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Serum cystatin C for acute kidney injury evaluation in children treated with aminoglycosides

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Abstract

Background—Serum cystatin C (CysC) is a more accurate glomerular filtration rate marker than serum creatinine (SCr) and may rise more quickly with acute kidney injury (AKI).

Methods—We performed a prospective cohort study of 81 noncritically ill children during 110 aminoglycoside (AG) treatments. We calculated area under the curve (AUC) for CysC to diagnose SCr-defined AKI and predict persistent AKI. SCr-AKI definition was based on the Kidney Disease: Improving Global Outcomes (stage 1: 50 % or 26.5 $\mu\text{mol/l}$ SCr rise from baseline; stage 2: SCr doubling); CysC-AKI was based on a modified version using CysC rise.

Results—SCr-AKI and CysC-AKI developed in 45 and 48 % treatments, respectively. CysC rise predicted stage 1 (AUC = 0.75, 95 % CI 0.60–0.90) and 2 (AUC = 0.85, 95 % CI 0.75–0.95) SCr-AKI 2 days before SCr-AKI attainment. The best combined sensitivity/specificity for percent CysC rise to predict stage 1 SCr-AKI was with a 44 % CysC rise (sensitivity = 65 %, specificity = 83 %). CysC rise on day of SCr-AKI development was associated with SCr-AKI 48 h (AUC = 0.73, 95 % CI 0.56–0.90) and 50 % persistent SCr rise at treatment end (AUC = 0.76, 95 % CI 0.61–0.90).

Conclusions—CysC is as an early AKI biomarker and predictive of persistent AKI on aminoglycoside treatment.

Keywords

Acute renal failure; Pediatric nephrology; Diagnostic testing; Early biomarker; Antibiotics; Nephrotoxicity

Compliance with ethical standards Institutional ethics boards approved the study before initiation. All procedures performed in the study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent/assent was obtained before participation.

Conflict of interest The authors declare that they have no conflict of interest.

Introduction

Acute kidney injury (AKI) is associated with mortality, morbidity, and longer hospital length of stay (LOS) in hospitalized patients [1–8]. Nephrotoxic medication use is a leading cause of AKI in hospitalized children [9–12]. Aminoglycosides (AG) are commonly used antibiotics that are known to be nephrotoxic. AG-nephrotoxicity results in renal tubular injury and recent studies have shown that some patients treated with AG or other nephrotoxins may be at risk for chronic kidney disease development [13–18].

The current reference standard test for AKI is based on evaluating serum creatinine (SCr) rise from baseline, indicating acute decrease in glomerular filtration rate (GFR). However, SCr rises late in AKI pathogenesis, up to 3 days after a defined insult [19, 20], impeding early intervention and minimization of further kidney injury. SCr concentration is affected by various factors such as age, sex, and muscle mass, reducing the specificity of SCr as an AKI biomarker. New AKI diagnostic biomarkers have been identified and studied in recent years, mostly consisting of urine markers of renal tubular injury [21–24].

Serum cystatin C (CysC), a filtration marker of GFR, is an endogenous protease inhibitor produced at a constant rate by nucleated cells and its concentration is not affected by age, gender, or muscle mass in children [25, 26]. In children, CysC is a more accurate estimate of GFR than SCr and is more diagnostic of reduced chronic kidney function [27, 28]. Thus, it is possible that acute CysC change may be more effective at detecting acute GFR changes with AKI than SCr (i.e., be a better reference test for AKI). Several studies have used CysC to define AKI [29–31], though the extent to which CysC-defined AKI and SCr-defined AKI are related, remains unclear. If CysC is a better reference standard for AKI definition, then AKI outcomes in clinical trials will be more accurately ascertained and novel AKI biomarkers could be evaluated with more confidence than currently feasible with SCr-defined AKI.

Some studies have evaluated CysC for early AKI diagnosis, similar to how urine AKI biomarkers have been studied. There is controversy as to whether CysC rises before SCr does with AKI [32]. Some North American and European hospitals have already incorporated CysC in the care of outpatients. However, to the best of our knowledge, there are no data evaluating the role of serum CysC in assessing AG nephrotoxicity or AKI in the non-critical care hospital setting, where most hospitalized children are admitted.

The goals of this study were to determine, in non-critically ill children treated with AG, the extent to which CysC (i) is an early diagnostic marker of AKI, and (ii) is associated with persistent acute kidney dysfunction. We also sought to compare AKI incidence and outcome associations of CysC-defined AKI to SCr-defined AKI.

Materials and methods

Design, setting, and patient selection

We conducted a prospective cohort study of inpatient, noncritically ill children treated with AG at the Montreal Children's Hospital (MCH), Canada from September 2008 to March 2012. In the final year, as a pilot evaluation for multi-center AG study, similar patients from

Cincinnati Children's Hospital Medical Center (CCHMC, Ohio, USA) were recruited. Inclusion criteria were hospitalization in a non-intensive care unit and initiating AG treatment with expected treatment duration ≥ 3 days. AG episodes were identified by daily communication with the Pharmacy department. Exclusion criteria were patient age <3 months or >18 years at treatment start, AKI evidence before AG initiation (SCr $\geq 50\%$ above baseline), known pre-existing renal condition or admission reason (e.g., renal infection, chronic renal diagnosis, dialysis, tubulopathy) and intravenous AG treatment in the 2 weeks before study. Individuals could be recruited into the study more than once, at separate hospital admissions. Patients were approached if they were within 48 h of first AG dose. Informed consent/assent was obtained before participation. After recruitment, treatment episodes lasting <3 days were excluded. At analysis, treatment episodes with less than one SCr and CysC value available at least every 5 treatment days on average, were also excluded (i.e., inadequate numbers of SCr and CysC levels on treatment). Institutional ethics boards approved the study before initiation. All procedures performed in the study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Figure 1 describes the study flow.

Clinical data collection

The following variables were evaluated: age at treatment start, gender, admission height and weight; admission and discharge diagnoses; treating service; AG type (e.g., gentamicin, tobramycin, or amikacin) and dosing/duration; treatment reason; past medical history; other medication taken before and during AG treatment; dialysis treatment; hospital LOS and mortality.

Biospecimen collection

At recruitment, leftover serum from blood collected during routine care was obtained from the biochemistry laboratory for pre-recruitment SCr and CysC measurement. Thereafter, during daily routine blood work, 1–3 cc blood was collected for up to 14 days of AG treatment. An attempt was made to measure serum AG levels on treatment days 3 and 8, when not measured during routine care. At analysis, only study days with both SCr and CysC results were considered for AKI definition.

Laboratory measurement

SCr was measured at the Montreal Biochemistry Laboratory using an IDMS-traceable assay. CysC was measured at the CCHMC Biomarker Laboratory, Ohio (PD, MB) using a nephelometer (Siemens BN-II, Siemens, AG; www.Siemens.com) (company-reported CV's $<1.1\%$) [23, 33]. Biomarker measurements were performed blind to clinical data.

SCr-AKI definition

SCr-AKI definition was based on the Kidney Disease: Improving Global Outcomes (KDIGO) definition [34]: stage 1 was a SCr rise to $\geq 50\%$ (within 7 days) or by ≥ 0.3 mg/dl ($26.5 \mu\text{mol/l}$, within 48 h) from baseline; stage 2 was doubling of baseline SCr; stage 3 was tripling of baseline SCr, SCr rise to ≥ 4.0 mg/dl ($353.6 \mu\text{mol/l}$), dialysis initiation, or

estimated GFR (eGFR) decrease to <35 ml/min/m². eGFR was calculated using the CKiD equation [35]. Baseline SCr was the lowest value within 3 months before AG start. When baseline SCr was unknown, a normal baseline eGFR (120 ml/min/1.73 m²) was assumed and estimated baseline SCr was back-calculated using the CKiD equation [36, 37]. The KDIGO urine output criteria were not feasible to consider, since patients were on non-critical care wards.

CysC-AKI definition

CysC-AKI definition was performed using a modified KDIGO definition, but using CysC rise instead of SCr rise from baseline. It was not possible to include the stage 1 criterion of “0.3 mg/dl (26.5 μ mol/l) rise from baseline”, since determining what raw CysC rise equates to a raw 0.3 mg/dl SCr rise would not be possible. Therefore, stage 1 CysC-AKI was only based on a 50 % CysC rise from baseline. To estimate baseline CysC, we calculated baseline SCr-based eGFR, followed by back-calculation of estimated baseline CysC, using a validated CysC eGFR equation [28]. In 62 % of AG-treatment episodes with a pre-AG CysC value available (using leftover serum drawn from routine care), the measured baseline CysC was used.

Persistent kidney dysfunction

This secondary outcome was defined in two ways. The first was the presence of SCr-AKI duration for at least 2 consecutive days after first evidence of SCr-AKI (i.e., SCr-AKI ≥ 48 h). The second method was defined using percent change in SCr from baseline at the end of AG treatment (SCr on the last day of AG or within 72 h of treatment end/baseline SCr), referred to as end of treatment persistent SCr rise. We evaluated the presence of a 15, 25, and 50 % end of treatment persistent SCr rise.

Statistical analysis

Continuous variables were compared between groups using Mann–Whitney or *t* tests, as appropriate. Categorical variables were compared using Chi-square or Fisher’s exact tests. Agreement level between SCr- and CysC-AKI definitions was evaluated by percent agreement and Kappa statistics.

CysC as an early SCr-AKI biomarker—The day of AG treatment that subjects first fulfilled criteria for SCr-AKI was identified in SCr-AKI patients and referred to as Day 0 SCr-AKI. Percent CysC rise from baseline was plotted daily from 3 days before SCr-AKI occurred (Day -3 SCr-AKI) to 2 days after SCr-AKI occurred (Day $+2$ SCr-AKI). For each of those days relative to first evidence of SCr-AKI (i.e., Days -3 to $+2$ SCr-AKI), the areas under the receiver-operating characteristic curves (AUC, 95 % confidence interval [CI]) of percent CysC rise from baseline were calculated to predict SCr-AKI (keeping in mind that percent SCr rise from Day 0 to Day $+2$ SCr-AKI is discriminatory and not “predictive”). For AUC calculation, one CysC value per non-AKI subject was randomly selected by using a computer generated selection process (to identify one random CysC value available per control subject). Sensitivity and specificity for different CysC percent rises were calculated and the most discriminatory value was estimated by calculating the *j*-statistic (sensitivity % + specificity % $- 100$ %).

CysC association with persistent kidney dysfunction—The AUC for CysC and SCr percent rise from baseline, measured on Day 0 of SCr-AKI (i.e., first day of SCr-AKI attainment), were calculated to predict (a) SCr-AKI duration ≥ 48 h, and (b) persistent SCr rise (≥ 15 , ≥ 25 , or ≥ 50 %) from baseline at the end of AG treatment.

AKI outcome association—Multivariate Cox proportional hazards regression was used to evaluate associations of SCr-AKI and CysC-AKI with LOS, controlling for age, gender, total AG hours, reason for taking AG, presence of documented infection and use of other nephrotoxins in the 2 weeks before treatment. These other variables were selected for inclusion in the model if they were associated with SCr-AKI and hospital length of stay (i.e., potential confounders) or if determined a priori to be potentially relevant (e.g., age, gender, total drug hours, infection). We did not include highly correlated variables within the model (e.g., AG type and reason for AG were highly correlated). Hazard ratios (HR, 95 % CI) were calculated; a HR < 1 indicated association with *longer* LOS.

Because we included more than one AG treatment episode per subject, we performed a sensitivity analysis whereby we only included the first AG episode for each subject (one episode per subject). We repeated the analyses described above, for CysC to predict AKI, persistent AKI and association with longer LOS. All results were almost identical in magnitude and direction, unless otherwise specified. Analyses were performed using STATA 10® statistical software package (College Station, TX, USA).

Results

Cohort characteristics and AKI incidence

Eighty-one children with 110 AG treatment episodes were included (Fig. 1). Patient characteristics, stratified by AKI status, are shown in Table 1. SCr-AKI occurred in 50 treatment episodes and CysC-AKI occurred in 53 episodes (not mutually exclusive). Treatment episodes with SCr-AKI were more likely to have febrile neutropenia as treatment reason, prior nephrotoxin use, and be treated on the hematology/oncology service (Table 1). CysC-AKI treatment episodes were more likely to occur in younger patients, with febrile neutropenia as treatment reason and be associated with a longer LOS (Table 1). At least one AG level was measured in 96 of the AG treatment episodes. Only six of these treatment episodes had an AG level above therapeutic dose; four of these high levels were measured during treatments associated with no SCr-AKI development; and two were from treatments associated with SCr-AKI development ($p < 0.05$, not shown).

SCr-AKI vs. CysC-AKI incidence and severity

All AG episodes were evaluated for occurrence of SCr-AKI (as per KDIGO definition) and of CysC-AKI (as per the modified KDIGO definition using CysC instead of SCr). There was moderate agreement (percent agreement = 73.6 %, kappa = 0.47, $p < 0.001$) between the presence and absence of any SCr-AKI and CysC-AKI. There were no instances of SCr-AKI whereby AKI was attained only by the criterion of $26.5 \mu\text{mol/l}$ SCr rise from baseline (the criterion which was not included in the CysC-AKI definition). However, agreement was lower (percent agreement = 57.3 %, kappa = 0.33, $p < 0.001$) when comparing SCr- and

CysC-AKI severity staging (Table 2). AKI staging disagreement was primarily for stage 1 AKI (detailed in Table 2). Fifty-seven percent (12/21) of patients with stage 1 SCr-AKI had no CysC-AKI; 50 % (11/22) of patients with stage 1 CysC-AKI had no SCr-AKI. Thirty-seven (34 %) patients had both SCr and CysC AKI. The majority of SCr-AKI (82 %) and of CysC-AKI (83 %) first appeared before AG treatment day 5. The difference between actual pre-AG CysC value and estimated baseline CysC was 0.07 ± 0.09 mg/l.

CysC as an early biomarker of AKI

Figure 2 demonstrates the CysC percent rise from baseline (expressed as fold-wise increase) from 3 days before to 2 days after the first day of SCr-AKI attainment (Day 0). Two days before SCr-AKI (Day -2), there was a median 1.5 fold rise in CysC. However, there was substantial variability as evidenced by wide interquartile range (Fig. 2).

Table 3 shows that percent CysC rise from baseline predicted stage 1 AKI and stage 2 AKI development, when measured in the 2 days before stage 1 or stage 2 AKI attainment (Days -1 and -2 SCr-AKI) and discriminated for stage 1 or 2 AKI (Day 0, +1 and +2). On Day-2 AKI, the area under the receiver operating characteristic curves (AUC) for percent CysC rise to predict stage 1 and 2 AKI were 0.75 and 0.85, respectively (Table 3). On Day 0 AKI, percent CysC rise discriminated for stage 1 and stage 2 SCr-AKI with AUCs of 0.77 and 0.94, respectively (Table 3).

A 10 % CysC rise on Day -2 of SCr-AKI predicted stage 1 AKI or worse with sensitivity = 71 % and specificity = 53 %; 25 % CysC rise on Day -2 predicted stage AKI with similar sensitivity but with specificity = 71 %. The best combined sensitivity and specificity for Day -2 percent CysC rise to predict stage 1 AKI was with a 44 % CysC rise (j-statistic = 0.48, sensitivity = 65 %, specificity = 83 %).

Of note, all AUCs were higher in treatment episodes where a pre-AG treatment CysC value was available, versus treatments for which baseline CysC was estimated (data not shown).

CysC and persistent kidney dysfunction

CysC percent rise from baseline on Day 0 of SCr-AKI, predicted SCr-AKI duration ≥ 48 h with an AUC of 0.73 (95 % CI 0.56–0.90). At AG treatment end (105/110 treatments with available data), in 64 (61 %), 52 (50 %) and 33 (31 %) episodes, patients had SCr ≥ 15 , ≥ 25 , and ≥ 50 % above baseline values, respectively. Figure 3 shows the AUC for CysC percent rise from baseline on Day 0 of SCr-AKI to predict persistent SCr rise ≥ 50 % above baseline at AG treatment end (AUC = 0.76, 95 % CI 0.61–0.90). Day 0 percent CysC rise predicted ≥ 15 and ≥ 25 % persistent SCr rise at treatment end with AUCs of 0.59 (95 % CI 0.39–0.79) and 0.67 (95 % CI 0.47–0.88), respectively. For comparison, percent SCr rise from baseline on Day 0 of SCr-AKI predicted a ≥ 15 , ≥ 25 , and ≥ 50 % end of treatment SCr rise with AUCs of 0.50 (95 % CI 0.32–0.68), 0.58 (95 % CI 0.41–0.74), and 0.68 (95 % CI 0.53–0.82), respectively.

AKI and hospital length of stay

CysC-AKI was associated with longer hospital LOS when controlling for other variables (age, gender, total AG hours, reason for taking AG, presence of documented infection and use of other nephrotoxins before treatment), with an adjusted hazard ratio of 0.59 (95 % CI 0.38–0.92, $p = 0.02$). Increased AG treatment duration in hours was also independently associated with longer LOS (adjusted HR = 0.996 (95 % CI 0.994–0.998), $p < 0.001$) and febrile neutropenia was associated with shorter LOS (adjusted HR = 1.55 (95 % CI 1.19–2.02), $p = 0.001$). SCr-AKI did not predict longer LOS when adjusted for other variables.

When only one AG episode per patient was used for these analyses, the relationship between CysC-AKI and prolonged hospital stay was stronger (adjusted p value 0.009), but there was no difference in the association between SCr-AKI and LOS (data not shown).

Discussion

This is the first prospective study critically examining the use of serum CysC in non-critical care pediatric patients treated with AG, a common setting associated with AKI. We found that CysC was an early biomarker of SCr-AKI, with patients having a median 1.5-fold rise from baseline 2 days prior to SCr-AKI development, and was predictive of persistent AKI. CysC-AKI was more strongly associated with longer LOS than SCr-AKI was, when controlling for other variables.

Prior to using CysC rise to define AKI, it is essential to understand how it differs from the SCr-AKI definition. In our study, there was considerable disagreement between SCr-AKI and CysC-AKI diagnosis and staging. Future studies with larger sample sizes should investigate the differences in characteristics between patients who are discordant between the two AKI definitions, such as diagnoses or medication, or use renal injury biomarkers to differentiate between patients with true renal cellular damage and those with non-AKI related SCr or CysC rise (e.g., volume depletion). Furthermore, to better understand how SCr- and CysC-AKI definitions are related, it is important to evaluate the rationale of using a 50 % rise cutoff in CysC, as is used in the Kidney Disease: Improving Global Outcomes (KDIGO) definition. We found that a cutoff of 44 % CysC rise was diagnostic of SCr-AKI, supporting the current 50 % rise cutoff value. CysC-AKI was more strongly associated with LOS, suggesting that it may more accurately reflect true kidney injury than SCr-AKI, though this requires confirmation. Unlike our previous studies in non-critically ill patients treated with AG [12], SCr-AKI was not associated with longer LOS in this study. This may be due to sample size constraints or one of our inclusion criteria, which was explicitly based on minimum treatment duration.

In patients with AKI, there was a rise in CysC before SCr rise, though there was considerable variability. The AUCs for early AKI diagnosis were similar or better to AUCs found in other studies of urine protein AKI biomarkers [23, 38]. Studies performed in other AKI settings have also suggested that CysC is an early AKI biomarker [32, 39–42]; in other words, that CysC rise with AKI occurs before SCr rise does. Why this is the case is unclear, as CysC and SCr are both markers of GFR. CysC may be a more accurate marker of GFR and is simply more specific to acute changes in GFR than SCr, being less affected by non-

renal factors (such as muscle mass, diet, medication) and exhibiting no tubular secretion; thus perhaps with acute changes in GFR, CysC is more sensitive and specific for detecting a GFR decrease. Despite this lack of clarity, CysC may potentially be used as an early biomarker of AKI, for inclusion in AKI treatment or AKI complication prevention studies (e.g., prevention of fluid overload) or in an early AKI biomarker panel.

CysC measured on the first day of SCr-AKI predicted persistent AKI. This is potentially very useful, and could be integrated into AKI prevention strategies (e.g., nephrotoxin avoidance/cessation) or may be used to stratify need for more intensive SCr monitoring. Of note, the percentage of patients with persistent SCr rise at end of AG treatment was higher than expected. This is highly relevant given recent research showing that some patients treated with nephrotoxins may be at higher risk for long-term chronic kidney disease [18]. This finding deserves further investigation in long-term follow-up studies, ideally identifying which patients are at increased risk for late outcomes.

There were limitations to this study. The sample size limited our ability to control for several other potential confounders of the AKI-outcome relationship and to evaluate CysC predictive value over and above a baseline clinical model. The study was not powered to explicitly evaluate more severe AKI (e.g., Stage 2 AKI or worse) as an outcome or as a predictor of clinical outcomes. Nearly all patients were from a single center, thus limiting generalizability. However, we demonstrated feasibility of performing such a study in non-ICU wards in another center, which is encouraging for future non-ICU AKI biomarker research. Another limitation was the lack of measured baseline (pre-AG) CysC values in a significant proportion of subjects. However, in order to obtain baseline CysC for all subjects, the study would have to be performed in a center where CysC is measured as part of routine care. This would be feasible, since lag-time from CysC measurement using current technology is not associated with significant lag time. An alternative method to estimate baseline CysC would be to retrospectively use the lowest CysC during treatment. However, we did not favor this method, as this is not reproducible in a prospective manner for future studies. Although our patient heterogeneity was a strength for generalizability, it is likely that certain patient subgroups differ in how well CysC performs, which we were unable to effectively evaluate. As a strength, the study was novel and performed on non-ICU wards. It is necessary to understand how new AKI biomarkers behave in non-critical patients if they are to be used in clinical care.

In conclusion, serum CysC may be used for early AKI diagnosis and to predict persistent AKI following aminoglycoside treatment in non-critically ill children.

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Fig. 1.

Subject selection flow diagram that depicts identification and selection of aminoglycoside treatment episodes for Montreal patients. Box A. Exclusion criteria are described in the text. Box B. Reasons included under “Not possible to recruit”: guardian unavailable, treatment changed early, renal problem discovered prior to recruitment. Box 4. Cincinnati patient study flow not detailed (pilot study); six treatment episodes from six individuals were included. Box D. Required number of serum creatinine and cystatin C values described in text. *AG* aminoglycoside; *Scr* serum creatinine; *CysC* cystatin C

Fig. 2.

Cystatin C relative rise from baseline, from 3 days before to 2 days after serum creatinine-defined acute kidney injury (SCr-AKI) first attainment. Box plots are shown depicting the rise in CysC from baseline (y-axis, fold-increase in CysC from baseline), which occurred in SCr-AKI patients, from 3 days before to 2 days after first evidence (Day 0) of SCr-AKI (x-axis). *Middle lines* in the boxes represent medians; *lower and upper box borders* represent 25th and 75th percentile values; *lower and upper stems* represent 5th and 95th percentile values. The figure only includes treatment episodes with SCr-AKI development. Numbers of subjects for Days -3, -2, -1, 0, +1, and +2 box plots are 9, 12, 18, 45, 43, and 36, respectively. *SCr* serum creatinine; *AKI* acute kidney injury; *CysC* cystatin C

Fig. 3.

Receiver operating characteristic (ROC) curve for percent cystatin C rise from baseline to predict persistent (≥50%) serum creatinine rise at the end of aminoglycoside treatment. The ROC curve plots sensitivity versus 1-specificity for various cutoffs of percent CysC rise from baseline, measured on the first day of SCr-AKI attainment, to predict a persistent ≥50% SCr rise above baseline at the end of AG treatment. *SCr* serum creatinine; *AG* aminoglycosides; *CysC* cystatin C; *SCr-AKI* serum creatinine-defined acute kidney injury; *AKI* acute kidney injury

Table 1

Patient characteristics comparing subjects with versus without acute kidney injury (AKI) defined by serum creatinine (SCr) and cystatin C (CysC)

Variable	SCr- No AKI (n = 60)	SCr-AKI [§] (n = 50)	CysC- No AKI (n = 57)	CysC-AKI (n = 53)
Continuous variables (mean ± SD, median)				
Age (years)	8.3 ± 5.3 (9.3)	7.8 ± 4.4 (7.63)	9.0 ± 5.3 (9.8) *	7.1 ± 4.3 (6.6) *
Days between hospitalization and AG start	6.6 ± 16.1 (0)	4.7 ± 14.5 (0)	3.6 ± (10.2) (0)	8.0 ± 19.3 (0)
Hours of AG treatment	172.4 ± 108.9 (143.5)	216.4 ± 265.2 (171.0)	169.9 ± 111.7 (139.8)	216.5 ± 257.2 (167.1)
AG day 1 dose (mg/kg/day)				
Gentamicin	6.5 ± 1.1 (6.4)	6.7 ± 0.8 (6.5)	6.5 ± 1.1 (6.3)	6.6 ± 0.7 (6.5)
Tobramycin	7.3 ± 1.7 (7.5)	6.9 ± 1.5 (7.5)	7.2 ± 1.8 (7.5)	7.1 ± 1.4 (7.5)
Amikacin	23.5 ± 0.5 (23.5)	20 ± 0 (20)	-	22.4 ± 2.1 (23.15)
Days of hospital stay	25.4 ± 38.9 (10.5)	22.94 ± 37.50 (9)	14.3 ± 15.1 (9) *	35.0 ± 50.7 (11) *
Categorical variables (n (%)) ^a				
Male gender [#]	30 (50)	24 (48)	24 (42)	30 (57)
AG (aminoglycoside) drug				
Gentamicin	11 (18)	3 (6)	10 (18)	4 (8)
Tobramycin	47 (78)	46 (92)	47 (82)	46 (87)
Amikacin	2 (3)	1 (2)	0 (0)	3 (6)
Reason for AG treatment				
Documented or suspected infection	18 (30)	8 (16)	12 (21)	14 (26)
Cystic fibrosis	6 (10)	0 (0)	6 (11)	0 (0)
Febrile neutropenia	36 (60) **	42 (84) **	39 (68) *	39 (74) *
Service				
General pediatric	12 (20)	1 (2)	10 (18)	3 (6)
Surgery	5 (8)	2 (4)	5 (9)	2 (4)
Hematology/Oncology	43 (72) **	47 (94) **	42 (74)	48 (91)
Documented or suspected infection ^b	21 (35)	20 (40)	19 (33)	22 (42)
Concurrent nephrotoxic medication (NTM) ^c	21 (35) *	29 (58) *	22 (39)	28 (53)
Concurrent use of steroid	11 (18)	8 (16)	9 (16)	10 (19)
Use of NTM in 2 weeks prior to AG treatment	32 (53) *	36 (72) *	36 (63)	32 (60)

* *p* value < 0.05 compared to non-AKI category

** *p* value < 0.01 compared to non-AKI category

[§]Thirty-seven patients had AKI defined by the SCr method and the CysC method

[#]Of 81 patients studied, 40 (49 %) were male

^aFisher's exact test was used to look for significance between categorical variables

^bInfections other than those associated with clinical settings of cystic fibrosis or febrile neutropenia

^cNephrotoxins included vancomycin, acyclovir, ganciclovir, cidofovir, cyclosporine, tacrolimus, cisplatin, carboplatin, ifosfamide, methotrexate, cyclophosphamide, non-steroidal anti-inflammatories, caspofungin, amphotericin B, aspirin

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Comparison of AKI staging between serum creatinine-defined (SCr-AKI) and CysC-defined AKI (CysC-AKI)

Table 2

AKI stages defined by CysC (CysC-AKI)	AKI stages defined by SCr (SCr-AKI) ^I				
	No AKI	Stage 1 AKI	Stage 2 AKI	Stage 3 AKI	Total
No AKI (CysC)	44	12	1	0	57
Stage 1 AKI (CysC)	11	4	7	0	22
Stage 2 AKI (CysC)	5	3	6	5	19
Stage 3 AKI (CysC)	0	2	1	9	12
Total	60	21	15	14	110

^I Percent agreement = 57.27 %, kappa statistics = 0.33, between SCr and CysC-AKI stages
Note: *shaded boxes* represent AKI stages for which there was agreement between AKI classifications
AKI acute kidney injury; SCr serum creatinine; CysC cystatin C

Table 3

Areas under the receiver operating characteristic curves (AUC): percent cystatin C (CysC) rise from baseline to predict and discriminate for development of stage 1 and stage 2 serum creatinine-defined acute kidney injury (SCr-AKI)

AUC [95 % CI]	Stage 1 SCr-AKI	Stage 2 SCr-AKI
3 days before AKI development	0.58 [0.36–0.79]	0.81 [0.62–0.99]
Day –3 Optimal % CysC rise (sensitivity %, specificity %) *	47 % (38 %, 85 %)	48 % (78 %, 85 %)
2 days before AKI development	0.75 [0.60–0.90]	0.85 [0.75–0.95]
Day –2 Optimal % CysC rise (sensitivity %, specificity %)	44 % (65 %, 83 %)	52 % (67 %, 85 %)
1 day before AKI development	0.66 [0.51–0.81]	0.83 [0.73–0.93]
Day –1 Optimal % CysC rise (sensitivity %, specificity %)	40 % (50 %, 81 %)	45 % (73 %, 83 %)
Day of AKI development	0.77 [0.67–0.86]	0.94 [0.90–0.99]
Day 0 Optimal % CysC rise (sensitivity %, specificity %)	63 % (53 %, 93 %)	71 % (96 %, 83 %)
1 day after AKI development	0.82 [0.74–0.91]	0.94 [0.90–0.99]
Day +1 Optimal % CysC rise (sensitivity %, specificity %)	22 % (79 %, 71 %)	28 % (100 %, 73 %)
2 days after AKI development	0.78 [0.68–0.87]	0.87 [0.77–0.96]
Day +2 Optimal % CysC rise (sensitivity %, specificity %)	17 % (84 %, 66 %)	37 % (85 %, 81 %)

AKI acute kidney injury; SCr-AKI acute kidney injury defined by serum creatinine; AUC area under the receiver operating characteristic curve

* The CysC % rise that results in the highest combined sensitivity and specificity (j-statistic)