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Acute kidney injury biomarkers predict an increase in serum milrinone concentration earlier than serum creatinine-defined acute kidney injury in infants after cardiac surgery

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Abstract

Background—Milrinone, an inotropic agent used ubiquitously in children after cardiac surgery accumulates in acute kidney injury (AKI). We assessed if urinary AKI biomarkers are predictive of an increase in milrinone concentrations in infants after cardiac surgery.

Methods—Multicenter prospective pilot study of infants undergoing cardiac surgery. Urinary AKI biomarkers were measured in the urine at specific time intervals after cardiopulmonary bypass initiation. AKI was defined using the Kidney Disease: Improving Global Outcomes serum creatinine criteria. Serum milrinone concentrations were measured at specific intervals following drug initiation, dose changes, and termination. Excessive milrinone activity was defined as a 20%

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increase in serum concentration between 6 and 36 hours after initiation. The temporal relationship between urinary AKI biomarker concentrations and a 20% increase in milrinone concentration was assessed.

Results—AKI occurred in 31 (33%) of infants. Milrinone clearance was lower in patients with AKI (4.2 L/h/70 kg vs. 5.6 L/h/70 kg; $p = 0.02$). Excessive milrinone activity was associated with development of serum creatinine-defined AKI (OR 3.0, 95% CI: 1.21–7.39, $p = 0.02$). Both tissue inhibitor metalloproteinase type 2 and insulin-like growth factor-binding protein type 7 (TIMP-2*IGFBP-7) 0.78 at 12 hours (OR 2.72, 95% CI: 1.01–7.38, $p = 0.04$) and kidney injury molecule 1 (KIM-1) 529.57 at 24 hours (OR 2.76, 95% CI: 1.06–7.17, $p = 0.04$) predicted excessive milrinone activity before a diagnosis of AKI.

Conclusions—In this pilot study, urine TIMP-2*IGFBP-7 and KIM-1 were predictive of AKI and excessive milrinone activity. Future studies that include a pharmacodynamics assessment of patient hemodynamics, excessive milrinone activity, and AKI biomarker concentrations may be warranted to integrate this concept into clinical practice.

Keywords

acute kidney injury; milrinone; biomarkers; infants; cardiac surgery

Introduction

Milrinone is a phosphodiesterase type 3 (PDE3) inhibitor prescribed for many pediatric and adult patients with acute decompensated heart failure. It is also used in children after cardiac surgery for the prevention of low cardiac output syndrome (LCOS), a clinical phenomenon characterized by tachycardia, hypotension, and end-organ dysfunction [1]. The onset of LCOS occurs within 12 hours after cardiac surgery in 25%–40% of cases. Inhibition of PDE3 from milrinone results in an increase in cyclic adenosine monophosphate (cAMP)-mediated delivery of calcium to the myocardium, which leads to improvement in both systolic and diastolic function, as well as vascular smooth muscle dilation, thereby reducing ventricular afterload [2–4].

Pediatric milrinone dosing is fairly standardized across most pediatric cardiac surgical centers. Typically, a loading dose of 25–50 $\mu\text{g/kg}$ is administered intravenously, followed by initiation of a continuous infusion ranging from 0.25 to 1.0 $\mu\text{g/kg/minute}$. Despite these dosing strategies, the actual dose–response relationship has not been well described in children, and we have previously noted marked variability in serum milrinone concentrations despite similar weight-based dosing strategies [5]. The uncertainty in dosing is confounded by differences in patient age, size, and ontological maturation of the kidneys, and that therapeutic drug monitoring is not routinely performed. Dosing is also particularly challenging in the setting of acute kidney injury (AKI) because the kidneys clear milrinone [5, 6]. It has been reported that the mean plasma half-life of milrinone was threefold higher in those with AKI compared to healthy individuals [7].

In comparison to other inotropes, the pharmacodynamic effects of milrinone are not seen for 30–60 minutes [8, 9] and persist for several hours after discontinuation of therapy. As the

length of the administration time increases, so does the effective half-life, which is approximately 36 minutes after 30 minutes of an infusion and 138 minutes after 10 hours of an infusion [10]. The half-life is increased even more in patients with AKI [11]. This is important because there is a sigmoidal relationship between plasma milrinone concentration and percent cardiac index improvement across a range of 100–300 ng/mL. Specifically, no hemodynamic benefits have been seen at concentrations above 500 ng/mL, but the risk of adverse effects, including hypotension and systemic vasodilation, increases [12, 13].

Unfortunately, AKI occurs in 20%–40% of children after cardiac surgery [14, 15]. Timely dose adjustments of milrinone are critical to avoid hypotension and further renal compromise due to excess milrinone activity. Currently, AKI is diagnosed by a rise in serum creatinine that has several limitations: it rises 24–48 hours after injury has already occurred, and its concentrations are affected by factors unrelated to renal disease [16–18]. AKI biomarkers have been used to diagnose AKI before a rise in serum creatinine in children after cardiac surgery [19, 20], to predict drug clearance [21] and demonstrate renal toxicity in drug development [22].

The signs and symptoms of milrinone accumulation in patients with impending AKI and those with worsening cardiac function after cardiac surgery have significant overlap. In addition, the effects of a milrinone dose titration may not be seen for 4–6 hours. Therefore, earlier assessment of milrinone accumulation is necessary in those at risk for AKI. The purpose of this study was to determine if a targeted assessment of urinary AKI biomarkers were predictive of excessive milrinone activity in children after cardiac surgery. We hypothesized that AKI biomarkers will increase prior to a 20% increase in serum milrinone concentration in children after cardiac surgery.

Materials and Methods

We performed a prospective multicenter observational study (NCT101966237) in infants (< 1 year of age) after cardiac surgery with cardiopulmonary bypass (CPB) and who would receive intra- and post-operative milrinone from October 2013 to January 2015. Following institutional review board approval, infants were enrolled from three centers. Informed consent was obtained for each patient. Exclusion criteria were: pre-existing significant congenital anomalies of the kidneys and urinary tract, need for extracorporeal life support (ECMO) prior to and after cardiac surgery.

Clinical data were collected pre-, peri- and post-operatively. Clinical outcomes including duration of ventilation, length of intensive care unit and hospital stay, and mortality were assessed. Serum creatinine measurements were performed as part of standard clinical practice pre-operatively and daily for the 3 days following surgery. Baseline serum creatinine was defined as the lowest value obtained within 72 hours prior to surgery. AKI was defined by the KDIGO serum creatinine criteria (all stages) [23] that occurred no sooner than the first post-operative day but within 72 hours after CPB. Severe AKI was defined by KDIGO Stage 2 or 3 criteria, as this threshold has been associated with increased morbidity and mortality in infants after cardiac surgery [14] and critically ill children in general [24]. Serum creatinine measures were performed in the early mornings on each of 3 consecutive

days after surgery. Surgical complexity was categorized according to the Society of Thoracic Surgeons–European Association for Cardiothoracic Surgery, Congenital Heart Surgery (STAT) mortality categories [25]. Post-operative urine output was reported in mL/kg/hour daily, where post-operative day 0 was from admission to the cardiac intensive care unit until 6 am the next day, and post-operative day 1 was from 6 am the day after surgery until the next day at 6 am.

Blood samples were collected at specific time intervals (in a plain red top vacutainer with no additive) from time 0 (termination of CPB), 15, 20, and 60 minutes and then at 3, 6, 8, 12, 24, 36, 48, and 72 hours to assess serum milrinone concentrations. In some patients, additional samples were obtained: 1) immediately preceding and then 2 hours following a milrinone dose change and 2) at 30 and 60 minutes, and 3 and 8 hours following termination of the milrinone infusion. Importantly, milrinone dosing was not standardized between patients, and thus, sampling was variable, with some patients having the medication discontinued early without a dose change, and in others, it was continued beyond the 72 hours of enrollment. Finally, patient size and institutional review board restrictions limited blood sampling to a maximum of 14 mL during the study period, thereby preventing collection of samples at all the specified time points for each patient. Blood samples were centrifuged at $2400 \times g$ for 10 minutes at 4 °C, the serum extracted and stored at –80 °C until quantification of milrinone was performed. Serum milrinone quantification was performed using liquid chromatography-mass spectrometry-mass spectrometry (LC/MS-MS) using the following methodology: Milrinone (Toronto Research Chemicals, Inc.; Ontario, Canada) and procainamide (Sigma-Aldrich) stock dimethyl sulfoxide solutions (0.05 mol/L) were prepared and taken to prepare working solutions used to probe mass-spectrometry-mass spectrometry conditions to develop the LC/MS-MS method. An Applied Biosystems Sciex 4000[®] (Applied Biosystems; Foster City, CA) equipped with a Shimadzu HPLC (Shimadzu Scientific Instruments, Inc.; Columbia, MD) and Leap auto-sampler (LEAP Technologies; Carrboro, NC) was used. Milrinone and procainamide (internal standard) were monitored via electro-spray ionization positive ion mode (ESI+) using the following conditions: i) ion-spray voltage of 5500 V; ii) temperature, 450 °C; iii) curtain gas (CUR; set at 10) and Collisionally Activated Dissociation (CAD; set at 12) gas were nitrogen; iv) Ion Source gas one (GS1) and two (GS2) were set at 40; v) entrance potential was set at 10.0 V; vi) quadruple one (Q1) and three (Q3) were set on low resolution; vii) dwell time was set at 200 ms; and viii) declustering potential (DP), collision energy (CE), and collision cell exit potential (CXP) are voltages (V). Compound settings were i) milrinone: $t_R = 3.6$ minutes, $212.1 \rightarrow 140.0$ m/z , DP = 101, CE = 45, CXP = 8 and ii) procainamide (IS) $t_R = 3.1$ minutes, $236.3 \rightarrow 163.1$ m/z , DP = 56, CE = 23, CXP = 10. Liquid chromatography employed an Agilent Technologies, Zorbax extended-C18 50 × 4.6 mm column, 5 μ particle size, equipped with a C18 column guard and operated at 40 °C with a flow rate of 0.4 mL/minute. The mobile phase consisted of A: 0.01 mol/L ammonium acetate, 0.1% formic acid in water; B: 50:50 ACN:MeOH. Between samples, the auto sampler was washed with a 1:1:1:1 mixture of ACN:MeOH:IPA:water containing 0.1% formic acid. The chromatography method used was 95% A for 0.50 minutes, brought to 95% B at 3.00 minutes and held for 4.00 minutes, ramped back down to 5% B at 8.00 minutes and held for 0.50 minutes. Next, human serum (lot# BRH947221) was purchased from

Bioreclamation LLC (Liverpool, NY) and used to prepare standard curves with serial dilutions creating 18 standard curve serum samples. Quality control samples were also prepared and used in the study. The standard curve serum samples were then placed into the freezer (~4 hours) and then transferred to a -80 ± 10 °C freezer (24 hours). This was conducted so that standard curve samples underwent one freeze thaw cycle, analogous to the clinical samples. Standard curve and clinical samples were extracted using an extraction solution containing internal standard 4:1 (1:1 ACN:MeOH) and water. In individual sets, the PK sample tubes were removed from the freezer and allowed to thaw on ice. The samples were prepared, and the supernatants transferred into individual wells of a 96-well plate. The 96-well plate was placed into the LEAP auto-sampler cool-stack (8.0 ± 1.0 °C) and samples (10 microliters) were analyzed via LC/MS-MS methods. The milrinone standard curve data were processed and fit to $1/x^2$ weighted linear regression analysis. The linear response range was between 0.7 and 2600 ng/mL ($R^2 = 0.99$). The limit of detection was 0.7 ng/mL, while the limit of quantification was 2.6 ng/mL. The accuracy and precision of the analytical method was evaluated using six determinations per concentration, and seven different analyte concentrations were probed ranging from 3.0 to 1200 ng/mL. The mean values were all less than 15% of the nominal value. Likewise, precision was assessed, and the coefficient of variation (CV) was less than 15%. The linear standard curves were used to compute drug concentrations. Pharmacokinetic parameter estimates were generated through Bayesian estimation using MW/Pharm (Version 3.82) as previously described [5]. An allometric power model was used to account for differences in body size. Steady state was assumed at 6 hours. A 20% increase in milrinone concentration between 6 and 36 hours after the start of the infusion was used to define a clinically significant change from the level obtained at 6 hours.

Neutrophil gelatinase-associated lipocalin (NGAL), tissue inhibitor metalloproteinase type 2 and insulin-like growth factor-binding protein type 7 (TIMP-2*IGFBP-7), kidney injury molecule 1 (KIM-1), interleukin-18 (IL-18), and liver-type fatty acid-binding protein (L-FABP) were measured on the urine samples collected at baseline (prior to CPB initiation) and then at 2, 6, 12, 24, 48, and 72 hours following CPB initiation. TIMP-2*IGFBP-7 was measured using the Astute[®] 140 meter (Astute Medical, Inc., San Diego, California). The authors have previously reported the cut-off points of TIMP-2*IGFBP-7 associated with AKI [20]. Urine NGAL was measured using a commercially available assay (NGAL ELISA Kit 036; Bioporto, Grusbakken, Denmark) that specifically detects human NGAL [26]. The intra-assay CV was 2.1% and inter-assay variation was 9.1%. Urine IL-18 (Kit 7625) and L-FABP (Kit Z-001) were measured using commercially available ELISA kits (Medical & Biological Laboratories Co., Nagoya, Japan and CMIC Co., Tokyo, Japan, respectively) per manufacturer's instructions. Intra-assay CV for IL-18 and L-FABP were 7.3% and 6.1% respectively. Interassay CV were 7.5% and 10.9% for IL-18 and L-FABP respectively. The urine KIM-1 ELISA was performed using validated commercially available reagents (Duoset DY1750, R & D Systems, Inc., Minneapolis, MN) as previously described [28]. Intra- and inter-assay CV's for KIM-1 was 2% and 7.8%, respectively [19, 28–30].

Statistical analyses were performed using SAS version 9.3 (Carey, North Carolina). The study was powered using Fisher's exact test with an assumption of 15%–20% rate of severe AKI, which is 4 times more likely to have at least a 20% increase in milrinone concentration

than those without severe AKI that occurs between 6 and 36 hours after CPB initiation at a type-I error of 0.05 and power of >95%. Normally distributed continuous variables were summarized with mean and standard deviation and were analyzed using t-tests. Non-normally distributed variables were summarized as median with interquartile range and were analyzed using Wilcoxon rank sum tests. Categorical variables were summarized using frequency and proportion and compared using chi-square or fisher exact tests as appropriate. Biomarkers and milrinone clearance values were then log transformed in logistic regression to assess their association with AKI. Receiver operating characteristics (ROC) curves were analyzed to identify optimal cut-off points for biomarkers and milrinone clearance predictive of AKI using the Youden index method. We then performed a chi-square analysis to determine the association between a 20% increase in serum milrinone concentration 6–36 hours after initiation of the infusion and biomarker elevation before AKI. Odds ratios and 95% confidence interval were reported. Multivariable logistic regression was performed to predict a 20% increase in milrinone concentration prior to the diagnosis of AKI. The following variables were included in the model: age, sex, peritoneal drain (yes vs. no), circulatory arrest (yes vs. no), prior surgery (yes vs. no), single ventricle (yes vs. no), cross-clamp time, STAT score, and total bypass duration. A p value of <0.05 was considered statistically significant. p Values were not adjusted for multiple tests.

Results

Eligible patients (n = 124) were screened, of which 98 infants were enrolled. Twenty-six patients declined to participate. Four patients were excluded from the final analysis as they did not receive milrinone or were cannulated to ECMO in the immediate post-operative period.

AKI occurrence after CPB

Serum creatinine-defined AKI occurred in 31 (33%) patients, of which 12 (13%) were classified as KDIGO stage 1, 16 (17%) as KDIGO stage 2, and 3 (3%) as KDIGO stage 3. The mean time to AKI diagnosis from CPB initiation was 25 ± 7 hours for all AKI groups. Demographics and intraoperative characteristics are shown in Table 1.

Milrinone clearance in patients with AKI

Two patients received milrinone prior to cardiac surgery. A loading dose of 25–50 µg/kg was given in 24% (n = 23) patients. These patients were all from a single center. There was no difference in milrinone pharmacokinetics and AKI among those who received a loading dose versus those who did not. We did not evaluate clinical outcomes among those who received a loading dose as the sample size was small (n = 23), and we would not be able to discern between the center effect versus the bolus effect. The median dose of milrinone used in the entire cohort was 0.5 µg/kg/minute on the day of surgery and post-operative day 1, and there was no difference in dosing strategies between those with and without AKI (p = 0.26 and p = 0.39). Allometrically scaled milrinone clearance estimates increased with increasing enrollment age in patients both with and without AKI and was below that reported for adults of 16.8 L/h/70 kg (Figure I) [31]. In this study, milrinone clearance was lower in patients with AKI (all stages) (4.2, IQR: 3.2–5.9 L/h/70 kg) compared to those without AKI (5.6,

IQR: 4–8.5 L/h/70 kg) ($p = 0.02$) (Figure II). The estimated mean steady-state concentration with a standard dose of 0.5 $\mu\text{g/kg/minute}$ was 215 ng/mL (IQR: 116–311 ng/mL). Sixty percent of patients were outside the documented target range of 180–300 ng/mL (32% below and 20% above) [12, 13, 32]. A milrinone clearance of 4.64 L/h/70 kg was associated with AKI (OR: 2.95, 95% CI: 1.21–7.18; $p = 0.02$).

Urinary AKI biomarkers during and after CPB and AKI (all stages)

Comparison of baseline biomarker values was no different in patients with and without AKI (Table 1). In addition, comparison of our baseline biomarker values was similar to those published by Krawczeski et al. (data not shown) [34]. The area under the curve (AUC) for TIMP-2*IGFBP-7 to predict serum creatinine-defined AKI at 12 hours was 0.71 (95% CI: 0.60–0.83) with a negative predictive value of 0.83 (95% CI: 0.698–0.925) ($p = 0.002$) at a cut-off point of 0.78. The AUC for KIM-1 to predict serum creatinine-defined AKI when measured at 6 hours after CPB initiation was 0.66 (95% CI: 0.54–0.78) with a negative predictive value of 0.89 (95% CI: 0.73–0.97) ($p = 0.03$) at a cut-off point of 137.37 pg/mL. A summary of the biomarkers and their cut-off points is shown in Table 2.

Measured serum milrinone concentration and AKI (all stages)

A greater proportion of patients with AKI vs. those without AKI had at least a 20% increase in serum milrinone concentration (21/31 (68%) vs. 26/63 (41%), $p = 0.03$). There was no difference among those who did and did not receive a loading dose. Using logistic regression, a 20% increase in serum milrinone concentration was associated with the subsequent development of serum creatinine-defined AKI (OR 3.0, 95% CI: 1.21–7.39, $p = 0.02$).

Association between AKI urinary biomarkers and a 20% milrinone concentration increase and AKI—

Using cut-off points for biomarkers described in Table 2, two urinary AKI biomarkers were predictive of subsequent 20% increase in milrinone concentration prior to serum creatinine-defined AKI. Both TIMP-2*IGFBP-7 0.78 at 12 hours after CPB initiation (OR 2.72, 95% CI: 1.01–7.38, $p = 0.04$) and KIM-1 529.57 at 24 hours after CPB initiation (OR 2.76, 95% CI: 1.06–7.17, $p = 0.04$) predicted a subsequent 20% increase in milrinone concentration before the diagnosis of AKI (all stages). There was no association between an increase in urine NGAL and an increase in milrinone concentration in patients with and without AKI. In a multivariable model, adjusting for age, gender, STAT score, history of prior surgery, circulatory arrest, presence of a PD drain, both TIMP-2*IGFBP7 and KIM-1 remained predictive of a 20% increase in milrinone concentration and AKI. The model predictive performance improved with an AUC of 0.79, compared to each biomarker alone. A summary of the unadjusted and adjusted models is summarized in Table 3.

In patients with severe AKI (KDIGO stage 2 or 3), both TIMP-2*IGFBP-7 and KIM-1 were predictive of a subsequent 20% increase in serum milrinone concentration. Using cut-off points derived for severe AKI (data not shown), TIMP-2*IGFBP-7 0.74 at 2 hours after CPB initiation (OR: 3.69, 95% CI: 1.69–12.44, $p = 0.03$) and TIMP-2*IGFBP-7 0.78 at 12 hours after CPB initiation (OR 3.94, 95% CI: 1.30–11.97, $p = 0.01$) were predictive of a

20% increase in serum milrinone concentration in patients with subsequent serum creatinine-defined AKI. Using KIM-1 cut-off points derived for predicting severe AKI (data not shown), KIM-1 ≥ 730.96 at 2 hours after CPB (OR: 2.8; 95% CI: 1.17–6.67, $p = 0.02$) and a KIM-1 ≥ 585.65 at 12 hours after CPB (OR: 3.61; 95% CI: 1.39–9.4, $p = 0.01$) predicted a subsequent 20% increase in serum milrinone concentration in patients with severe (stage 2 or 3) AKI. In a multivariable model, adjusting for age, gender, STAT score, history of prior surgery, circulatory arrest, presence of a PD drain, both TIMP-2*IGFBP7 and KIM-1 remained predictive of a 20% increase in milrinone concentration and AKI. The model predictive performance improved with an AUC of 0.80, compared to each biomarker alone. A summary of the unadjusted and adjusted models is summarized in Table 4.

Association between AKI urinary biomarkers and milrinone clearance—Using cut-off points described in Table 2, there was a significant association between TIMP-2*IGFBP-7 ≥ 0.78 at 12 hours after CPB initiation and lower milrinone clearance (4.8 IQR: 3.3–6.2 L/h/70 kg) compared to those with TIMP-2*IGFBP-7 < 0.78 (6.4, IQR: 4–11.4 L/h/70 kg) ($p = 0.05$). Milrinone clearance was also lower in patients with TIMP-2*IGFBP-7 ≥ 1.46 at 24 hours after CPB (4.6, IQR: 3.3–6 L/h/70 kg) compared to those with TIMP-2*IGFBP-7 < 1.46 (5.8, IQR: 3.8–8.9 L/h/70 kg) ($p = 0.03$). The temporal relationship between the increase in urinary AKI biomarker and decrease in milrinone clearance could not be assessed because clearance is measured along a continuum.

Discussion

We performed a pilot pharmacokinetic study to explore the relationship between an increase in AKI biomarkers and an increase in serum milrinone concentration prior to a rise in serum creatinine corresponding with AKI. Milrinone clearance in the current study was lower than previously published reports of adults (16.8 L/h/70 kg) [31] and children (18.9 L/h/70 kg) [33]. More than half of patients had serum milrinone concentrations outside the reported therapeutic range (180–300 ng/mL), with 32% being above and 20% below, suggesting that current dosing strategies are not reliable for critically ill children requiring milrinone therapy after cardiac surgery. Similar to the authors' prior work [5], milrinone clearance in infants was significantly lower in patients with AKI. We observed an increase in TIMP-2*IGFBP-2 and KIM-1 that were predictive of serum creatinine-defined AKI, and the increase in AKI biomarkers occurred prior to a greater than 20% increase in serum milrinone concentrations. This suggests that AKI biomarkers may play a role in providing an early assessment of drug accumulation, prior to a rise in serum creatinine.

Dose adjustment of milrinone in children with AKI is based on adult recommendations, which are dependent upon changes in estimated creatinine clearance. In the present study, we demonstrate that even before creatinine rises, and thus a decline in glomerular filtration rate (GFR) is clinically evident, infants have elevated milrinone concentrations that are out of range, which may put them at risk for excessive vasodilation, hypotension, and resultant exacerbation of AKI. Importantly, unlike most inotropic medications where dose adjustment results in an almost immediate clinical response, the effects of a milrinone dose adjustment may take several hours, putting the patient at risk for ongoing hypotension and vasodilation, and thus further kidney injury. The relationship between an AKI biomarker increase and

impaired drug clearance exists for other medications, including tobramycin [21]. Downes et al. reported that an increase in urine NGAL was associated with impaired tobramycin clearance in patients with cystic fibrosis, including patients who never developed an increase in serum creatinine [21]. In addition, KIM-1 expression has been detected after exposure to a variety of nephrotoxic medications, even when serum creatinine concentrations do not increase, and is considered to be a highly sensitive biomarker for detecting AKI during drug development [22].

There continues to be a paucity of data on milrinone pharmacokinetics in kidney disease. Adult studies have evaluated the relationship between milrinone dose and AKI and found that patients with a GFR of less than 30 mL/minute/1.73 m² had milrinone concentrations threefold higher than the recommended upper limit [13]. Patients receiving maintenance intermittent hemodialysis had milrinone concentrations 10–20 times the upper limit of the therapeutic range with widely fluctuating levels measured before and after hemodialysis sessions [13]. In the cardiac surgical population, volume overload may further dilute serum creatinine resulting in missed cases of AKI and the inability to adjust nephrotoxic medications appropriately [34]. These data highlight the importance of non-serum creatinine-based monitoring to avoid significant nephrotoxicity and other drug-related adverse events.

AKI urinary biomarker assessment is used frequently in the area of drug development, and many candidate molecules are dismissed early due to excessive toxicity as defined by an increase in biomarker excretion. KIM-1 (a marker of proximal tubular injury) and TIMP-2*IGFBP-7, (markers of the presence of an arrest in the cell cycle) facilitate localization of injury to a specific area within the kidney. The same is true for other biomarkers such as NGAL, IL-18, and L-FABP. It is plausible that urinary biomarkers could also be used to assess for nephrotoxicity in patients at risk for AKI, especially when serum drug monitoring is not available, and before an anticipated rise in serum creatinine. This pharmacokinetic study highlights how an elevation of TIMP-2*IGFBP-7 and KIM-1 were predictive of both a greater than 20% increase in serum milrinone concentration, impaired milrinone clearance, and subsequent serum creatinine-defined AKI. This is particularly important, as therapeutic drug monitoring for milrinone is not widely available.

The strengths of this study are as follows: it is a multicenter investigation, and the rate of AKI is similar to previously published reports [14, 15]. It offers potential to allow for individualized dose adjustments for patients at risk for AKI if our pharmacokinetic results corroborate with a future pharmacodynamics study that assesses for an association between poor patient hemodynamics and AKI biomarker elevation prior to an increase in milrinone concentration and subsequent AKI. Another strength is that the population studied is homogenous, and it is likely that biomarker cut-off points and optimal drug levels may need to be specifically developed in this population. Finally, TIMP-2*IGFBP-7 and NGAL are available on a clinical testing platform, with a turnaround time of approximately 45 minutes, making their use in clinical care possible. There are, however, several important limitations in the study. This was a pharmacokinetic study, and we did not assess patient hemodynamics in relation to elevations of biomarkers or milrinone concentrations. AKI was defined using serum creatinine criteria only, and sampling was not performed at the same time as urine

biomarkers. It is therefore possible that a higher serum creatinine may have occurred sooner than reported. Use of urine output criteria has not been studied in the post-operative cardiac surgery cohort. This does not detract from the fact that stage 2 AKI based on serum creatinine alone is still associated with poor outcomes in this population. Milrinone therapeutic drug monitoring and TIMP-2*IGFBP-7 are not available for clinical use across all centers limiting the translation of the study results to practice. Additionally, a KIM-1 clinical testing platform is still under development. Since we included only patients less than a year of age, our results may not be applicable to a broader, more heterogeneous population of children undergoing cardiac surgery.

Conclusion

We performed a prospective pilot concept study to assess the ability of AKI biomarkers to predict an increase in milrinone serum concentration and AKI. We found that TIMP-2*IGFBP-7 at 12 hours and KIM-1 at 6 hours after CPB initiation were predictive of AKI and an increase in serum milrinone concentrations, which may suggest excessive milrinone activity. Future studies that include a pharmacodynamics assessment of impaired patient hemodynamics and excess milrinone activity in conjunction with AKI biomarkers are necessary before dose adjustment of milrinone can empirically be performed. In addition, future studies that include older children and adults receiving milrinone are needed to demonstrate the utility of AKI urinary biomarkers for predicting excess milrinone activity.

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Non-standard abbreviations

AKI

acute kidney injury

KDIGO

kidney disease improving global outcomes

TIMP-2*IGFBP-7

tissue inhibitor metalloproteinase-2 and insulin like growth factor binding protein 7

KIM-1

kidney injury molecule 1

PDE3

phosphodiesterase type 3

LCOS

low cardiac output syndrome

cAMP

cyclic adenosine monophosphate

CPB

cardiopulmonary bypass

Society of Thoracic Surgeons

European Association for Cardiothoracic Surgery, Congenital Heart Surgery (STAT)
mortality categories

mL

milliliters

g

gravity

°C

degrees Celsius

LC/MS-MS

liquid chromatography-mass spectrometry-mass spectrometry

CUR

curtain gas

CAD

collision activated dissociation

GS1

source gas 1

GS2

source gas 2; volts

Q1

quadrupole 1

Q3

quadrupole 3

ms

milliseconds

DP

declustering potential

CE

collision energy

CXP

collision cell exit potential

V

voltages

NGAL

neutrophil gelatinase associated lipocalin

L-FABP

liver-type fatty acid binding protein

IL-18

interleukin-18

CV

coefficient of variation

References

1. Hoffman TM, Wernovsky G, Atz AM, et al. Efficacy and safety of milrinone in preventing low cardiac output syndrome in infants and children after corrective surgery for congenital heart disease. *Circulation*. 2003; 107:996–1002. [PubMed: 12600913]
2. Baruch L, Patacsil P, Hameed A, et al. Pharmacodynamic effects of milrinone with and without a bolus loading infusion. *Am Heart J*. 2001; 141:266–273. [PubMed: 11174341]
3. Duggal B, Pratap U, Slavik Z, et al. Milrinone and low cardiac output following cardiac surgery in infants: is there a direct myocardial effect? *Pediatr Cardiol*. 2005; 26:642–645. [PubMed: 16193374]
4. Meyer S, Gortner L, Brown K, et al. The role of milrinone in children with cardiovascular compromise: review of the literature. *Wien Med Wochenschr*. 2011; 161:184–191. [PubMed: 21360291]
5. Gist KM, Mizuno T, Goldstein SL, et al. Retrospective evaluation of milrinone pharmacokinetics in children with kidney injury. *Ther Drug Monit*. 2015; 37(6):792–6. [PubMed: 25860636]
6. Stroshane RM, Koss RF, Biddlecome CE, et al. Oral and intravenous pharmacokinetics of milrinone in human volunteers. *J Pharm Sci*. 1984; 73:1438–1441. [PubMed: 6502494]
7. Larsson R, Liedholm H, Andersson KE, et al. Pharmacokinetics and effects on blood pressure of a single oral dose of milrinone in healthy subjects and in patients with renal impairment. *Eur J Clin Pharm*. 1986; 29:549–553.
8. Loh E, Elkayam U, Cody R, et al. A randomized multicenter study comparing the efficacy and safety of intravenous milrinone and intravenous nitroglycerin in patients with advanced heart failure. *J Card Fail*. 2001; 7:114–121. [PubMed: 11420762]
9. Zuppa AF, Nicolson SC, Adamson PC, et al. Population pharmacokinetics of milrinone in neonates with hypoplastic left heart syndrome undergoing stage I reconstruction. *Anesth Analg*. 2006; 102:1062–1069. [PubMed: 16551899]
10. Anderson BJ, Holford NH. Tips and traps analyzing pediatric PK data. *Paediatr Anaesth*. 2011; 21:222–237. [PubMed: 21320233]
11. Taniguchi T, Shibata K, Saito S, et al. Pharmacokinetics of milrinone in patients with congestive heart failure during continuous venovenous hemofiltration. *Intensive Care Med*. 2000; 26:1089–1093. [PubMed: 11030165]

12. Bailey JM, Hoffman TM, Wessel DL, et al. A population pharmacokinetic analysis of milrinone in pediatric patients after cardiac surgery. *J Pharmacokinet Pharmacodyn*. 2004; 31:43–59. [PubMed: 15346851]
13. Cox ZL, Calcutt MW, Morrison TB, et al. Elevation of plasma milrinone concentrations in stage D heart failure associated with renal dysfunction. *J Cardiovasc Pharmacol Ther*. 2013; 18:433–438. [PubMed: 23695773]
14. Blinder JJ, Goldstein SL, Lee VV, et al. Congenital heart surgery in infants: effects of acute kidney injury on outcomes. *J Thorac Cardiovasc Surg*. 2012; 143:368–374. [PubMed: 21798562]
15. Li S, Krawczeski CD, Zappitelli M, et al. Incidence, risk factors, and outcomes of acute kidney injury after pediatric cardiac surgery: a prospective multicenter study. *Crit Care Med*. 2011; 39:1493–1499. [PubMed: 21336114]
16. Doi K, Yuen PS, Eisner C, et al. Reduced production of creatinine limits its use as marker of kidney injury in sepsis. *J Am Soc Nephrol*. 2009; 20:1217–1221. [PubMed: 19389851]
17. Narayanan S, Appleton HD. Creatinine: a review. *Clin Chem*. 1980; 26:1119–1126. [PubMed: 6156031]
18. Samra M, Abcar AC. False estimates of elevated creatinine. *Perm J*. 2012; 16:51–52.
19. Krawczeski CD, Goldstein SL, Woo JG, et al. Temporal relationship and predictive value of urinary acute kidney injury biomarkers after pediatric cardiopulmonary bypass. *J Am Coll Cardiol*. 2011; 58:2301–2309. [PubMed: 22093507]
20. Gist KM, Goldstein SL, Wrona J, et al. Kinetics of the cell cycle arrest biomarkers (TIMP-2*IGFBP-7) for prediction of acute kidney injury in infants after cardiac surgery. *Pediatr Nephrol*. 2017; 32(9):1611–1619.
21. Downes KJ, Dong M, Fukuda T, et al. Urinary kidney injury biomarkers and tobramycin clearance among children and young adults with cystic fibrosis: a population pharmacokinetic analysis. *J Antimicrob Chemother*. 2017; 72:254–260. [PubMed: 27585963]
22. Waring WS, Moonie A. Earlier recognition of nephrotoxicity using novel biomarkers of acute kidney injury. *Clin Toxicol (Phila)*. 2011; 49:720–728. [PubMed: 21970770]
23. KDIGO AKI Work Group. KDIGO clinical practice guideline for acute kidney injury. *Kidney Int Suppl*. 2012; 2:1–138.
24. Kaddourah A, Basu RK, Bagshaw SM, et al. Epidemiology of acute kidney injury in critically ill children and young adults. *N Engl J Med*. 2017; 376:11–20. [PubMed: 27959707]
25. O'Brien SM, Clarke DR, Jacobs JP, et al. An empirically based tool for analyzing mortality associated with congenital heart surgery. *J Thorac Cardiovasc Surg*. 2009; 138:1139–1153. [PubMed: 19837218]
26. Bennett M, Dent CL, Ma Q, et al. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. *Clin J Am Soc Nephrol*. 2008; 3:665–673. [PubMed: 18337554]
27. Chaturvedi S, Farmer T, Kapke GF. Assay validation for KIM-1: human urinary renal dysfunction biomarker. *Int J Biol Sci*. 2009; 5:128–134. [PubMed: 19173034]
28. Ascher SB, Scherzer R, Peralta CA, et al. Association of kidney function and early kidney injury with incident hypertension in HIV-infected women. *Hypertension*. 2017; 69:304–313. [PubMed: 27993956]
29. Liu KD, Siew ED, Reeves WB, et al. Storage time and urine biomarker levels in the ASSESS-AKI study. *PLoS One*. 2016; 11:e0164832. [PubMed: 27788160]
30. Parikh CR, Devarajan P, Zappitelli M, et al. Postoperative biomarkers predict acute kidney injury and poor outcomes after pediatric cardiac surgery. *J Am Soc Nephrol*. 2011; 22:1737–1747. [PubMed: 21836147]
31. Butterworth JF, Hines RL, Royster RL, et al. A pharmacokinetic and pharmacodynamic evaluation of milrinone in adults undergoing cardiac surgery. *Anesth Analg*. 1995; 81:783–792. [PubMed: 7574011]
32. Paradis M, Jiang X, McLachlan AJ, et al. Population pharmacokinetics and dosing regimen design of milrinone in preterm infants. *Arch Dis Child Fetal Neonatal Ed*. 2007; 92:F204–209. [PubMed: 16690639]

33. Ramamoorthy C, Anderson GD, Williams GD, et al. Pharmacokinetics and side effects of milrinone in infants and children after open heart surgery. *Anesth Analg*. 1998; 86:283–289. [PubMed: 9459233]
34. Basu RK, Andrews A, Krawczeski C, et al. Acute kidney injury based on corrected serum creatinine is associated with increased morbidity in children following the arterial switch operation. *Pediatr Crit Care Med*. 2013; 14:e218–224. [PubMed: 23439467]

Figure I. Age-related increase in milrinone body weight normalized clearance with age
Younger infants had a lower milrinone clearance, including patients with and without AKI.
The dotted line represents the reported mean clearance for adults of 16.8 L/h/70 kg [31].

Figure II.
Comparison of milrinone clearance in patients with and without AKI.

Table 1

Demographics, intraoperative and post-operative characteristics among patients with and without AKI.

	Overall (n = 94)	AKI (n = 31)	No AKI (n = 63)	p
Age (days), mean $\pm$ SD	154.2 $\pm$ 85.7	148.1 $\pm$ 87.2	157.2 $\pm$ 85.4	0.63
Gender (Male)	63 (67)	23 (74)	40 (63)	0.30
Weight (kg), median (IQR)	6.0 (4.7–6.8)	6.1 (4.9–6.8)	6.0 (4.6–6.7)	0.51
Single ventricle disease, n (%)	33 (35)	7 (23)	26 (41)	0.07
STAT mortality score, n (%)				0.33
1	3 (3)	2 (6)	1 (2)	
2	38 (40)	13 (42)	25 (40)	
3	21 (22)	5 (16)	16 (25)	
4	24 (26)	10 (32)	14 (22)	
5	8 (9)	1 (3)	7 (11)	
CPB time (minutes), median (IQR)	149 (99–187)	162 (123–205)	143 (88–186)	0.12
Cross-clamp time (minutes), median (IQR)	71.5 (41–117)	101 (62–147)	57 (33–94)	0.005
Circulatory arrest used, n (%)	17 (18)	6 (19)	11 (17)	0.82
Peritoneal drain present, n (%)	28 (30)	13 (42)	15 (24)	0.07
Baseline urine TIMP-2*IGFBP-7 (ng/mL) ² /1000	0.51 (0.12–1.38)	0.28 (0.07–0.78)	0.62 (0.12–1.56)	0.34
Baseline urine NGAL (ng/mL)	7.77 (2.01–21.2)	8 (2.22–44.6)	5.63 (1.98–18)	0.16
Baseline urine IL-18 (pg/mL)	87.7 (37.8–363)	75.9 (39.2–165)	106 (37.8–428)	0.34
Baseline urine KIM-1 (pg/mL)	221 (60.9–528)	300 (63.2–818)	221 (20.7–524)	0.42
Baseline urine FABP (ng/mL)	3.42 (1.68–8.14)	2.97 (1.5–3.6)	3.71 (1.92–9.89)	0.12
Maximal VIS score, median (IQR)	8 (6–12)	8 (5–14)	8 (7–11.50)	0.60
Urine output (mL/kg/h), mean $\pm$ SD				
Post-operative day 0	2.6 $\pm$ 1.4	2.0 $\pm$ 1.1	2.9 $\pm$ 1.4	0.001
Post-operative day 1	2.8 $\pm$ 1.4	2.1 $\pm$ 1.1	3.1 $\pm$ 1.4	<0.001
Milrinone clearance (L/h/70 kg), median (IQR)	5.13 (3.7, 7.06)	4.2 (3.2, 5.9)	5.6 (4, 8.5)	0.02
Duration of ventilation (days), median (IQR)	1.1 (0.7–2.1)	1.1 (0.8–3.1)	1.1 (0.7–1.7)	0.19
ICU LOS (days), median (IQR)	4 (3–7)	4 (3–9)	4 (2–7)	0.32
Hospital LOS (days), median (IQR)	8 (5–14)	8 (6–21)	8 (5–12)	0.48
Death, n (%)	3 (3)	0 (0)	3 (5)	0.55

SD = standard deviation, kg = kilograms, IQR = interquartile range, n = number, % = percent, STAT = Society of Thoracic Surgeons–European Association for Cardiothoracic Surgery, Congenital Heart Surgery (STAT) mortality categories, CPB = cardiopulmonary bypass, VIS = vasoactive inotrope score, mL/kg/h = milliliters per kilogram per hour, L/h/70 kg = liters per hour per 70 kilograms (normalized to body weight), ICU = intensive care unit, LOS = length of stay, TIMP-2*IGFBP7 = tissue inhibitor metalloproteinase type 2 and insulin-like growth factor-binding protein type 7, NGAL = neutrophil gelatinase associated lipocalin, IL-18 = interleukin 18, KIM-1 = kidney injury molecule 1, L-FABP = liver type fatty acid binding protein.

Table 2

Biomarker test characteristics and cut-off values for prediction of AKI

	Cut-off point	Sensitivity	Specificity	PPV	NPV	AUC	p > Chi-Square
TIMP-2*IGFBP-7 ((ng/mL)²/1000)							
2 hour	1.03	0.59	0.71	0.43	0.82	0.60	0.33
6 hour	0.66	0.68	0.49	0.38	0.77	0.58	0.26
12 hour	0.78	0.69	0.69	0.50	0.83	0.71	0.002
24 hour	1.46	0.41	0.85	0.60	0.73	0.63	0.04
Urine NGAL (ng/mL)							
2 hour	163.88	0.35	0.84	0.47	0.76	0.55	0.58
6 hour	58.85	0.72	0.43	0.35	0.78	0.51	0.77
12 hour	58.25	0.46	0.80	0.52	0.76	0.64	0.04
24 hour	34.03	0.62	0.79	0.61	0.79	0.71	0.003
Urine IL-18 (pg/mL)							
2 hour	1587.7	0.91	0.15	0.31	0.80	0.47	0.72
6 hour	51.26	0.23	0.88	0.46	0.72	0.51	0.91
12 hour	72.74	0.46	0.68	0.41	0.73	0.50	0.66
24 hour	182.54	0.84	0.30	0.40	0.77	0.54	0.45
Urine KIM-1 (pg/mL)							
2 hour	372.16	0.65	0.63	0.43	0.81	0.63	0.23
6 hour	137.37	0.85	0.53	0.44	0.89	0.66	0.03
12 hour	417.21	0.71	0.51	0.41	0.79	0.61	0.08
24 hour	529.57	0.84	0.41	0.44	0.82	0.60	0.18
Urine L-FABP (ng/mL)							
2 hour	32.66	0.70	0.54	0.40	0.80	0.61	0.08
6 hour	109.30	0.65	0.52	0.38	0.77	0.57	0.22
12 hour	58.27	0.75	0.43	0.38	0.79	0.50	0.92
24 hour	14.60	0.48	0.75	0.52	0.72	0.60	0.16

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Ng = nanograms, pg = picograms, mL = milliliter, PPV = positive predictive value, NPV = negative predictive value, AUC = area under the curve, TIMP-2⁹IGFBP-8 = tissue inhibitor of metalloproteinases-2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP-7), NGAL = neutrophil gelatinase-associated lipocalin, IL-18 = interleukin-18, KIM-1 = kidney injury molecule 1, L-FABP = liver-type fatty acid-binding protein.

Table 3

Unadjusted and adjusted models for biomarkers and other confounding factors related to an increase in serum milrinone concentration prior to the diagnosis of AKI

Variable	Odds Ratio (95% Confidence Interval)	p
Unadjusted		
TIMP2-IGFBP-7 >0.78	2.72 (1.01–7.38)	0.04
KIM-1 >529.57	2.76 (1.06–7.17)	0.03
Multivariable Model (AUC=0.788)		
TIMP-2*IGFBP-7 >0.78	2.29 (0.74–7.14)	0.15
KIM-1 >585.6	3.74 (1.22–11.47)	0.02
Prior surgery (Yes vs. No)	0.15 (0.05–0.47)	0.001
Circulatory arrest (Yes vs. No)	4.37 (1.16–16.48)	0.03
Peritoneal drain (Yes vs. No)	0.18 (0.05–0.6)	0.005

TIMP-2*IGFBP7 = tissue inhibitor metalloproteinase type 2 and insulin-like growth factor-binding protein type 7, KIM-1 = kidney injury molecule 1, AUC = area under the curve, AKI = acute kidney injury. Age, gender, and STAT score were included in the multivariable model but are not reported as they were not found to be independent predictors of an increase in serum milrinone concentrations prior to the diagnosis of AKI (all stages).

Table 4

Unadjusted and adjusted models for biomarkers and other confounding factors related to an increase in serum milrinone concentration prior to the diagnosis of severe AKI (KDIGO stage 2 and 3).

Variable	Odds Ratio (95% Confidence Interval)	p
Unadjusted		
TIMP2-IGFBP-7 >0.78	3.94 (1.30–11.97)	0.01
KIM-1 >585.65	3.24 (1.19–8.80)	0.02
Multivariable Model (AUC=0.804)		
TIMP-2*IGFBP-7 >0.78	3.58 (1.02–12.50)	0.05
KIM-1 >585.6	3.97 (1.36–11.58)	0.01
Prior surgery (Yes vs. No)	0.16 (0.05–0.49)	0.001
Circulatory arrest (Yes vs. No)	4.94 (1.25–19.53)	0.02
Peritoneal drain (Yes vs. No)	0.24 (0.08–0.78)	0.02

TIMP-2*IGFBP7 = tissue inhibitor metalloproteinase type 2 and insulin-like growth factor-binding protein type 7, KIM-1 = kidney injury molecule 1, AUC = area under the curve, AKI = acute kidney injury. Age, gender, and STAT score were included in the multivariable model but are not reported as they were not found to be independent predictors of an increase in serum milrinone concentrations prior to the diagnosis of severe AKI (KDIGO stage 2 and 3).