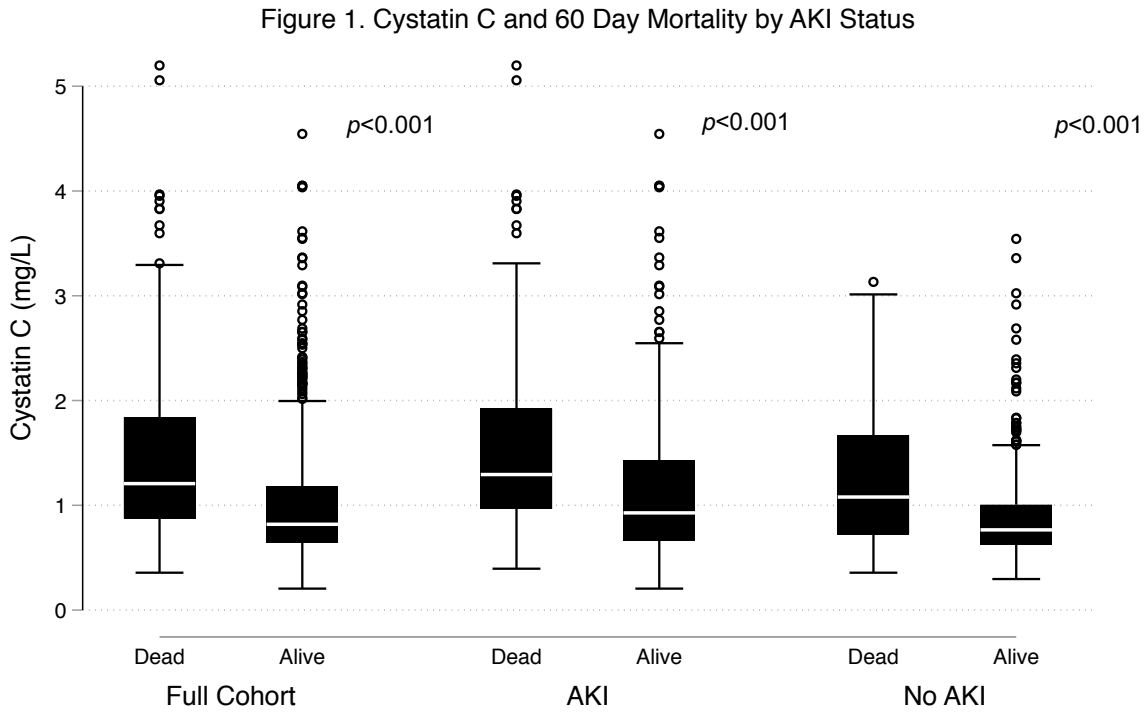
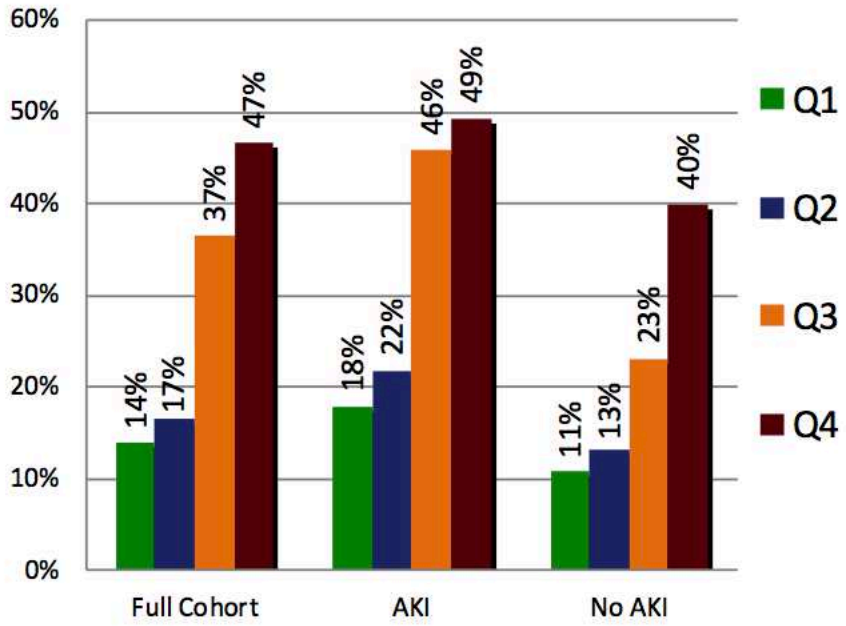
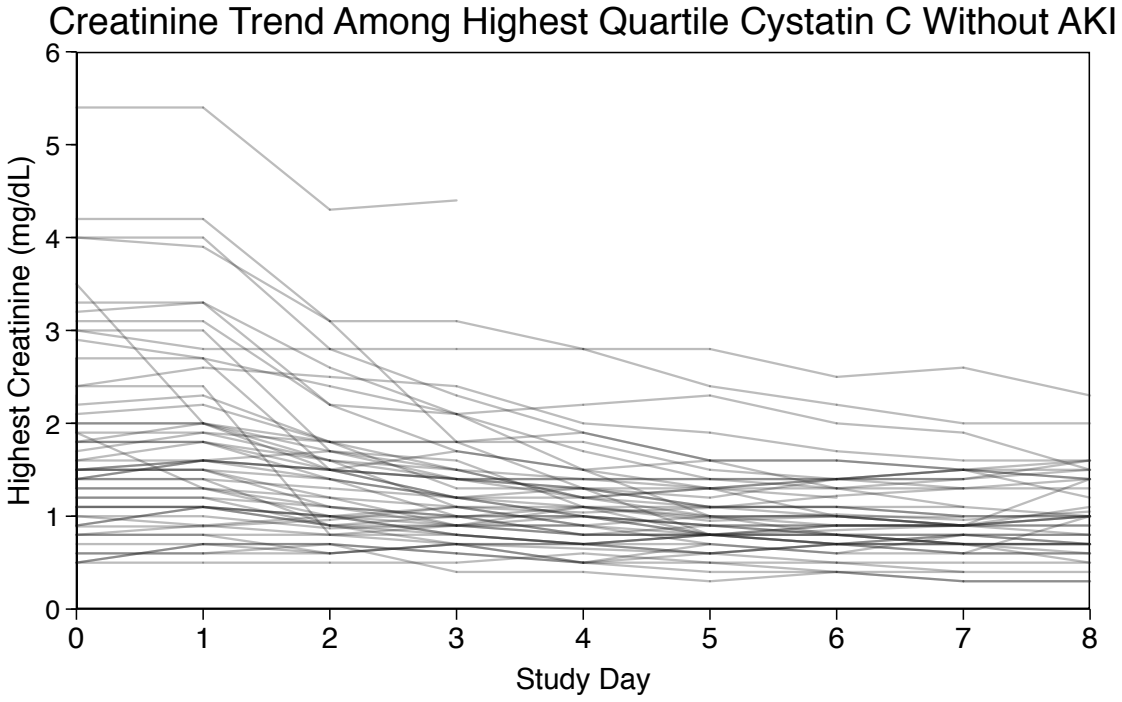


Figure 2. Crude 60 day Mortality Rate by Cystatin C Quartile



462 Figure 3.
463



464
465
466 **Figure Legends**

467

468 Figure 1. Box and whisker plots of the raw plasma cystatin C data in the full cohort and
469 stratified by Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury
470 (AKI) status show that cystatin C is higher among those who died by 60 days compared
471 to those who survived. This difference was statistically significant by Wilcoxon ranksum
472 testing in the full cohort and both strata of AKI status. The cystatin C quartile cut offs
473 were established in the full cohort and applied to the data stratified by AKI status.

474

475 Figure 2: Bar graphs of the crude 60-day mortality rate by plasma cystatin C quartile in
476 the full cohort and in the strata defined by Acute Kidney Injury (AKI) by Kidney Disease:
477 Improving Global Outcomes (KDIGO) criteria. The cystatin C quartile cut offs were

established in the full cohort and applied to the data stratified by AKI status. Crude mortality rates are similar in each quartile between the full cohort and the stratified data.

Figure 3. Creatinine trends plotted for each of the 65 subjects in the highest quartile of plasma cystatin C that did have Acute Kidney Injury (AKI) by Kidney Disease: Improving Global Outcomes (KDIGO) criteria show that the creatinine trajectories for these individuals are down trending or plateaued in the first eight days after enrollment.

Figures

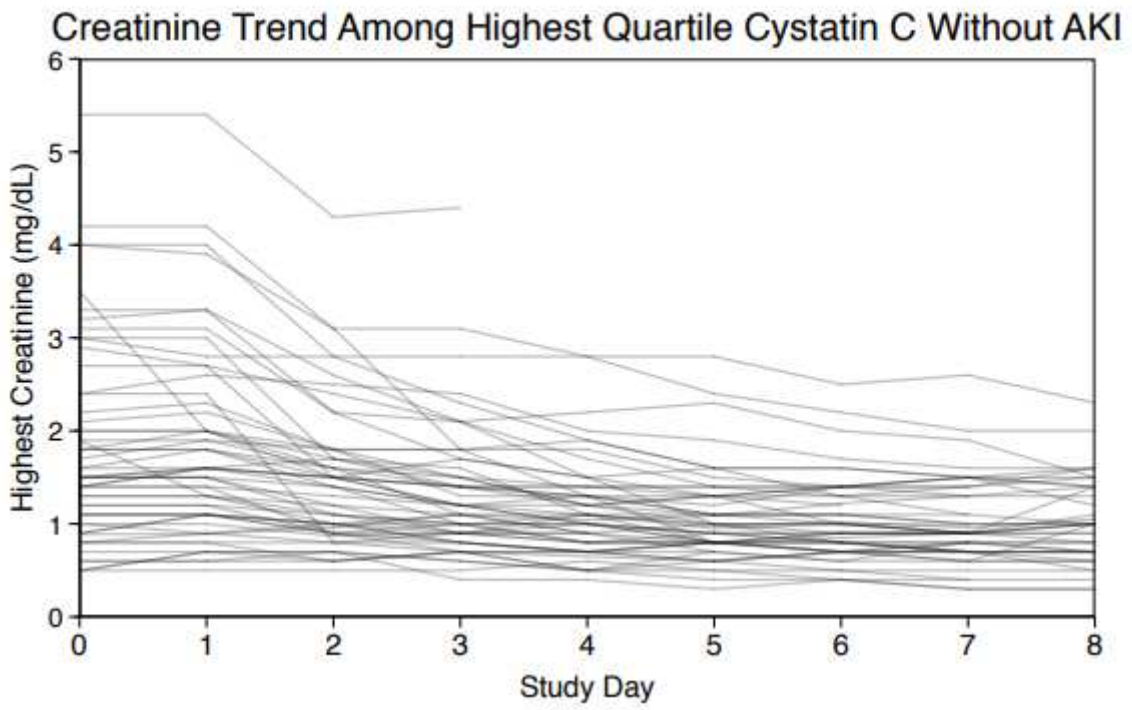


Figure 1

Creatinine Trend Among Highest Quartile Cystatin C Without AKI

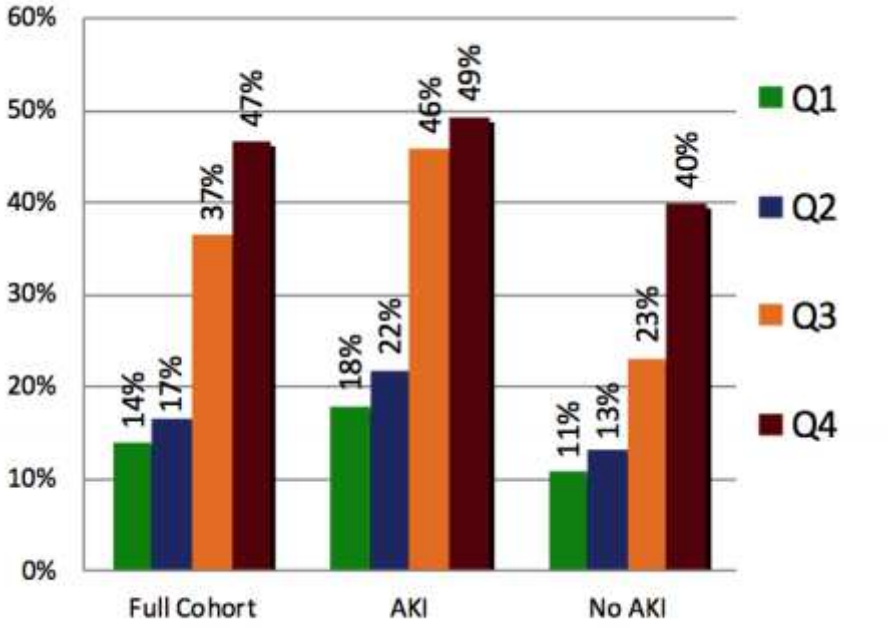


Figure 2

Crude 60 day Mortality Rate by Cystatin C Quartile

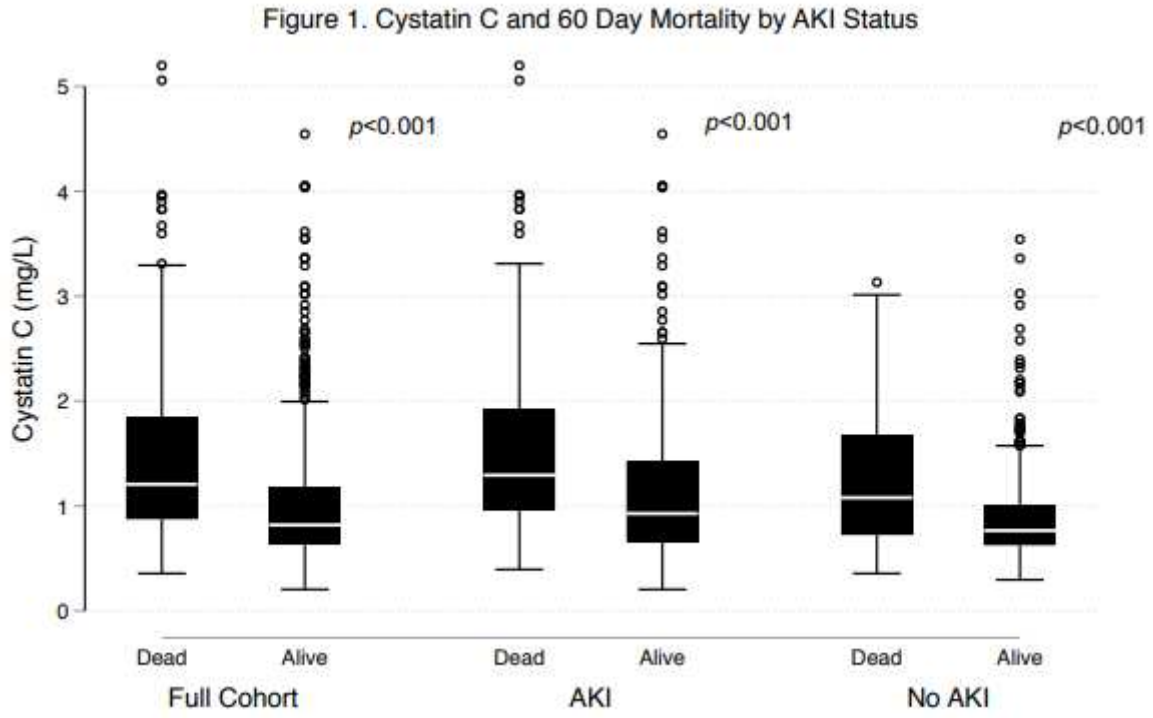


Figure 3

Cystatin C and Mortality by Acute Kidney Injury Status.