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Proof of Concept: The Use of Renal Biomarkers in Critically Ill Pediatric Patients for Therapeutic Drug Monitoring

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PROOF OF CONCEPT: THE USE OF RENAL BIOMARKERS IN CRITICALLY ILL PEDIATRIC PATIENTS FOR THERAPEUTIC DRUG MONITORING

By

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ABSTRACT

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Acute kidney injury (AKI) is a serious and common complication in critically ill pediatric patients. The incidence of pediatric AKI continues to increase, especially in patients who undergo surgical correction of congenital heart defects. Serum creatinine and urine output are the most commonly used tools to assess renal function, with international guidelines standardizing AKI-definitions based upon these parameters. However, changes in serum creatinine can occur 24 hours or later after a renal insult event, delaying the diagnosis and potential interventions to reverse injury. It is critical to identify endogenous renal biomarkers within the pediatric population that are both timely and accurate in the early stages of AKI. Further, it is important to begin to examine the relationship of AKI biomarkers and glomerular filtration rate (GFR), independent of creatinine. The potential use of renal biomarkers to estimate GFR can further direct clinicians to adjust medications that are renally eliminated, especially medications that require therapeutic drug monitoring. There are a growing number of pediatric medications that warrant therapeutic drug monitoring to optimize clinical outcomes. Milrinone, a phosphodiesterase inhibitor which increases cardiac output following cardiac surgery, is such an agent. Emerging evidence suggests that milrinone has a targeted therapeutic range, yet therapeutic drug monitoring is rarely performed despite

the inter-subject variability between dose and concentration. Early identification of patients developing AKI through the use of renal biomarkers can assist clinicians in initiating therapeutic drug monitoring, especially for those medications with a narrow therapeutic index or in those agents where a therapeutic range has not been formally established. In addition, the adoption of AKI biomarkers into pharmacokinetic modeling programs has the potential to optimize milrinone therapeutic drug monitoring. This proof-of-concept dissertation begins to explore the potential use of renal biomarkers in pediatric AKI to guide therapeutic drug monitoring of milrinone, the challenges of conducting pediatric clinical research, and the need for future clinical studies to assess GFR in the pediatric patient population.

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LIST OF ABBREVIATIONS

AKI	acute kidney Injury
ADH	anti-diuretic hormone
AKI	acute kidney injury
AKIN	Acute Kidney Injury Network
AUC	area under the curve
BUN	blood urea nitrogen
Cl	clearance
CrCl	creatinine clearance
GFR	glomerular filtration rate
HLHS	hypoplastic left heart syndrome
IL-18	interleukin-18
IQR	intra-quartile range
KDIGO	Kidney Disease: Improving Global Outcomes
KIM-1	kidney injury molecule
L-FABP	liver-type fatty acid binding protein
NGAL	neutrophil gelatinase-associated lipocalin
PICU	Pediatric Intensive Care Unit
pRIFLE	Pediatric Risk, Injury, Failure, Loss, End Stage Renal Disease
RRR	relative risk reduction
T1/2	half-life
TDM	therapeutic drug monitoring
TOF	Tetralogy of Fallot
Vd	volume of distribution

CHAPTER 1: INTRODUCTION AND PROOF OF CONCEPT

Pediatric acute kidney injury (AKI) is a pathophysiologic condition that remains difficult to diagnose and treat. The incidence of pediatric AKI appears to be increasing, with more cases being multifactorial in nature as compared to primary disease.[1] Further, it is estimated that approximately 5% of hospitalized pediatric patients and up to 50% of patients in the pediatric intensive care unit (PICU) experience AKI.[1] The morbidity and mortality associated with pediatric AKI remains high, with prolonged hospitalization, an increase of in-hospital mortality rates, and an increased risk of developing chronic renal disease.[1] It is the belief that through earlier intervention, disease progression can be mitigated.

Unfortunately, the current renal assessment parameters routinely used in pediatric clinical practice (e.g., serum creatinine and urine output) are unreliable. There is considerable need to find "bedside" markers of renal function that can appropriately represent a changing physiologic system. Further, it is important that these markers can not only differentiate normal versus abnormal function, but they must also account for pediatric ontogeny to truly be applicable to the pediatric patient population.

The "proof of concept" of this dissertation focuses on the use of renal biomarker for the early detection of pediatric AKI in critically ill pediatric patients. More specifically, the goal is to provide an epistemological foundation for future pharmacologic research to use pediatric renal biomarkers as a marker of glomerular filtration rate to adjust medications dosing during AKI. By definition, "proof of concept' describes research that is cutting edge and the potential to be extended or scalable. The adoption of AKI renal biomarkers into routine clinical care (especially in the PICU) has a tremendous opportunity to appropriately identify disease progression, introduce therapeutic interventions, and limit disease progression by

altering medications appropriately; this adoption can help identify patients that warrant therapeutic drug monitoring (TDM).[2] While most TDM agents have commercially available assays (e.g., aminoglycosides), others do not. To personalize medications where a therapeutic range exists or has the potential to exist, appropriately identifying pediatric patients at risk for developing AKI and then adjusting dosing regimens based upon drug concentrations to prevent or limit adverse events seems paramount.

1.1 The Need for a New "Gold Standard" for Identifying Patients with AKI

Serum creatinine is a long-standing clinical "gold standard" of renal function; however, it is an unreliable indicator of renal function in the pediatric patient. In addition to variable endogenous production, its rise after renal insult is often delayed and does not indicate the full level of renal damage. Current international guidelines use a combination of serum creatinine and urine output to stage renal dysfunction.[3-5] The creation of international consensus guidelines has been critical in the definition of pediatric AKI, a disease with variable underlying pathophysiology. However, the key clinical indicators defining AKI (serum creatinine and urine output) are fundamentally flawed. Clinicians frequently understand the limitations of these 2 indicators during renal function decline and rely on "experience" to guide diagnosis and treatment. Medications are not commonly adjusted during acute renal decompensation, nor in recovery. Pharmacologic agents with a known therapeutic range are monitored during these renal changes, but TDM is initiated only after a rise in serum creatinine or a decrease in urine output has already occurred. Thus, any pharmacologic changes are already delayed 24-48 hours after the intrinsic damages have occurred within the kidney. It is important to identify new renal biomarkers, or a panel of biomarkers, that can be routinely monitored in critically ill children to screen for those patients who are at risk of developing AKI. Identifying new renal biomarkers that can describe the location and extent of kidney

injury can facilitate the diagnosis, potential therapeutic interventions, and adjustment of existing medications.

1.2 Role of Therapeutic Drug Monitoring in Critically Ill Children

Several medications used in critically ill children require TDM. Aminoglycosides and vancomycin are the most frequently pharmacokinetically monitored medications in the PICU. Anti-convulsant therapy (e.g., phenobarbital, carbamazepine), immunosuppressant therapy (e.g., tacrolimus and cyclosporine), antiarrhythmic therapy (e.g., flecainide) are other examples of medications that warrant routine TDM. Medications that are primarily renally eliminated may be a stronger correlate to underlying renal function than serum creatinine or urine output. For example, aminoglycosides clearance has been demonstrated to be an excellent indicator of renal function, especially after an acute renal insult.[6]

There continues to be a growing number of pharmacologic agents that have the potential to utilize TDM in critically ill children. The impact of augmented renal clearance of antibacterial therapy in extremely sick pediatric patients may warrant the future use of TDM. As clinicians attempt to minimize bacterial resistance to commonly used antibiotics, the judicious use of these agents becomes paramount; thus, the application of TDM for antibiotics such as meropenem may be commonplace in the future. The emergence of a milrinone therapeutic range has also been discovered.[2] Milrinone plasma concentrations between 100-300 ng/mL appear to optimize cardiac index in pediatric patients following cardiac congenital repair; however, routine TDM is not performed in this patient population. The most obvious barrier to TDM is the lack of commercially available assays that can be routinely and timely used in clinical practice. Cardiac centers would be required to develop a laboratory assay for routine use or would be required to send the specimens out to a commercial lab, delaying results. If an

institution-specific drug assayed is developed, the pragmatic limitations of monitoring all cardiac patients on milrinone may be cost prohibitive (i.e., it would be extremely difficult to staff a clinical laboratory 24/7 to run milrinone concentrations). To mitigate this issue, it would be important to identify cardiac patients that would be at risk for developing milrinone toxicity. Milrinone is primarily renally eliminated; therefore, patients who develop AKI would be at the greatest risk for developing supra-therapeutic concentrations. By appropriately identifying patients in AKI, milrinone plasma concentrations could be obtained and continuous infusion dosing could be modified. This is just one example of utilizing AKI biomarkers to identify patients requiring adjustments of medication regimens thru TDM, a method that could be extended to other agents.

The ultimate goal of utilizing AKI biomarkers in TDM would be the creation of a "creatinine clearance" equivalent of glomerular filtration rate (GFR) utilizing AKI biomarkers. Renally-eliminated medications are frequently adjusted based upon creatinine clearance (e.g. Schwartz equation) but suffer from the limitations as described previously.[7] The creation of a "biomarker-based GFR calculator" could assist clinicians to manage pharmacologic alterations during the major stages of renal injury: initiation of the injury, extension of the injury, maintenance of the ongoing injury and resolution. The potential application of this "biomarker-based GFR calculator" would allow the clinician to have real-time information regarding renal function, allowing them to adjust renally-eliminated medications to avoid further renal damage (e.g., tacrolimus) or adjust medications that require TDM (e.g., aminoglycosides, milrinone). This calculator could be scientifically validated through pediatric clinical research studies to predict medication clearance throughout the continuum of AKI. Once validated, pediatric dosing references could be updated to recommend to clinicians the appropriate dosing adjustments to alter medication in AKI patients, based upon biomarker values.

1.3 Summary

The early identification and potential therapeutic interventions of pediatric AKI is paramount in reducing the morbidity and mortality associated with the disease. The current guidelines to diagnose disease and estimate GFR (using formula-derived creatinine clearance equations) do not adequately address a rapidly changing pathophysiologic condition. Thus, there is tremendous clinical need to identify “bedside” biomarkers to guide therapy, especially those medications that require or have the potential to require therapeutic drug monitoring. The proof-of-concept presented in the remaining chapters begins to examine the potential opportunities to improve this clinical practice. The first step in developing a pediatric-specific “biomarker-based GFR calculator” would be to identify potential biomarkers in the pediatric patient population. Chapter 2 provides an updated overview of the renal biomarkers currently being studied in pediatric cardiac patients, a group with extremely high rates of AKI. Chapter 3 describes the use of milrinone in congenital heart disease and makes the argument that milrinone serves as an example of a potential “non-traditional” TDM agent. Chapter 4 provides an interim analysis of an ongoing IRB-approved pediatric clinical research study “A pilot study evaluating the relationship between neutrophil gelatinase-associated lipocalin (NGAL) and milrinone plasma concentrations in pediatric patients following corrective surgery for congenital heart disease”. Chapter 5 outlines the challenges and ethical considerations of conducting pediatric clinical research during the COVID-19 pandemic. Finally, Chapter 6 discusses the future application of renal biomarkers in pediatric pharmacology.

Chapter 2: The Use of Renal Biomarkers in Pediatric Cardiac Patients with Acute Kidney Injury

2.1 Introduction

The assessment of acute renal injury (AKI) in critically ill pediatric patients has evolved over the past decade, with the goal to identify changes in renal function expeditiously and accurately for diagnostic and therapeutic interventions. Acute kidney injury occurs in 6% of pediatric hospitalized patients and 50% of pediatric patients in intensive care.[1] The development of AKI has been demonstrated to increase the risk of mortality and contributes to morbidities such as prolonged hospitalization, increased ventilation days, increased risks of acid-base disorders and endocrine abnormalities, and the potential development of chronic renal failure. Acute kidney injury has a significant economic impact, with annual projected cost of $5-20 billion in the US alone.[8] Historically, the true prevalence of AKI has been difficult to ascertain due to varying definitions of the disease. The definition of AKI has been standardized and has been expanded to encompasses a broad array of clinical conditions, including pre-renal azotemia, acute tubular necrosis, acute interstitial nephritis, acute glomerular disease, and acute post-renal obstructive nephropathy.[3-5]

Acute kidney injury leads to a rapid decline in the kidney's regulatory, secretory, and excretory functions, resulting in a decrease in glomerular filtration rate (GFR) and the retention of nitrogenous and other metabolic waste. In addition, medications that are renally eliminated have diminished clearance, resulting in drug-induced toxicities. To prevent these complications, it is important to identify early markers of AKI to implement appropriate treatment strategies. Urine output and serum creatinine are the primary indicators of acute kidney injury in both adult and pediatric patients. However, these markers have been found to be less sensitive when compared to actual renal function,

with greater than 50% of kidney function loss required before significant changes in serum creatinine are observed.[7] There remains a tremendous clinical need to identify biomarkers of AKI that can be efficiently incorporated into the care delivery model to allow for early detection, localization, and treatment of AKI. The emergence of renal biomarkers as early indicators of renal dysfunction is beginning to be utilized in clinical care. This chapter will provide a brief review of renal function in pediatric patients and the limitations with current clinical markers, the definition and classifications systems of pediatric AKI, and the emerging role of biomarkers specific to pediatric patients in diagnosing AKI. Finally, a review of key pediatric cardio-thoracic surgery studies will examine the role of AKI biomarkers in the congenital heart disease patient.

2.2 Methods

A literature search was performed in the following databases: Medline (Pubmed), SCOPUS (Elsevier), Embase (OVID), and Cochrane Central Register of Controlled Trials (CENTRAL), from January 1990 to present. An additional search was carried out using Google Scholar. Databases were searched using the following key-words: “pediatrics”, “renal function”, “acute kidney injury”, “AKI”, neutrophil gelatinase-associated lipocalin”, “NGAL”, kidney-injury molecule”, “liver-type fatty acid binding protein”, “interleukin-18”. Articles were limited to English-language studies published in peer reviewed journals, with additional publication identified from review articles published. Exclusion criteria were previous chronic kidney disease, adult patients, and transplant patients.

2.3 Clinical Markers of Pediatric Renal Function

Serum creatinine is the most commonly used marker of renal function and is a surrogate marker of glomerular filtration rate. While administration of an exogenous

marker is considered the “gold standard” (e.g., inulin) to measure glomerular filtration rate, it is rarely done in routine clinical practice. Thus, the use of urine output and serum creatinine to estimate glomerular filtration remains the mainstay in the care delivery model, despite their inaccuracy and delayed diagnostic value in AKI. Challenges associated with the interpretation of serum creatinine specific to pediatric patients includes: the influence of age, sex, nutritional status, and muscle mass composition on endogenous creatinine values[4]; serum creatinine does not allow specific location or extent of kidney damage; there is a delay of approximately 24-36 hours in serum creatinine values when compared to the time of kidney injury.[9] The delay in creatinine rise is believed to be multifactorial, including the rate of endogenous creatinine generation, the rate of renal “reserve” to compensate for diminishing renal function, and the degree of renal compromise.[7,9]

Despite creatinine’s limitations, it is a mainstay to estimate glomerular filtration in both pediatric and adult patients due to its ease and availability. Serum creatinine values are often incorporated into equations (e.g., Cockcroft-Gault, Schwartz) to calculate creatinine clearance (CrCl), an estimate of GFR (Table 1). [10] The Schwartz equation was developed in 1976 and is a frequently used standard to estimate GFR in children.[10] The Schwartz equation incorporates “k-values” to account for covariables such as age, sex, and height to standardize for ontogeny and patient size.[10] While often used in clinical practice, evidence suggests that the Schwartz equation overestimates GFR by 20-40%.[11] The Conahan-Barratt equation was developed at approximately the same time as Schwartz equation and uses serum creatinine to estimate GFR calculated with the variables of height and serum creatinine (along with the constant 0.43). Despite being available for 40 years, this equation has never been widely adopted into practice.

Table 1: Equations to Estimate CrCl and eGFR in Adult and Pediatric Patients

Cockcroft Gault	CrCl = (140 – age) x lean body weigth (kg) (x0.85 if female) Serum creatinine (mg/dL) x 72
Schwartz Equation	CrCl (mL/min) = (k x height in cm)/SCr (k=0.45 for infants 1-52 weeks of age, k=0.55 for children 1-12 years of age, k=0.55 for females 13-18 years of age, k=0.7 for males 13-18 years of age).
Bedside IDMS-traceable Schwartz	eGFR (mL/min/1.73 m2) = (0.413 x height in cm)/SCr
Counahan-Barratt	eGFR (mL/min/1.73 m2) = (0.43 x height in cm)/SCr
Correction time urine CrCl method	CrCl (mL/min) = (1.73/BSA) x (urine Cr x total urine volume)/plasma Cr x duration of collection in minutes

In 2009, the Schwartz equation was modified to the “Bedside IDMS-traceable Schwartz equation” to address the standardization of serum creatinine measurements among laboratories utilizing the IDMS-traceable methodology. However, there remains insufficient evidence that the use of the new Bedside IDMS-traceable Schwartz equation is a better predictor for GFR in pediatric patients, especially when used to adjust medication dosing. A study by Padgett et al compared the Schwartz, Conahan-Barratt, and Bedside IDMS-traceable Schwartz equation in patients who simultaneously received either a 12- or 24-hour timed urine for creatinine clearance.[11] A total of 91 stable pediatric patient (5 infants, 43 children, and 43 adolescents) without underlying renal dysfunction nor AKI were enrolled in the study. When compared to timed urine collection, the correlation to equation-based CrCl and GFR values was 0.71 for the Schwartz equation and 0.72 for both Bedside IDMS-traceable Schwartz and Conahan-Barratt.[11] These data further support that the use of serum creatinine equations to estimate GFR must be cautiously interpreted in clinical care, especially during periods of rapidly changing renal function.

Urine output adjusted for weight per unit of time (mL/kg/hr) is frequently utilized to assess renal function, often combined with serum creatinine. A summary of pediatric urine output descriptions can be found in Table 2. While urine output is an important clinical marker, there may be contributing factors which alter the clinical presentation. Diminished cardiac output, hypovolemia, prolonged fasting, and factors contributing to stress (e.g., pain) may stimulate the production of anti-diuretic hormone, diminishing urine output independent of intra-renal insult. Thus, it is critical that a sufficient time for urine collection is accounted for (>6 hours), with the goal duration of 12 hours for appropriate renal assessment. Finally, weight-based evaluation of urine output must take in consideration the potential role of obesity, especially in adolescents. Intravascular

circulatory volume is independent of body weight in children and adolescents; thus, weight adjusted urine volumes may be falsely reduced in obese patients.

Table 2: Urine Output Values in Pediatric Patients

Normal Urine Output	0.5-1.5 ml/kg/hr
Oliguria (infant)	< 1 mL/kg/hr
Oliguria (child)	< 0.5 mL/kg/hr
Anuria	No urine production
Polyuria	≥3 mL/kg/hr

2.4 Acute Kidney Injury Classifications in Pediatrics

The current clinical guidelines to standardize diagnosis and staging of AKI in pediatric patients are primarily based upon 3 classifications: 1) the Pediatric Risk, Injury, Failure, Loss, End Stage Renal Disease (pRIFLE) criteria; 2) the Acute Kidney Injury Network criteria (AKIN); 3) the Kidney Disease: Improving Global Outcomes (KDIGO) classification.[3-5] A summary of the classifications can be found in Tables 3-5. In 2007, the Acute Dialysis Quality Initiative Group established the adult RIFLE criterion to assist in stratifying intensive care patients for risks of morbidity, mortality, hospital length of stay, and health care costs. These clinical markers were re-evaluated for the pediatric patient population in 2011 and the pRIFLE criterion were established.[3] The pRIFLE classification system combines urine output along with CrCl as determined by the Schwartz equation. The pRIFLE criteria suffer from several limitations including: 1) the extrapolation of critically ill adult patients to pediatrics patients; 2) validation of the criterion in a limited sample of 150 pediatric patients; and 3) lack of baseline creatinine clearance in the pediatric sample population.

The Acute Kidney Injury Network (AKIN) consensus guidelines were developed to expand upon the pRIFLE guidelines, with small changes in criteria 1.[4] The AKIN guidelines, however, were not validated due to a lack of prospective studies. The KDIGO criteria were established in 2012 as an international, multidisciplinary, clinical practice guideline for AKI.[5] These evidenced-based clinical practice guidelines are the most widely used standards in staging AKI in both adult and pediatric patients, although they were not originally intended for use in neonates. The National Institute of Diabetes and Digestive and Kidney Disease guidelines modified the KDIGO classification to include neonates.[12] While the pRIFLE, AKIN, and KDIGO guidelines established a standardized

framework for the diagnosis of pediatric AKI, the staging criteria inherently suffer from the limitations described with serum creatinine and urine output.

Table 3: Pediatric RIFLE (pRIFLE) Description and Criteria [7]

Category	Estimated CrCL (using Schwartz equation)	Urine Output
Risk	Decrease by 25%	<0.5 ml/kg/hr x 6-12 hours
Injury	Decrease by 50%	<0.5 ml/kg/hr >12 hours
Failure	Decrease by 75% or <35 mL/min/1.73	<0.3 ml/kg/hr for >24 hours or anuria >12 hours
Loss	Loss of renal function >4 weeks	
End-Stage	End-stage renal disease	

Table 4: Acute Kidney Injury Network (AKIN) Description and Criteria [8]

Stage	Serum Creatinine	Urine Output
1	Increase >0.3 mg/dL or 1.5- to 2-fold from baseline	<0.5 ml/kg/hr x 6 hours
2	Increase >2- to 3-fold from baseline	<0.5 ml/kg/hr x 12 hours
3	Increase >3-fold from baseline or >4.0 mg/dL with acute increase of >0.5 mg/dL	<0.3 ml/kg/hr for x 24 hours or anuria x 12 hours

Table 5: KDIGO Description and Criteria [9]

Stage	Serum Creatinine	Urine Output
1	1.5-1.9 x baseline or >0.3 mg/dL increase	<0.5 ml/kg/hr x 6-12 hours
2	2-2.9 x baseline	<0.5 ml/kg/hr >12 hours
3	3 times baseline or increase in serum creatine >4 mg/dL or Initiation of Renal Replacement Therapy (RRT) or A decrease in eGFR to <35 ml/min per 1.73 m2 in patients < 18 years of age	<0.3 ml/kg/hr for >24 hours or anuria >12 hours

2.5 Role of Biomarkers in Pediatric Acute Kidney Injury

The early diagnosis and treatment of AKI is paramount in reducing AKI-associated morbidity and mortality. As previously discussed, routine diagnostic markers to identify AKI (serum creatinine, urine output) are not sensitive, specific, nor timely in a patient with rapidly declining renal function; thus, there remains the need to identify biomarkers associated with early-stage AKI. A biomarker is defined as a parameter of biochemical, genetic, or physiologic change that indicates the presence, severity, and/or progress of a disease. Since a biomarker can be measured in urine, serum, plasma or other bodily fluids, the ideal pediatric AKI biomarker should be: noninvasive (being able to be done at the bedside or in a clinical lab), highly sensitive and specific, able to monitor kidney function independent of age, and should not have interference with drugs or nutritional products unique to pediatric patients.[13] In addition, the ideal biomarker should demonstrate a wide dynamic range and values that optimize risk stratification. Biomarkers used for renal impairment should be able to identify the primary location of the injury, identify the duration of kidney failure (acute versus chronic), identify the underlying etiology, and monitor the response to interventions.[13] A summary of AKI biomarkers and associated location of injury can be found in Table 6, with the time course for biomarker expression following an insult shown in Figure 1.

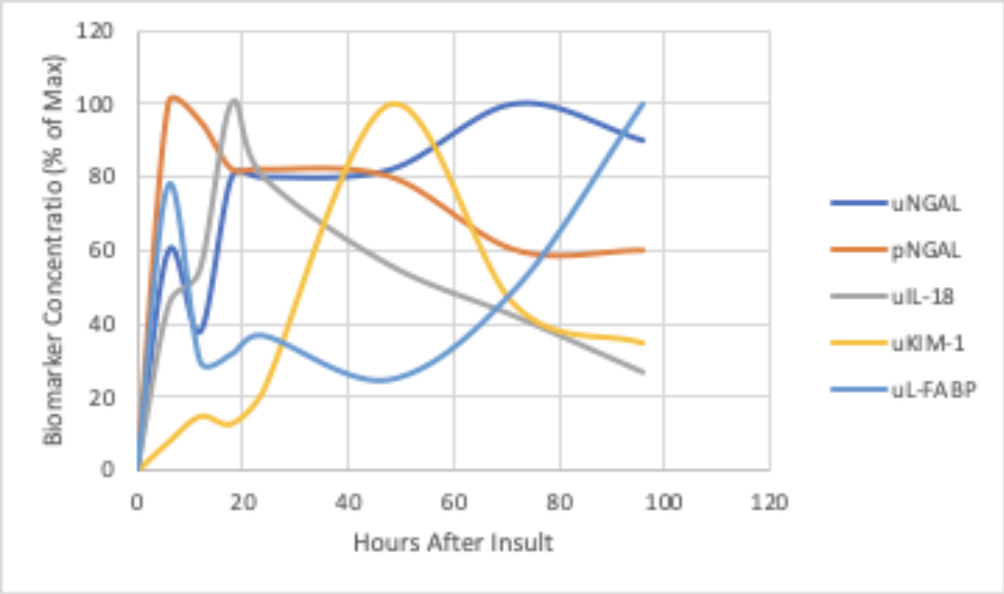
Traditionally, diagnostic tests and biomarkers are initially discovered and studied in adult patients with the eventual translation to pediatric patients. However, the extrapolation of biomarkers to pediatrics is confounded by growth and development and may not be appropriate. Kidney function is composed of 3 key elements: glomerular filtration, tubular secretion, and tubular resorption. Age-dependent renal changes associated with ontogeny often result in physiologic differences as compared to adults. For example, development of glomerular filtration rate, tubular excretion, and tubular reabsorption are markedly different in a 32-week gestational newborn as compared to a

1-year-old.[9] Ontogeny-derived differences in normal physiologic functions can lead to variations in biomarker values, independent of pathophysiologic conditions. Thus, the application of AKI biomarkers in adults may not be translatable to pediatric patients. Even within the field of “pediatrics”, there can be significant physiologic differences between a newborn as compared to adolescents. These differences can be further exacerbated by pediatric cardiac patient, especially following corrective surgery. The impact of cardio-pulmonary bypass, volume expanders, and the invasiveness of cardio-thoracic surgery can further alter endogenous biomarker production. It is important that continued research examines the influence of maturation, development, and underlying congenital anomalies on renal physiology and pathophysiology.[14] While considerable research remains, the following discussion highlights several biomarkers currently under review in pediatric cardiac patients. Neutrophil gelatinase-associated lipocalin (NGAL) has been the most extensively studied AKI biomarker in the pediatric cardiothoracic patient population to date, with other biomarkers having been examined in this patient population.

Table 6: Renal Biomarker Injury Location[7]

Kidney Location	**AKI Biomarker**	**Specific Location**
Tubule	L-FABP Cystatin C IL-18 KIM-1	Proximal Tubule
Tubule	NGAL	Loop of Henle
Glomerulus	Cystatin C Creatinine	Bowman's capsule

Figure 1: Renal Biomarker Expression Following Renal Insult[7,9]



2.6 Neutrophil Gelatinase-Associated Lipocalin (NGAL)

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa protein found primarily in human neutrophils, with limited expression in the liver, spleen, and kidneys. Its primary function is to serve as a bacteriostatic agent in the endogenous immune response, depriving bacteria of iron which inhibits their growth; additional physiologic functions include scavenging iron and inducing epithelial cell growth.[14] The NGAL protein exists in 3 structural forms: a monomer, a homodimer, and a heterodimeric form, which is conjugated to gelatinase. The heterodimeric and monomeric forms are the predominant forms in the tubular epithelial cells, with the homomeric form primarily in neutrophils. Intravascular NGAL undergoes extensive renal glomerular filtration due to its low molecular weight and positive charge, with secretion in the proximal tubule in response to ATP depletion.[13] The thick ascending limb and collecting duct produce intrarenal NGAL.

Intrarenal NGAL concentrations are upregulated following an ischemic or nephrotoxic kidney injury. An increase in NGAL concentrations is detectable in the urine within 3 hours after injury, peaking at 6 hours following injury.[14] Elevated NGAL concentrations can increase 10-fold in serum and 100-fold in urine after acute injury, with clinical studies demonstrating that increases in plasma and urine NGAL are a powerful and independent predictor of AKI independent of underlying etiology, including the pediatric patient population.[13-22] Chronic kidney disease, cardiorenal syndrome, anemia, pregnancy, and cancer can also increase NGAL concentrations.[19]

2.7 Kidney Injury Marker-1 (KIM-1)

Kidney injury marker-1 (KIM-1) is a transmembrane protein that contains Ig domains and extracellular mucin, which appears to have a role in the molecular and cellular biology associated with AKI. Expression of KIM-1 is low in the normal kidney but

is upregulated after ischemia-reperfusion injury and proliferates to de-differentiated epithelial cells of the proximal tubule 48 hours (about 2 days) after injury.[14] During injury, the extracellular component is shed from the membrane in a matrix metalloproteinase-dependent manner which serves as the biomarker after renal ischemic or toxic injury.[14] It is believed that KIM-1acts as a phagocyte, engulfing apoptotic bodies and necrotic debris. Since KIM-1 appears to play a role in renal recovery and tubular regeneration, it is understandable why the timing of peak change would occur 2-3 days after injury. Similar to NGAL, KIM-1 demonstrates age-dependent differences in urine concentrations. Healthy children 3-5 years of age demonstrate a mean concentration of 336 pcg/mL, while adolescents are similar to that observed in adults (515 pcg/mL).[18] Unlike NGAL, there does not appear to be significant difference in values based upon gender. Due to the latent timeframe in KIM-1 expression, its role in AKI may be more limited to kidney recovery as compared to acute kidney injury; however, additional research is warranted in the pediatric patient population.

2.8 Interleukin-18 (IL-18)

Interleukin 18 (IL-18) is a 22-kD member of the interleukin cytokine family which regulates innate and adaptive immunity. IL-18 is produced by macrophages, mononuclear cells, and non-immune cells including proximal tubule cells. During AKI, IL-18 concentrations double as a result of its role as an important pro-inflammatory cytokine mediator in acute ischemia AKI.[15] The increased IL-18 concentrations are a result of the release from proximal tubule cells and not immune-mediated carriers. The rise in urine IL-18 occurs within the first 6 hours of renal insult but does not peak until 12-18 hours.[22] A rise of urine IL-18 concentrations appears to be more specific for acute tubular necrosis as compared to other etiologies. With the delayed increase of IL-18, the potential use of targeted therapies to ablate the pro-inflammatory properties of IL-18 are

currently being explored.[23] A meta-analysis of IL-18 concentrations in adults found it a predictive AKI biomarker in various settings including cardiac surgery and intensive care units for children, adolescents, and adults.[23] The 95th percentile of IL-18 concentrations in 368 pediatric patients was 87.9 pg/mL.[23] Similar to NGAL, IL-18 appears to have gender related difference in adolescents, with females > 10 years of age demonstrating higher concentrations than males (273 pg/mL vs 71 pg/mL).[19]

2.9 Liver Fatty Acid Binding Protein (L-FABP)

Liver fatty acid-binding (L-FABP) protein was first discovered in the 1970's as a binder to long chain fatty acids and other lipids, responsible for transportation of fatty acids to mitochondria or peroxisomes. While L-FABP is expressed in the liver, it can also be found in the intestine, pancreas, lungs, stomach, and kidneys. This 14-kDa protein is expressed in normal and diseased kidneys in both the convoluted and proximal tubules and appears to play an important role in kidney injury and repair; thus, it is often considered a reno-protective protein.[15] When exposed to renal hypoxia, L-FABP expression is upregulated to decrease the severity of renal ischemia-reperfusion injury.[22] Patients who develop AKI demonstrate a rise in L-FABP concentration within 6 hours of insult, with a gradual decrease until 12 hours after the event.[19] Of note, an increase in L-FABP concentrations resumes at 80 hours (about 3 and a half days) after insult. The secondary increase during the maintenance and repair stage of AKI is reflective of the renal protective nature of this protein. The 95th percentile value of L-FABP is 17 ng/mL across all pediatric age groups.[19] Pediatric studies have demonstrated a slight decrease in L-FABP values with older adolescents; however, there remains considerable variation in concentrations throughout childhood.[19]

2.10 Cystatin C

Cystatin C is a 13-kDA protein that serves as an endogenous inhibitor of cysteine proteinase and is generally found in all nucleated cells. Cystatin C is freely filtered by the glomerulus and reabsorbed in the proximal tubule[15] The concentration of cystatin C in the blood is related to an individual's GFR and can serve as a stronger indicator of GFRs between 60-90 mL/min as compared to creatinine. Serum cystatin C is not impacted by muscle mass and does not transfer across the placenta, making it a viable option as a renal marker in the first few days of life.[21] Cystatin C has been demonstrated to be superior to creatinine in animal models and chronic kidney disease, however its role in AKI remains uncertain.[15,21,24] Covariables such as diabetes, corticosteroids, inflammation, hyperbilirubinemia, hyperthyroid, and rheumatic factor appear to impact cystatin C concentrations.[24] Cystatin C urine concentrations vary significantly in pediatric patients. Higher mean values (1354 ng/mL) are observed in preterm neonates <26 weeks gestational age compared to neonates >30 weeks gestational age (209 ng/mL).[24-27] Similar studies in premature neonates demonstrated higher concentrations in premature infants and a general decline to adult values at approximately 1 year of age.[24-27] The age-related changes in cystatin C may be due to the anatomical length of the renal tubules, with the absolute proportion reabsorbed likely to be less than that seen in adults.

2.11 Renal Biomarkers in Healthy Pediatric Patients

NGAL has been the most extensively studied AKI renal biomarker in pediatric patients, especially patients with congenital heart disease. Studies have evaluated plasma (pNGAL) and urine NGAL (uNGAL) concentrations in both healthy and non-healthy pediatric patients, ranging from prematurity to adolescents.[7,9,13-14,16-22] Studies conducted in healthy pediatric patients demonstrate both age and gender influences on

endogenous NGAL concentrations. Premature neonates demonstrate higher median uNGAL values (298 ng/mL; IQR, 196-240) compared to term infants (45 ng/mL; IQR, 33-211) and older children (20 ng/mL; IQR, 3-27).[19] Nephrogenesis is complete at 36-week gestation and continues postnatally; thus, higher uNGAL concentrations are reflective of immature nephron development, most likely due to greater glomerular filtration and the inability of the proximal and distal tubules to resorb filtrated NGAL. Data in older children and adolescents appear mixed. Children 3-4 years of age (3.6 ng/mL; IQR, 1.9-2.5) and age 5-9 years (4.5 ng/mL; IQR, 2.1-9.4) demonstrate lower uNGAL concentrations compared to children 10-14 years (7.6 ng/mL; IQR, 3.3-21.7) and 15-18 year (12.1 ng/mL; IQR, 6.4-270).[19] In addition, uNGAL concentrations appear to be higher in females as compared to males when adjusted for age, although these results are mixed. Females greater than 10 years of age appear to have higher uNGAL concentrations, possibly due to contamination from vaginal secretions that contain neutrophils. Data for pNGAL studies in healthy pediatric patients are sparse, with 1 study solely evaluating plasma concentrations of NGAL in infants 0-6 years of age.[28] Wheeler et al describes a median pNGAL concentration of 80 ng/mL, independent of age and gender.[28] Urine levels of NGAL have been the most extensively studied renal biomarker in the pediatric cardiac patient population. Studies consistently demonstrate that NGAL is an early predictor of AKI. A pooled sensitivity and specificity analysis for 9 pediatric studies was conducted by Filho and colleague and demonstrated a uNGAL sensitivity = 0.76 (95% CI = 0.62-0.86) and specificity = 0.93 (95% CI = 0.88-0.96).[16] In addition, the pooled analysis of pNGAL demonstrated a sensitivity = 0.8 (95% CI = 0.64-0.94) and specificity 0.87 (95% CI = 0.74-0.94). Thus, NGAL appears to be an appropriate marker of AKI in the pediatric patient population.

Kidney-injury molecule-1 demonstrates similar age variation as that seen with NGAL. KIM-1 is lower in 3–4-year-old subjects (336 pg/mL; IQR, 217-513) and 5-9 years

subjects (386 pg/mL; IQR, 217-620) as compared to 15–18-year adolescents (515 pg/mL; IQR, 195-866).[19] Multivariate analysis has demonstrated a 3.8% (95% CI=1.6-5.9) increase in KIM-1 urine concentrations for each increase in year of age. Interleukin-18 also demonstrates 2.2% increase for each year of age (95% CI=0.4-4.0), but also demonstrates a 49% increase based upon female gender. IL-18 concentrations appear to be higher in 10–14-year subjects (25.4 pg/mL; IQR, 15.6-37.3) as compared to 3–4-year children (18 pg/mL; IQR, 10-30).[19] L-FABP demonstrates an inverse relationship between age and urine concentration, with a 3.5% decrease in concentration for each increase in year (95% CI: -5.6 to –1.4). Mean L-FABP concentrations have been shown to approximate 3.4 ng/mL, with slightly higher concentration in children 3-5 years of age (5.2 ng/mL) as compared to 15–18-year adolescents (3.8 ng/mL).[19] Finally, cystatin C demonstrates significant differences in urinary values among preterm neonates as compared to term infants, Higher urinary cystatin C concentrations (1354 ng/mL) are demonstrated in preterm neonates as compared to term neonates (209 ng/mL).[22] Serum cystatin C demonstrate similar patterns, with preterm neonatal values ranging from 1500-1750 ng/mL with a significant decrease in serum concentrations at 4-11 months (1000 ng/mL), which remains stable throughout adolescents (800 ng/mL).[24]

2.12 Renal Biomarkers in Pediatric AKI Secondary to Cardiac Surgery

The pediatric cardiac surgery patient population has been the most extensively studied AKI pediatric population to date. Cardiac surgery patients are more likely to develop AKI, baseline serum creatinine information is available prior to surgery, sampling (urine and blood) is often performed within the context of the surgery, and the timing of AKI after surgery (onset 48-72 hours after surgery) can be predicted.[29-31] A

summary of key studies evaluating renal biomarkers in pediatric cardiac patients is presented below.

2.12.1 Fadel Study

Fadel et al examined pNGAL concentrations in 40 pediatric patients (2 to 78 months of age) who received cardio-pulmonary bypass during pediatric cardiac surgery.[17] Subjects were divided into 2 groups: 1) 19 patients who developed pRIFLE-defined category II or III AKI; 2) 21 patients who did not develop AKI or were categorized as pRIFLE I. Serum creatine and NGAL concentrations were obtained at 2-, 12-, and 24-hours following surgery. Mean pNGAL concentrations in patients with AKI were statistically higher compared to those patients without AKI at 2 hours (154 vs 66 ng/dL; $p<0.0001$), 12 hours (201 vs 84 ng/dL; $p<0.0001$), and 24 hours (238 vs 108 ng/dL; $p<0.0001$) following surgery. Further, there were strong correlations between pNGAL concentrations and AKI at 2 hours ($r^2=0.893$), 12 hours ($r^2=0.873$), and 24 hours ($r^2=0.795$) following surgery. A cutoff level of 100 ng/mL at 2-hour and 125 ng/mL at 12-hour recorded the highest accuracy (95% for both), sensitivity (100% and 89.5%, respectively) and specificity (90.5% and 100%, respectively) for diagnosing AKI. The correlation of serum creatinine with NGAL concentrations was statistically significant at 24-hours after surgery (a predictable outcome due to the delayed rise in serum creatinine following renal insult). The results of this study demonstrated that pNGAL could be an early marker of AKI following cardiopulmonary bypass in the pediatric patient population

2.12.2 Krawczeski Study

Krawczeski et al investigated the pattern and potential value of 4 urinary biomarkers for predicting cardiac surgery-associated AKI: NGAL, IL-18, L-FABP, and

KIM-1.[18] Urine samples were obtained prior to cardiopulmonary bypass and at 2, 6, 12, and 24-hours following bypass initiation. In addition, concurrent serum creatinine concentrations were obtained. Acute kidney injury was defined as a >50% increase in serum creatinine from baseline within 48 hours after bypass. The results of 220 patients demonstrated that AKI occurred in 27% of patients, with uNGAL significantly increased at 2 hours in those patients with AKI. Elevated levels of IL-18 and L-FABP were demonstrated at 6-hours, while KIM-1 levels were increased at 12-hours. At 6 hours, uNGAL, IL-18, and L-FABP were significant predictors of AKI, with NGAL demonstrating the highest discrimination. At 12 hours, uNGAL demonstrated the highest predictive AUC compared to the other 3 biomarkers. At 24 hours, all 4 biomarkers were significantly higher than the control group. The uNGAL concentration at 2 hours was independently correlated with ventilator days and hospital length of stay (p=0.001). Finally, the investigators examined the combination of biomarkers to determine if the combination of biomarkers enhanced the predictive abilities. The authors did note that combination of the renal biomarkers led to an overall improvement in AKI prediction. The combination of uNGAL and IL-18 provided the best predictability of AKI at 6 hours, with a combination of all 4 biomarkers demonstrating the best predictability at 24 hours.

2.12.3 Baek Study

Baek and colleagues examined AKI in 30 patients with congenital heart disease who underwent cardiopulmonary bypass surgery.[21] Urine and blood samples of NGAL, KIM-1, and IL-18 were collected at baseline, 6, 24 and 48 hours after surgery. Serum and urine creatinine and blood urea nitrogen (BUN) were also collected. Acute kidney injury was defined by the KDIGO criteria, although the specific stage was not identified. Of the 30 patients (mean age =1.14 months), 12 patients developed AKI within 48 hours after surgery, with 8 developing AKI within 24 hours after surgery. The investigators'

decision to collect urine creatinine was an attempt to standardize AKI biomarker with urine creatinine values, accounting for ontogenic differences in renal tubule structure and function. The median baseline uNGAL concentrations was 400 ng/dL at baseline, 357 ng/dL at 6 hours, 275 ng/dL at 24 hours, and 257 ng/dL at 48 hours in the 12 patients with AKI, which were not statistically different than the control group. Of note, the NGAL/Cr ratio ranged from 34 ng/mg at baseline to 40 ng/mg at 48 hours. KIM-1 urine concentrations ranged from 26 ng/dL at baseline to 195 ng/dL at 24 hours in the AKI cohort, subsequently falling to 62 ng/dL at 48 hours. Urine KIM-1/serum creatinine ratio met AKI criteria at 24 hours post-surgery (15 ng/mg) but did not at 48 hours following surgery. There was no statistical significance demonstrated with IL-18 concentrations through all 4 time periods (range: 6 ng/dL at baseline vs 17 ng/dL at 6 hours) nor with IL-18/Cr ratio. Urine NGAL/serum creatinine ratios and IL-18/serum creatinine ratios did not demonstrate a significant trend for the first 48 hour following surgery. While the investigators concluded that KIM-1/Cr concentrations could be considered a strong predictor for AKI in younger children, the baseline uNGAL levels were considerably higher in this cohort compared to other studies.[7,9,18] Thus, there are concerns that the higher baseline NGAL concentrations could not differentiate between AKI and non-AKI patients. The concept of standardizing urine biomarkers to another endogenous biomarker (e.g., urine creatinine) remains an interesting concept that warrants further investigation in the future.

2.12.4 Yoneyama Study

Yoneyama and colleagues evaluated 103 patients (<18 years of age) to evaluate urinary biomarkers in patients who underwent cardiac surgery.[29] Acute kidney injury was defined as >50% increase in serum creatinine from baseline. Urine L-FABP and NGAL were obtained at intensive care admission, 4, 12, and 24 hours after surgery. Area under

the curve were calculated at each assessment time. Approximately 50% of patients developed AKI by the second post-operative day, with univentricular status, aortic cross-clamp time, and interoperative fluid balance independently associated with AKI development. Urine NGAL concentrations were statistically elevated in the AKI cohort compared to the non-AKI group at intensive care admission (45 vs 5 ng/dL), 4 hours (15 vs 5 ng/dL), 12 hours (22 vs 8 ng/dL) and 24 hours (23 vs 8 ng/dL). The uNGAL AUC ranged from 0.9 upon intensive care admission to 0.62 at 12 hours (4-hour AUC = 0.8; 24-hour AUC=0.73). A statistically significant increase in L-FABP was demonstrated at all time points as compared to non-AKI cohort: baseline (55 vs 20 ng/dL), 4 hours (40 vs 15 ng/dL), 12 hours (50 vs 20 ng/dL) and 24 hours (40 vs 5 ng/dL). The L-FABP AUC ranged from 0.82 upon intensive care admission to 0.63 at 12 hours (4-hour AUC = 0.78; 24-hour AUC=0.72). Patients with higher uNGAL concentrations at baseline and at 4 and 24 hours after ICU admission demonstrated longer intubation days, intensive care duration, and hospitalization compared to control group. The authors concluded that uNGAL and L-FABP can be useful biomarkers to detect AKI in the pediatric cardiac patient population. Further, this study was unique compared to previous AKI studies due to its analysis of clinical outcomes, demonstrating worsening clinical outcomes with elevated uNGAL concentrations.

2.13 Summary

Acute kidney injury continues to be a common and serious condition, especially in the pediatric patient population undergoing congenital cardiac repair. Currently used clinical parameters (e.g., serum creatinine and urine output) are delayed and unreliable in relation to the renal insult. Thus, diagnostic and therapeutic interventions are often hampered, contributing to significant morbidity and mortality in this patient population. Enhanced understanding of the factors associated with AKI have led to the investigation

of renal biomarkers that can more timely identify AKI: NGAL, KIM-1, L-FABP, IL-18, and cystatin C. Each biomarker has a unique profile that when evaluated in aggregate, can assist in the diagnosis and treatment of AKI. As demonstrated in Table 7, the timing of biomarker expression following renal insult, the association between AKI and biomarker values in the pediatric patient, the ability to identify the location of the renal insult, and the commercial availability of assay kits all play an important role in determining their place in therapy.

AKI occurs in 4 phases: 1) initiation of the injury; 2) extension of the injury; 3) maintenance of the ongoing injury; and 4) resolution.[19] Early identification of renal injury can facilitate therapeutic treatments to facilitate resolution. For example, during the initiation phase, therapeutic interventions such as vasodilator therapy, antioxidant therapy, and/or iron chelation therapy may assist in limiting disease progression. Since uNGAL appears to be an early biomarker of AKI, the routine use of uNGAL in post-operative patients could more rapidly identify patients progressing to AKI. Renal tubules undergo re-perfusion related death, with amplified inflammation during the extension phase. Biomarkers such as IL-18 and L-FABP could facilitate therapies with anti-inflammatory and antiapoptotic interventions. Finally, patients in the maintenance/resolution phases of the AKI demonstrate simultaneous renal cell death and regeneration. The use of KIM-1 may be the best biomarker to measure progression and the impact of therapeutic interventions such as growth factors and stem cells.

The adoption of renal biomarkers into routine pediatric clinical practice still warrants significant research. Challenges associated with utilizing biomarkers include the variability of reference values based upon age and gender, as demonstrated by NGAL and IL-18. This variability Illustrates the importance of growth and development on renal physiology and pathophysiology. The standardization of age cohorts based

upon renal ontogeny (e.g., neonates, 1-month to 2 years of age, and >2 years of age) could assist in establishment of standardized reference ranges.

The use of urine sampling is particularly advantageous in pediatric patients, especially neonates. Reducing the need for repeated blood draws, especially in the post-operative period, is an attractive diagnostic option when screening for the potential development of disease (such as AKI). The use of an AKI urine panel that includes the aforementioned biomarkers has the potential to impact the outcomes associated with pediatric AKI. This is particularly relevant when considering concomitant pharmacologic therapy. Kidney function and renal clearance are important factors that can alter medication pharmacokinetics, leading to drug accumulation and adverse events. Earlier detection of AKI could help prevent drug-related kidney injury (acute and chronic) due to possibly avoiding repeated dosing of offending agents. Future pediatric studies are warranted to study the relationship between AKI biomarkers, glomerular filtration, and pharmacokinetic parameters. It is the goal that earlier identification and treatment strategies can reduce prevalence of AKI in this vulnerable patient population.

Table 7: Summary of AKI Biomarkers

AKI Biomarker	Detection Time After Renal Injury	Advantage	Disadvantage
NGAL	2-4 hours	- Acute phase biomarker - Blood and urine sampling available - Commercially available test - Approved for clinical use in Europe - Extensive research in pediatric critically ill patients following cardiac surgery - Provides injury location within kidney (Loop of Henle)	-Values vary based upon age and sex - Not approved for clinical use in the US.
KIM-1	12-24 hours	- Late phase biomarker -Potential role when used in combination with another biomarker - Provides injury location within kidney (proximal tubule)	- Inconsistent association with pediatric AKI in cardiac surgery patients -Values vary based upon age and sex - No commercially available kit
Interleukin-18	6-24 hours	- Late phase biomarker -Provides injury location within kidney (proximal tubule)	-Inconsistent association with pediatric AKI in cardiac surgery patients -Values vary based upon age and sex - No commercially available kit
L-FABP	1 hour after ischemic tubular injury	- Acute phase biomarker -Provides injury location within kidney (proximal tubule)	-Inconsistent association with pediatric AKI in clinical studies - No commercially available kit
Cystatin C	12-24 hours	-Functional marker of kidney function -Blood and urine sampling available -Commercially available test - Approved for clinical use in Europe and US	- Does not provide injury location within kidney

Chapter 3: Milrinone Therapy in Pediatric Congenital Heart Disease

3.1 Introduction

Low cardiac output syndrome is a common and serious complication seen in patients that undergo congenital heart repair. It is projected that low cardiac output syndrome occurs in 25% of pediatric heart surgery patients, often between 6 to 18 hours after surgery.[33] Patients with low cardiac output syndrome have a predictable decrease in cardiac output due to impaired myocardial contractility that increases systemic vascular resistance by 25% and pulmonary vascular resistance by 40% as compared to baseline.[33] Compromised cardiac output combined with elevated vascular resistance leads to peripheral endothelial ischemia and reperfusion injury, increasing length of hospitalization and higher rates of post-surgical complications, including nosocomial infections.[2]

Pharmacologic management following congenital heart repair is focused on optimizing cardiac contractility, improving diastolic dysfunction, and appropriately managing volume to optimize preload and decrease afterload. Adrenergic agonists such as dopamine and dobutamine, along with the vasodilator nitroglycerin, have been used to maintain hemodynamic stability following corrective surgery. However, catecholamines are associated with increased myocardial consumption, tachycardia, increased afterload, and can be proarrhythmic. Further, catecholamines can depress myocardial function due to down regulation of the beta-adrenergic receptors.[33] Amrinone, a phosphodiesterase III inhibitor, was the first dipyridine used in pediatric low cardiac output syndrome patients as adjuvant therapy to catecholamine infusions. However, the development of milrinone led clinicians to use it as the inodilator of choice due to its enhanced potency compared to amrinone (10 to 75 times greater) and

reduced incidence of thrombocytopenia.[34] Milrinone has become the phosphodiesterase inhibitor of choice over the past 20 years; yet, significant research is warranted to optimize the dose-concentration-response relationships, especially in relation to renal function in pediatric patients.

3.2 Methods

A literature search was performed in the following databases: Medline (Pubmed), SCOPUS (Elsevier), Embase (OVID), and Cochrane Central Register of Controlled Trials (CENTRAL), from January 1990 to present. An additional search was carried out using Google Scholar. Databases were searched using the following key-words: “pediatrics”, “milrinone”, “cardiac surgery”, “cardiothoracic study”, “congenital heart disease”, “pharmacokinetics”, “congestive heart failure”, “acute kidney injury”, “AKI”, “renal biomarkers”, and “kidney injury”. Articles were limited to English-language studies published in peer reviewed journals, with additional publication identified from review articles published.

3.3 Pharmacology of Milrinone in Pediatric Patients

Milrinone is a phosphodiesterase III (PDE-III) inhibitor that demonstrates both inotropic and vasodilatory properties, with little or no chronotropic activity.[34] The PDE-III inhibition decreases the hydrolysis of cyclic adenosine monophosphate (cAMP), which increases intracellular calcium concentrations in cardiac and vascular smooth muscle.[34] The increased intracellular concentration of cAMP facilitates the uptake of calcium from the sarcoplasmic reticulum during muscular excitation/contraction coupling. Thus, milrinone administration results in increased cardiac output and stroke volume, decreased systemic vascular resistance, and decreased intracardiac filling pressures with no significant changes in heart rate and myocardial oxygen demand. To date, there

have been no studies examining ontogeny-related differences in milrinone pharmacologic mechanism of action.

3.3.1 Pharmacokinetics of Milrinone in Pediatric Patients

The pharmacokinetics of milrinone in pediatric cardiac patients has been widely investigated.[35-43] While milrinone is completely absorbed from the gastrointestinal tract, it is administered as a continuous infusion due to its short elimination half-life and clinical requirement for continuous administration. Milrinone exhibits a bi-phasic distribution, with a noticeably short alpha phase volume of distribution (Vd). Beta-phase Vd is age-dependent and pathophysiologic dependent, with patients in congestive heart failure demonstrating a larger volume of distribution as compared to those without.[35] Infants after cardiac surgery demonstrate a Vd= 0.8$\pm$0.4 L/kg, whereas children demonstrate a slightly lower Vd 0.7 + 0.2 L/kg.[35-39] Normal Vd in adult patients after cardiac surgery =0.4$\pm$ 0.1 L/kg, while patients with congestive heart failure 0.45 L/kg.[34] Milrinone is 70% protein bound, although the exact protein has not been established. Approximately 10-12% of milrinone is hepatically metabolized. Of the 5 metabolites, the O-glucuronide metabolite is the major metabolite, which is excreted in the urine. Milrinone is primarily excreted as unchanged drug in the urine (80-90%).[34-37] The elimination half-life in infants after cardiac surgery = 3.15 $\pm$ 2 hours, while older children have a slightly lower half-life (1.86 $\pm$ 2 hours) after surgery. [35-39] In comparison, the elimination half-life in adults after cardiac surgery = 1.69 $\pm$0.18 hours, while adult patients with congestive heart failure have a slightly longer half-life (2.4 hours).[32,34] The average clearance of milrinone in infants after cardiac surgery = 3.8 $\pm$ 1mL/kg/minute, which is slightly lower than in children following cardiac surgery (5.9 $\pm$ 2 mL/kg/minute).[35-39] A study by Lindsay et al demonstrated significantly higher milrinone clearance in pediatric patients in septic shock (10.6 $\pm$5.3 ml/kg/minute) as compared to those reported in pediatric heart repair

patients.[36] In comparison, adult patients demonstrate lower milrinone clearance as compared to pediatric patients in both adults following cardiac surgery (2 ±0.7 mL/kg/minute) and in adults with congestive heart failure (2.2 mL/kg/minute).[32,34]

Milrinone is administered as an intravenous continuous infusion, initiated immediately after corrective cardiac surgery to prevent low cardiac output syndrome. Clinical studies have utilized a continuous infusion of 0.5 mcg/kg/min (range 0.25-0.75 mcg/kg/min), with or without a loading dose of 50-75 mcg/kg. [35-43] Adult dosing is comparable, with a 50 mcg/kg bolus followed by a continuous infusion of 0.25 mcg/kg/min (range 0.375-0.75 mcg/kg/min). [32-34] Since renal clearance is the major route of elimination, renal dosing adjustment guidelines have been provided by the manufacturer (Table 8). [44] To date, there have been no pediatric studies investigating milrinone dose reduction with impaired renal function. Rather, pediatric clinicians often empirically reduce milrinone infusion rates if renal function appears compromised.

3.4 Clinical Trials of Milrinone in Pediatric Congenital Heart Disease Patients

The emergence of milrinone therapy in pediatric patients began in the mid-1990's, with several case series and small studies describing the use of milrinone in pediatric congenital heart disease patients and pediatric septic shock syndrome.[33,36,39] While demonstrating benefit, these studies were insufficiently powered to assess effectiveness compared to adrenergic agents with or without concomitant amrinone therapy. The first large study evaluating milrinone therapy in pediatric congenital heart disease patients was the multi-institutional PRIMACORP study.

Table 8: Milrinone Dosing in Adults with Renal Impairment [44]

Cl_{cr} (mL/min)	0.375 mcg/kg/min	0.5 mcg/kg/min	0.75 mcg/kg/min
50	0.25 mcg/kg/min	0.375 mcg/kg/min	0.5 mcg/kg/min
40	0.125 mcg/kg/min	0.25 mcg/kg/min	0.375 mcg/kg/min
30	0.0625 mcg/kg/min	0.125 mcg/kg/min	0.25 mcg/kg/min
20	Alternate Therapy	0.0625 mc/kg/min	0.125 mcg/kg/min
10	Alternate Therapy	Alternate Therapy	0.0625 mcg/kg/min
5	Alternate Therapy	Alternate Therapy	Alternate Therapy

3.4.1 PRIMACORP Study

The first randomized, double-blind, placebo-controlled study evaluating milrinone therapy in pediatric congenital heart patients with low cardiac output syndrome was the PRIMACORP study (PRophylactic Intravenous use of Milrinone After Cardiac OperRation in Pediatrics).[42] The 31-center study randomized pediatric patients ≤ 6 years of age undergoing congenital heart repair to one of three treatment arms: 1) low dose milrinone (25 mcg/kg bolus followed by 0.25 mcg/kg/min continuous infusion); 2) high dose milrinone (75 mcg/kg bolus followed by 0.75 mcg/kg/min continuous infusion); or 3) placebo. Patients were excluded from the study if: body weight < 2 kg, premature birth (<36 WGA), renal dysfunction (creatinine >1.5 mg/dL 48 hours prior to surgery), and low cardiac output syndrome or hypotension on arrival to the intensive care unit from the operating room. The primary end point was a composite variable of either death or the development of low cardiac output syndrome requiring additional pharmacological support or other support administered within the 36 hours after receiving drug. Patients were defined as having low cardiac output syndrome if exhibiting the following clinical signs and symptoms: tachycardia, oliguria, poor perfusion, or cardiac arrest. Additional pharmacological or widespread support was defined as mechanical support of circulation (e.g., extracorporeal life support, mechanical pacing) or an increase in the amount of pharmacological support relative to baseline (>100% over baseline or the addition of a new inotropic agent). Secondary end points were similar to the primary endpoint of death or development of low cardiac output syndrome but were focused on presentation later in the hospital course (>36 hours – 30 days). Additional variables analyzed included duration of mechanical ventilation, length of hospital stay, total urine output and renal function at end of study (defined by creatinine clearance). Cardiovascular hemodynamic parameters were measured at the start of the study and every 4 hours up to 36 hours, with percent changes in systolic and diastolic pressures as compared to baseline.

Finally, lactate levels and arterial/venous oximetry concentrations were collected at baseline and every 4 hours for 36 hours.

A total of 242 patients were enrolled in the study, with 238 patients receiving milrinone therapy. Eleven patients had major protocol violations, with the remaining 227 patients receiving milrinone per protocol and included in the final analysis (75 patients in placebo arm; 79 patients in low dose arm; 73 patients in high dose arm). A summary of patient demographics is included in Table 9. The primary endpoint of death within 36 hours of surgery demonstrated no deaths in placebo nor either treatment arm. The second primary end point, low cardiac output syndrome requiring treatment within the first 36 hours following surgery, occurred in 26.7% of patients in the placebo group, 17.7% within low-dose milrinone group (relative risk reduction [RRR]=34% vs placebo; p=0.023) and 9.6% in high-dose milrinone group (RRR=64%; p=0.007). There was not a significant difference in the secondary end point of death after 36 hours for any group. However, there was a significant reduction in the composite variable of low cardiac output syndrome by the final visit, with the high-dose milrinone cohort demonstrating a 48% RRR of low cardiac output syndrome compared to placebo. The most common clinical symptoms associated with low cardiac output syndrome were: cool extremities (72.7%), oliguria (54.5%), tachycardia (31.8%), widened arterial-mixed oxygen difference (45.5%) and metabolic acidosis (22.7%). Treatment approaches for low cardiac output syndrome consisted of escalation of existing pharmacological support (84%) or initiation of extracorporeal membrane oxygenation (4.5%). There was no significant difference amongst the 3 arms in mechanical ventilation days, duration of hospitalization, urine output, creatinine clearance, serum lactate concentrations, differences between arterial and mixed venous oxygen saturation. However, the percentage of patients who had a prolonged hospital stay (>15 days) was greater in the placebo group (23.3%) as compared to the low-dose milrinone (8.2%) and high-dose

milrinone groups (13.5%). The overall rates of serious adverse effects were not significantly different among the treatment groups, including thrombocytopenia, arrhythmias, and hypotension. A secondary analysis comparing patients with low cardiac output syndrome and without demonstrated a significantly longer duration of mechanical ventilation (3.1 vs 1.4 days; p=0.001) and hospital stay (11.3 vs 8.9 days; p=0.016).

In summary, the PRIMACORP study remains the largest pediatric cardiovascular study performed to date. Prophylactic high-dose milrinone administration (75 mcg/kg bolus followed by continuous infusion 0.75 mcg/kg/min) following cardiac surgery resulted in a 64% RRR in the development of low cardiac output syndrome as compared to placebo. There was not a statistically significant RRR in the development of low cardiac output syndrome in patients receiving low dose milrinone as compared to placebo. In addition, there was no significant difference in the incidence of adverse effects (e.g. thrombocytopenia, arrhythmias, or hypotension) among the 3 treatment arms. The largest limitation to the PRIMACORP study was the diagnosis of low cardiac output syndrome. Since this syndrome is a constellation of clinical signs and symptoms, standardization across 31 centers can be complex, especially in the absence of a definitive diagnostic marker (e.g., thermodilution measurements of cardiac index). The primary aims of the study were to assess milrinone's ability to prevent the development of low cardiac output syndrome, not the diagnosis of low cardiac output syndrome; thus, clinicians were able to utilize their clinical judgement if the patient warranted significant escalation of support. A second limitation of the study, per the authors, was the exclusion of patients with biventricular repair and other staged reconstruction. While not listed specifically as an exclusion criterion, potentially high-risk patients were not included in the study. Regardless, the PRIMACORP study was the first large randomized, clinical study that demonstrated the prophylactic use of high-dose milrinone

reduced the risk of developing low cardiac output syndrome after pediatric congenital heart surgery.

Table 9: Patient Summary of PRIMACORP Study[42]

	Placebo (n=75)	Low Dose (n=79)	High Dose (n=73)
Age (months)	8.3 ± 14.8	5.9 ±10.2	8.6 ±16.5
Weight (kg)	6.1± 4.1	5.7± 3.3	5.9± 4.0
Female (%)	52.0	39.2	46.6
Surgical Repair			
Tetralogy of Fallot	23	31	19
CAVC	17	15	13
Arterial Switch	10	13	14
VSD	6	7	4
Mitral Valve	7	3	3
DORV	5	4	4
TAPVR	1	4	7
Truncus Arteriosus	5	1	5
Ross Procedure	1	1	4
CPB Time (min)			
Cross Clamp Time	76.3 ± 36.5	70.1± 30.9	77.8± 29.7
CPB Time	132.0 ± 50.6	122.9± 56.3	124.0± 46.6
Total Support Time	136.5 ± 52.1	120.5±54.2	131.6 ± 48.3

CAVC = complete atrioventricular canal; VSD = ventricular septal defect; DORV = double outlet right ventricle; TAPVR = total anomalous pulmonary venous return;CPB = cardiopulmonary bypass.

3.4.2 Bailey Study

Bailey and colleagues evaluated the pharmacokinetic profile of milrinone in 20 children (3-22 months of age) undergoing congenital heart repair.[35] The purpose of the study was to evaluate the pharmacokinetics of milrinone in pediatric patients after cardiac surgery and to determine an optimal dosing regimen based upon milrinone pharmacokinetics. Patients either received a single bolus dose (50 mcg/kg) or a bolus dose plus a continuous infusion of 0.5 mcg/kg/min following surgery and weaned from cardiopulmonary bypass. Patient were initiated on inotropes at the discretion of the attending anesthesiologists, with the following hemodynamic parameters assessed at baseline: heart rate and rhythm, systemic blood pressure, left arterial pressure, central venous pressure, and cardiac output. Arterial blood samples were obtained at 10, 15, 20, 30, 45, 60, 90, 120, 180, 240, 360, 600, and 960 minutes following initiation of milrinone therapy (either bolus or continuous infusion). Milrinone plasma concentrations were measured by high performance liquid chromatography (HPLC), with the pharmacokinetic parameters analyzed using NONMEM. Pharmacokinetic analysis within NONMEM initially estimated a first-order model. However, when a subroutine modeling program was utilized to predict drug plasma concentrations (NMVCLDRG-Palo Alto Veteran's Medical Center), two- and three-compartment models were evaluated. The effect of body mass on milrinone clearance was linearly modeled for either weight or body surface area, with age and dopamine administration as covariates. The model's ability to predict observed data was measured by performance error (Cmeasured-Cpredicted)/(Cpredicted), with the optimal model being (-2 X logarithm of the likelihood of the results) and the median absolute performance error.

A total of 15 patients followed the "per protocol" dosing as outlined previously. Two patients who were originally expected to receive only the 50 ug/kg bolus also received a continuous infusion at 0.5 mcg/kg/min because the attending physician

deemed it clinically necessary. Another 3 individuals had an increase in their continuous infusion rate to 0.7 mcg/kg/min due to clinical necessity. Following the 50 mcg/kg loading dose, a 12% decrease in mean blood pressure and an 18% increase in cardiac output was measured ($p<0.05$). There were non-significant changes in heart rate, left arterial pressure, and central venous pressure. The mean milrinone plasma concentration obtained at 5 minutes following the loading dose was 235 ng/mL, which corresponded to the timing of these hemodynamic measurements. A three-compartment pharmacokinetic model (using the linear function of "weight") improved the objective function and decreased the absolute performance error as compared to either a 1-compartment or 2-compartment model. After correcting for weight, clearance was noted to increase with age. The addition of body surface area or dopamine dose as covariables did not improve the model. A summary of the pharmacokinetic variables is presented in Table 10, which demonstrates the model parameters and standard errors of the parameter estimates and estimates of interindividual variation. Of note, the investigators reported that 95% of the study subjects demonstrated pharmacokinetic parameters that lie between the typical value minus twice the percentage estimate of interindividual variation and the typical value plus twice the interindividual variation.

The optimal pharmacokinetic model adjusted volume of distribution for weight and elimination clearance on weight and age. There was an increase in elimination clearance with increasing age, which remained when adjusting for weight. Thus, weight-normalized clearance could be linearly modeled with age (defined in months) from 3 – 22 months of age. For example, the predicted clearance for a 3-month-old was 2.6 ml x min x kg as compared with a 22-month-old (5.6 ml x min x kg). The determination of the optimal dosing regimen based upon pharmacokinetic parameters is related to target concentration. For the purposes of this study, a plasma concentration >200 ng/mL was targeted for optimal inotropic effect. A 50 mcg/kg loading dose would be expected to

achieve a concentration of 230 ng/mL immediately following the bolus. Based upon population pharmacokinetic parameters, a loading dose of 3 mcg/kg/min for 30 minutes followed by a maintenance infusion of 0.5 mcg/kg/min would be projected to maintain a plasma concentration between 200-250 ng/mL.

In summary, Bailey and colleagues demonstrated several key concepts. The use of a 3-compartment model to describe milrinone pharmacokinetics in the pediatric patient population was unique, considering most milrinone modeling in both adults and pediatric patients had utilized a 2-compartment model.[38, 39, 42] In addition, the investigators noted an increase in cardiac index immediately after surgery that correlated to a plasma concentration of 235 ng/mL. This correlation was the first study examining the potential role of desired therapeutic outcome with plasma concentrations. Finally, Bailey and colleagues demonstrated that clearance can be associated with age, independent of weight.

Table 10: Pharmacokinetic Parameters of Milrinone-Bailey[35]

Parameter	Typical Value	Interindividual Variation (%)
Vd 1 (ml)	190(10) *weight	20
Vd 2 (ml)	204 (14) *weight	3
Vd 3 (ml)	457(124) *weight	50
CL1 (ml x min $^{-1}$)	2.5(1.4) *weight* [1+0.058(0.038) *age]	32,12
CL2 (ml x min $^{-1}$)	14.5(1.7) *weight	1
CL 3(ml x min $^{-1}$)	5(2.5) *weight	120

3.4.3 Ramamoorthy Study

Ramamoorthy et al. evaluated the pharmacokinetics and side effect profile of milrinone in 19 infants and children < 13 years of age.[39] The purpose of this prospective, open-label, dose escalating study was to describe the pharmacokinetic profile of milrinone in this patient population and to examine the side effect profile. Patients were considered eligible for the study if there was clinical indication for inotrope support following surgery and baseline platelet counts >100,000 cells/mm^{-3} upon admission into the PICU. Patients received one of two dosing strategies: 1) 25 mcg/kg loading dose and continuous infusion initiated at 0.25 mcg/kg/min, with a 25 mcg/kg bolus dose 30 minutes after the initiation of the continuous infusion; 2) 50 mcg/kg loading dose followed by an infusion rate of 0.5 mcg/kg/min, with a 25 mcg/kg bolus 30 minutes after start of infusion and an increase in the infusion rate to 0.75 mcg/kg/min.

Blood samples were obtained 30 minutes after each loading dose, followed by samples obtained after 22 and 24 hours while on continuous infusion. Finally, samples were obtained after therapy was discontinued at the following times: 5, 10, 20 and 60 minutes, and 3, 5, and 7 hours. Milrinone was measured in cardio-pulmonary bypass circuits by spiking milrinone in previously used cardio-pulmonary bypass circuits in which patients did not receive milrinone. The circuits contained waste blood that was circulated at 2.5 mL/min, with milrinone injected proximal to the oxygenator into the circuit and samples were collected distal to the oxygenator at 1, 10, and 20 minutes. The milrinone dose used (0.3 mg/L of prime volume) was calculated to yield a plasma concentration of 300 ng/mL. Milrinone pharmacokinetic parameters were analyzed using NONMEM, fitting both a one- and a two-compartment model. The two-compartment model had 4 parameters: clearance, central volume, peripheral volume, and intercompartmental clearance. Covariates added to the model were age, gender, body weight, body surface area, duration of bypass, and duration of milrinone infusion. To examine milrinone side

effects, blood samples were evaluated for platelet count, liver enzymes, and kidney function prior to and following initiation of milrinone therapy.

Nineteen children were enrolled in the study: 12 infants <1 year of age and 7 children (aged ≥1 year of age and < 13 years). The average patient age was 3 ± 3.7 years, average weight was 12 kg ± 12, cardiopulmonary bypass time 120 ± 24 minutes, and milrinone infusion duration 40 ± 20 hours. Milrinone concentrations after discontinuing therapy fit the two-compartment model best in 18/19 patients. Milrinone pharmacokinetic parameters for all patients (representing 247 samples) are presented in Table 11.

Table 11: Pharmacokinetic Parameters of Milrinone-Ramamoorthy [39]

Pharmacokinetic Parameter	Mean + SD
Cpss (ng/mL) Low Dose High Dose	 113 ± 39 206 ± 74
CL (mL/kg/min)	4.5 ± 1.8
T ½	2.7 ± 2.1
Vd (L/kg)	0.83 + 0.4

There was considerable variation in the pharmacokinetic parameters when patient covariates were not included. The addition of body weight or body surface area (BSA) significantly impacted both CL and V. Age as a stand-alone variable was a significant factor but was not significant when weight and BSA were controlled. Neither duration of infusion nor dose significantly influenced CL or half-life, nor did gender or serum creatinine. Mean cardio-pulmonary bypass circuit milrinone concentrations were 343 ng/mL at 1 minute, 320 at 10 minutes, and 324 at 20 minutes. Platelet count decreased significantly in both the low dose and high dose milrinone groups; however, there was no difference between groups. At 24 hours of therapy, 21% of patients were thrombocytopenic, at 36 hours this rate increased to 47%. However, of the 19 patients receiving milrinone, only 2 infants required platelet transfusions. Two patients developed arrythmias during milrinone administration, one in each dosing group. One patient (low dose infant) who developed junctional ectopic tachycardia had a milrinone concentration =178 ng/mL. The second patient with arrhythmia demonstrated ectopic tachycardia, with a corresponding milrinone plasma concentration =345 ng/mL. There was no significant difference in BUN, serum creatinine, nor ALTs as compared to baseline.

In contrast to the study by Baily[35], milrinone was best represented by a two-compartment pharmacokinetic model. Clearance was significantly lower in infants (3.8 mL x kg x min) compared to children (5.9 mL x kg x min). Pharmacokinetic analysis showed a strong correlation between body weight/BSA and CL and Vd in children. When adjusted for body weight or BSA, the large inter-subject variability of Cl and Vd were reduced. The half-life was not significantly different than that described in adults, but the altered clearance reflects marked differences in Vd compared to adults. Approximately 60% of patients experienced thrombocytopenia, with 2 patients requiring platelet transfusion. Thrombocytopenia appears to be related to the duration of milrinone administration rather than dose or concentration. Thus, serial platelet monitoring should

ensue while on therapy. Finally, it does not appear that milrinone binds appreciably to cardio-pulmonary bypass circuitry.

3.4.4 Zuppa Study

Zuppa and colleagues conducted a blinded, randomized pharmacokinetic study of milrinone in neonates with hypoplastic left heart syndrome (HLHS).[38] The objectives of this study were to describe the plasma concentration-time profile in neonates with HLHS, to develop a pharmacokinetic model that assessed the impact of cardio-pulmonary bypass circuits and micro-filtration on milrinone concentrations, and to analyze the milrinone clearance following surgery. Patients were randomized to either 100 mcg/kg or 250 mcg/kg bolus, followed by standardized continuous infusion 0.5 mcg/kg/min. The unit was primed with an average of 345 mL of a combined plasmalyte, 5% albumin, and whole blood solution to achieve a hematocrit of 30%. Milrinone was administered into the venous reservoir of cardio-pulmonary bypass. After weaning from bypass with dopamine, all patients underwent micro ultra-filtration. Upon admission into the PICU, milrinone continuous infusion was initiated at 0.5 mcg/kg/min. Milrinone samples were obtained from the CPB circuit or arterial catheter immediately before and at 5, 10, 15, and 20 minutes after bolus, in addition to a sample obtained at the end of micro ultra-filtration. Blood samples were obtained before the start of the continuous infusion, at 3, 6, 9 and 24 hours after initiation of therapy, and immediately before discontinuation of therapy. Pharmacokinetic parameters were analyzed by NONMEM, utilizing a two-compartment model. Models were corrected for weight-normalized clearance, volumes of central compartment, volume of peripheral compartment, and intercompartmental clearance. Volume was assumed to be consistent across all 4 levels. A mixed-effect model was used to characterize the impact of coronary-pulmonary bypass, micro ultra-filtration, and post-operative administration.

A total of 16 neonates were enrolled in the study. Eight patients received the 100 mcg/kg loading dose while the remaining 8 received the 250 mcg/kg bolus. There was no significant difference between groups in gestational age, weight, age at surgery, baseline creatinine, CPB time, nor total support time. One patient developed ventricular tachycardia in the high bolus group, while 1 patient developed hypotension in the low bolus group. A loading dose of 100 mcg/kg resulted in a peak plasma concentration =287 mcg/kg, while a loading dose of 250 mcg/kg resulted in a peak concentration of 662 ng/ml. All patients demonstrated an increase in plasma concentrations while receiving micro ultra-filtration, with drug accumulation observed over the first 12 hours postoperatively. Despite micro ultra-filtration drug clearance of 3.3 mL x kg x min, there was no decease in milrinone plasma concentration; thus, the increase in milrinone levels were due to the concentrating effects seen by ultrafiltration. During the first 12 hours follow surgery, milrinone CL was significantly impaired and improved at 12 hours. Steady state clearance was 2.6 ml x kg x min, slightly lower than older patients.

In summary, a loading dose of 100 mcg/kg on cardio-pulmonary bypass resulted in plasma milrinone concentrations comparable to 50 mcg/kg in infants not receiving bypass The use of post-operative micro ultra-filtration increases milrinone plasma concentration by 35% due to its concentrating effect of systemic blood flow. Due to a decrease in renal function within the first 12 hours following surgery, continuous infusion rates of 0.2 mcg/kg/min may be considered in neonates with HLHS to achieve plasma concentrations of approximately 200 ng/ml. However, as urine function increases post operatively, continuous infusion dosing may need to increase for optimal inotropic support.

3.4.5 Modeling Summary

A summary of milrinone pharmacokinetic modeling studies can be found in Table 12. In addition to the aforementioned studies, several investigators have examined the covariables impacting volume and clearance within milrinone pharmacokinetic modeling. Hornik and colleagues, utilizing a 1-compartment model, demonstrated that age (defined as maternal post-menstrual age), weight, and creatinine clearance were significant factors impacting milrinone clearance.[40] Gist and colleagues utilized a 2-compartment pharmacokinetic model and observed a 0.7 correlation between milrinone clearance and creatine clearance.[47]

In summary, children have higher milrinone clearance rates as compared to adults, but the clearance is quite variable. The inter-subject variability of the pediatric dose-concentration relationship results in inconsistent serum concentrations, as demonstrated by Guerra.[2] The covariables of age and weight signify the importance of renal development and ontogeny on milrinone clearance. Concomitant renal function was defined as an important covariable in both the Hornik and Gist studies,[40,47] further impact the milrinone dose-concentration relationship. While these findings should not be surprising since milrinone is primarily renally eliminated, the inter-subject variability demonstrated with milrinone standardized dosing underscores the complexity in treating pediatric cardiac-repair patients.

Table 12: Milrinone Pharmacokinetic Modeling Summary

Study	Age Group Sample Size	Samples	Pharmacokinetic Parameters	Significant Findings
Bailey[35]	Neonates; n=46 1-24 months n=93 >24 months; n=18	462	Vd_β = 0.482 L/kg Cl=6.29 ml/min/kg	3-Compartment Model -adjusted for age and weight to characterize pharmacokinetic model Cl=2.42 x weight x (1+0.058*age)
Ramamo-orthy[39]	1 month – 13 yrs; n=19	247	Vd_β = 0.83L/kg Cl=4.5 ml/kg/min	2-compartment Model -weight, age, and BSA significant covariates for milrinone clearance
Zuppa[38]	Neonates: n=16	80	Vd_β = 0.72 L/kg Cl=2.6 ml/kg/min	2-compartment Model -weight and age significant covariates for milrinone clearance; Mico ultra filtration during surgery improved milrinone Cl following surgery
Hornik[40]	Neonates; n=13 1-24 months; n=19 2 yrs-12 yrs; n=29 >12 yrs; n=13	111	Vd = 0.46 L/kg Cl=3.8ml/kg/min	1- compartment Model - weight, age, and CrCL significant covariates for milrinone clearance Cl=15.9(wt[kg]/70)(0.75)x (Post menstrual age (1.12)/67 (1.12) +PMA) x (CrCl)/117)
Gist[47]	21 Days – 21 yrs; n=11	33	Vd_β -0.74 L/kg Cl=4.72 ml/kg/min	2-Compartment Model - milrinone clearance 0.7 correlation to CrCl

3.5 Emergence of Therapeutic Range in Milrinone Concentrations in Pediatrics

The inotropic and vasodilatory effects of milrinone are dose and concentration dependent.[33-34] The hemodynamic evidence in pediatric patients demonstrated by Bailey et al suggested that optimal inotropic activity may be dependent upon plasma concentrations, with an improvement in cardiac index with plasma concentrations approximately 230 ng/mL.[35] In addition, data in adult patients suggested that plasma concentrations between 100-300 ng/mL optimized cardiac output in patients with congestive heart failure.[45] Milrinone dosing is empiric, with a range in continuous infusion rates from 0.25-0.75 mcg/kg/min, titrated to effect. The diagnosis of low cardiac output syndrome remains subjective and based upon poor perfusion, poor oxygen saturations, and low systemic and central blood pressures. However, excessive vasodilation due to milrinone toxicity can present in the same manner. Thus, the need for vasopressor support to treat low systemic blood pressure can be either due to LCOS where milrinone concentrations need to be increased or due to low blood pressures secondary to milrinone toxicity as a result of excessive vasodilation. The problem is further compounded by two additional factors. As noted in the Ramamoothry study, there is considerable variability in clearance among pediatric patients, even when correcting for age and weight.[39] This variability, coupled with a patient population having undergone heart repair, can result in either subtherapeutic or toxic milrinone plasma concentrations. Since a commercially available milrinone assay to guide dosing is not available, clinical decision making may be challenging due to confusion as to whether a dosage increase, or decrease may be warranted.

3.5.1 Guerra Study

Guerra and colleagues studied the incidence of milrinone blood levels outside the therapeutic range (100-300 ng/mL) in pediatric patients who received milrinone therapy following surgical repair of congenital heart disease.[2] In addition, the study examined the concentration-effect and concentration-toxicity associated with milrinone plasma concentrations. The prospective cohort study examined milrinone plasma concentrations in children $\leq$ 2 years of age who received milrinone therapy following surgery for congenital heart disease. Milrinone dosing was at the discretion of the attending physician to prevent the development of low cardiac output syndrome. Blood samples were obtained at 4 intervals: 9-12 hours after surgery, 18-24 hours after surgery, 40-48 hours after surgery, and at the end of the infusion. Blood samples were categorized as within the therapeutic range (100-300 ng/mL) or outside the therapeutic range. Values in excess of 300 ng/mL were considered supratherapeutic while plasma concentrations < 100 ng/ml were considered subtherapeutic. In addition, signs of low cardiac output syndrome were reviewed for patients that were considered subtherapeutic, while patients with supratherapeutic concentrations were assessed for seizures, feeding intolerance, thrombocytopenia, hypotension, and/or arrhythmias. The occurrence of potential milrinone toxicity or ineffectiveness was recorded every 2 hours for the first 72 hours of milrinone therapy.

A total of 65 patients were enrolled in the study, consisting of 220 plasma concentrations. Median age was 3 months and weight = 4.5 kg. The median milrinone continuous infusion rate was 0.5 mcg/kg/min. Forty-eight percent of milrinone plasma concentrations were within the therapeutic range, while 52% were outside the range. A total of 78 samples (36%) were subtherapeutic, with 36 samples (16%) supratherapeutic. The median milrinone plasma concentrations within each patient (from the minimum to the maximum concentration throughout therapy) was 82 (46-185) ng/mL,

with a range/mean ratio of 59% demonstrating significant inter-patient variability. Low cardiac output syndrome occurred in 92% of patients, with arrythmias in 13 (20% of patients). When analyzed by sample time period, there was a statistically significant association between arteriovenous oxygen difference of 30% and toxic milrinone concentrations. There were no significant associations with the other clinical markers of toxicity or efficacy. However, when the data were reviewed as repeated measures rather than per time period, there was a statistically significant association between supratherapeutic milrinone concentrations and low cardiac output syndrome.

The findings from this study highlight the variability in the dose-concentration relationship of milrinone in pediatric cardiac surgery patient. With only 50% of milrinone plasma within the therapeutic range, the remaining 30% of concentrations were subtherapeutic and 20% potentially toxic. Patients with concentrations > 300 ng/mL were more likely to experience low cardiac output syndrome due to excessive vasodilation of milrinone. The authors concluded that the current dosing strategy of milrinone may be sub-optimal and future protocolized study should investigate the clinical implications non-therapeutic milrinone concentrations and the potential for real-time monitoring.

3.6 Role of Renal Dysfunction Impacting Milrinone Concentrations in Children

As scientific evidence supports a therapeutic range for milrinone plasma concentrations, factors influencing the dose-concentration relationship continue to be explored. Since milrinone is primarily excreted as unchanged drug in urine, dosing alterations are warranted in patients with renal dysfunction to achieve targeted concentrations. Milrinone dosing guidelines exists for adult patients with renal dysfunction, but no such guidelines exist in pediatrics. Clinically, practitioners decrease milrinone continuous infusion rates if a patient appears to be experiencing AKI. The

degree of dosage reduction is practitioner dependent. Clinical studies are beginning to examine the dose-concentration relationship in pediatric patients with AKI.

3.6.1 Gist Study

Gist and colleagues performed a retrospective evaluation of 11 patients who had acute kidney injury (AKI) with or without continuous renal replacement therapy (CRRT).[46] The purpose of this study was to evaluate milrinone plasma concentrations and pharmacokinetics in children with renal dysfunction. Milrinone therapeutic drug monitoring was the standard of care at the institution where this retrospective analysis occurred; thus, milrinone plasma concentrations were available. In addition, demographic and clinical data were collected that included: medical history, gender, age, weight, baseline and follow-up serum creatinine values, and milrinone dosing regimen. Population pharmacokinetic analysis was performed using Bayesian estimation using a 2-compartment model. Clearance and volume of distribution were scaled to body weight to account for differences in body size. A total of 11 patients with 33 milrinone samples were included in this study. The frequency of sampling varied between patients, with a median of 2 samples per patient. Patient age ranged from 3 weeks to 20 years of age. Median milrinone concentrations ranged from 640-860 ng/mL, with 72.7% of concentrations outside the targeted range of 180-300 ng/mL Approximately 49% of plasma concentration were supratherapeutic, while 24% were subtherapeutic. Three patients received CRRT, with a total of 11 samples obtained. Five of the samples (30%) were supratherapeutic while 1 was subtherapeutic. All 11 patients had a diagnosis of AKI based upon clinical diagnosis. Milrinone clearance increased with age; however, when normalized for weight, clearance did not change appreciably. There was large inter-patient variation in clearance (2.91 -13.6 L/h per 70kg), although there was no difference between patients who received CRRT as compared to those that did not

(range: 2.8-7.2 L/h per 70 k). There was a significant correlation between milrinone clearance and estimated creatinine clearance (R^2=0.7).

3.7 Summary

Milrinone therapy continues to be a mainstay treatment in pediatric patients undergoing congenital heart repair to prevent or treat low cardiac output syndrome. While routinely used in practice, optimizing milrinone dosing is often based upon clinical presentation. This optimization can be further complicated in patients with clinical or sub-clinical renal dysfunction, due to the extensive nature in which milrinone is renally eliminated. The interaction between renal function and milrinone dosing as demonstrated in the milrinone package insert underscores this interaction; yet a targeted, scientific approach to renally-adjusted milrinone therapy in pediatric patients remains elusive.

The large variability in the dose-concentration relationship in pediatric patients suggests that TDM should be considered to optimize cardiac output in the post-operative pediatric patient. Although a therapeutic range of milrinone has not been established as a standard of practice, the emerging evidence suggests that concentrations between 100-300 ng/mL optimizes cardiac output and prevents excessive dilation or pro-arrhythmic potential. Optimal sampling strategies need to be further investigated to determine the appropriate therapeutic drug monitoring approach. In addition, the incorporation of key physiologic indicators (e.g., renal biomarkers) should also be studied to best understand the extent that covariables impact the milrinone dose-concentration relationship.

Chapter 4: A Pilot Study Evaluating the Relationship between Neutrophil Gelatinase-associated Lipocalin (NGAL) and Milrinone Plasma Concentrations in Pediatric Patients Following Corrective Surgery for Congenital Heart Disease (An Interim Analysis)

4.1 Introduction

Milrinone is a phosphodiesterase type-3 inhibitor that exerts inotropic and vasodilatory pharmacologic effects, increasing cardiac index and decreasing left ventricular filling pressure. Initially indicated for adult patients with low cardiac output, milrinone is currently used in pediatric patients following cardiac surgery for the prevention or treatment of low cardiac output syndrome.[2,32,35-44] Several studies have demonstrated that achieving a target milrinone plasma concentration between 100-300 ng/mL is associated with optimal inotropic and vasodilatory response.[2,45] Further, it has also been shown that plasma concentrations $\geq$300 ng/mL may result in excessive vasodilatory effects and potential pro-arrhythmic activity.[2,45,48] Routine therapeutic drug monitoring has not been performed, to date, to achieve target concentrations and milrinone dosing has been largely empiric despite the variable relationship between milrinone dose and concentration.[2,45-48] A key factor impacting milrinone plasma concentrations is renal function, since 80-85% of the drug is excreted unchanged in the urine. In children, AKI occurs in 50% of critically ill post- operative cardiac patients;[21,29-31] therefore, it could be expected that this population would have altered milrinone drug clearance, yet dosing adjustments rarely occur. Two major factors preventing dosing adjustments are the unavailability of milrinone plasma concentrations to guide dosing and assessment of pediatric renal function as it relates to AKI.

Assessment of renal function is complicated in pediatric patients, especially those less than 5 years of age. While 12- and 24-hour timed urine collection is

considered the clinical “gold standard’, they are rarely used in clinical practice. Instead, urine output and creatinine clearance equations (e.g., Schwartz) are used to estimate GFR, with subsequent adjustments to medication therapy. Evidence suggests that biomarkers such as NGAL, KIM-1, LFABP, and IL-18 may be better indicators of AKI, including pediatric patients.[7,9,13-21] In particular, NGAL has been the most extensively studied biomarker in post-operative pediatric cardiac patients, due its predictability in determining AKI in patients following cardio-pulmonary bypass within the first 24 hours following surgery. [17,21,29-31] Clinical evidence suggests that routine use of NGAL in high-risk patient populations (e.g., critically ill patients) may become the standard of practice in the near future, especially within 72 hours following cardiac surgery.[49]

Early identification of post-operative pediatric patients developing AKI can lead to treatment alterations to prevent morbidity and mortality. The potential use of NGAL to identify these patients could lead to pharmacologic adjustments consistent with deteriorating renal function. This pilot study would be the first of its kind to examine the association between milrinone plasma concentrations and pNGAL and uNGAL concentrations in pediatric patients, while also comparing both NGAL concentrations to the Schwartz equation to estimate glomerular filtration rate. The *hypothesis* of this study is that NGAL will identify pediatric congenital heart patients that are at risk for accumulating milrinone plasma concentrations > 300 ng/mL. The *specific aims* are: 1) to determine milrinone plasma concentrations in PICU patients following surgical correction of congenital heart disease lesions; 2) to assess the potential correlation of milrinone plasma concentrations to pNGALand uNGAL concentrations; 3) to assess pNGAL and uNGAL concentrations and correlate with creatinine clearance (using Schwartz equation).

4.2 Materials and Methods

4.2.1 Patient Selection

Pediatric patients ≤5 years of age admitted to Children's Hospital and Medical Center, Omaha, Nebraska and scheduled to undergo repair of congenital heart lesion requiring milrinone therapy within the first 24 hours following cardiac surgery were considered eligible for the study. Patients were considered ineligible for the study if: 1) parental informed consent could not be obtained or if the parent/guardian refused to participate in the study; and/or 2) renal insufficiency was present prior to surgery (defined as pRIFLE category III or IV). The research protocol was approved the UNMC/Children's Hospital Institutional Review Board. Patient demographic information was collected by the Principal Investigator and Research Nurse Coordinator by utilizing the EPIC© electronic medical record (Madison, WI). Demographic information collected included: age, weight, height, and type of congenital heart lesion. Clinical information (serum electrolytes, complete blood counts with platelets, clotting studies, liver function tests) ascertained as part of routine clinical care following surgery was also collected. Creatinine clearance was calculated using the Schwartz-equation as shown in Table 1. Milrinone dose (including loading/bolus doses) and concurrent medications were documented. Clinical evidence of low cardiac output syndromes (as described by the clinical team), cardiac arrhythmias, or additional support modalities (e.g., extra-corporeal membrane oxygenation, continuous renal replacement therapy) were collected. Finally, patient outcome (survival to discharge or death) was documented.

4.2.2 Milrinone Therapy

Milrinone therapy was initiated at the discretion of the pediatric cardiothoracic surgeon, cardiologist, and/or intensive care physician for the prevention or treatment of LCOS following cardiac surgery. Milrinone continuous infusion of 0.5 mcg/kg/min was

initiated in the operating room prior to transfer to the PICU. The cardiothoracic surgeon, based upon clinical discretion, could administer a loading dose of 75 mcg/kg in the OR. Milrinone was diluted in 5% dextrose to a final concentration of either 200 mcg/mL or 400 mcg/mL, based upon patient weight and administered as a continuous infusion by the MedFusion 3500™ (Medex, Inc., Duluth, GA) syringe pump. Milrinone continuous infusion dosing adjustments were made based upon clinical symptoms of low cardiac output syndrome at the discretion of the physician. Milrinone was discontinued at the discretion of the medical team.

4.2.3 Blood and Urine Sample Collection and Analysis

Blood (2.5 mL) and urine (3 mL) samples were collected prior to surgery, and 12-, and 24-hours following surgery. Subsequent samples were collected with routine clinical labs every 12 hours until milrinone was discontinued or the patients received a total of 5 days of therapy. A single blood and urine collection occurred within 12 hours following milrinone discontinuation. Blood samples were collected through an existing central line into K_2EDTA vacutainer tubes containing sodium heparin; urine samples were collected from the catheter placed during surgery or bagged urine. All samples were transferred the Children's Hospital and Medical Center Clinical Laboratory or CHRI laboratory for processing, with samples stored at 4 degrees Celsius post collection (for a maximum of 16 hours) before processing. Stability of plasma and urine NGAL concentrations have been demonstrated over this time period.[50] Blood samples were centrifuged for 15 minutes at 3500 rpm. Plasma was separated and transferred to pre-labeled plastic storage tubes where plasma and urine samples were stored at -80 degrees Celsius until batched for analysis. Plasma NGAL and uNGAL were assayed by solid phase sandwich ELISA, using commercially available reagents following manufacturer's guidelines (R&D Systems DLCN20, Oxford, UK). The human lipocalin-

2/NGAL Quantikine ELISA kit demonstrates a sensitivity of 0.04 ng/ml within the assay range of 0.2 - 100 ng/mL in serum, plasma, saliva, and urine, and has been validated in an evaluation of NGAL measurements.[50-51]

Milrinone plasma sample analysis was performed in the UNMC College of Pharmacy Pediatric Pharmacology Research Unit after thawing. The assay was performed utilizing validated LC-MS/MS (Sciex 5000 triple quadrupole mass spectrometer), with standard calibrators and quality control samples spiked to micro-centrifuge tubes. Internal standard (amrinone) was added to track the analyte of interest through the extraction and instrumentation process. All samples were extracted using 50:50 ACN:MeOH to precipitate any proteins and other large molecules. A LC-MS system with a phenyl column for stationary phase (along with an isocratic mobile phase of 35:65 methanol:50mM ammonium pH 6.0) separated the analytes for detection at a flow rate of 0.35 mL/min. The analytical range of the assay was 5 ng/mL- 500 ng/mL, with the lower limit of quantitation (LLOQ) at 5 ng/mL. Calibration standards were run in duplicate at the front and back of the batch. Standards were required to back-calculate within +/-15% of their theoretical value (+/-20% for LLOQ) in order to be used in the regression. Quality control samples were processed in duplicate at all 3 concentration levels and a total number of quality control samples were required to be ≥10% of the total of unknowns on the batch. The standard operation procedure was validated in accordance with guidelines set forth by FDA.

4.3 Statistical Analysis

The full pilot clinical study aims to enroll 30 patients. A sample size of 30 produces a two-sided 95% confidence interval to determine the rate of individuals who would fall outside the therapeutic range of 100-300 ng/mL and achieves 80% power to detect a potential correlation between AKI biomarkers and milrinone concentrations

and/or creatinine clearance values. [52] All future analyses will be conducted using intent-to-treat criterion, including linear models (one-way ANOVA or linear regression) for continuous variables, student's t-test for parametric data, and Kruskal Wallis test and Wilcoxon-Mann-Whitney test for non-parametric data.

Descriptive statistics of the interim analysis (described herein) used non-parametric median and inter-quartile range (25% and 75%, respectively) to summarize outcome variables. Group differences were assessed with the Mann-Whitney U test for non-parametric data. A p-value of ≤ 0.05 was considered statistically significant. Spearman correlation was calculated for the associations between continuous variables, with p-values for null hypothesis of correlation being zero.[52] Paired samples were used for correlation analysis, with unpaired data excluded from analysis. Finally, milrinone concentrations below the LLOC were excluded from final analysis.

4.3.1 Pharmacokinetic Analysis

One- and 2-compartment clearance (Cl) models were considered as base models and were fit using the nonparametric adaptive grid (NPAG) algorithm within the Pmetrics package version 1.5.0 (Los Angeles, CA) for R version 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria). Model performance was evaluated and compared utilizing a regression of observed vs. predicted concentrations, visual plots of parameter estimates, and the rule of parsimony (which states that given the same outcome, the simplest model is the preferred one). The initial estimate of parameter weighting was accomplished using the inverse of the assay variance.[54] The observation variance was proportional with a scalar (lambda) to assay variance. The best-fit model was utilized to obtain median maximum a posteriori probability (MAP) Bayesian milrinone plasma concentration estimates at 12-minute intervals over the study period, generated from each patient's measured milrinone concentrations, exact dose, and

dosing schedule. In addition, patient-specific covariables (e.g., age, weight, BSA, serum creatinine, calculated creatinine clearance) were added to the model to best predict milrinone pharmacokinetic parameters (exploratory). Bayesian posteriors for each patient were used to plot observed vs. predicted plots for each patient.[54]

4.4 Results

A total of 8 patients have been enrolled to date, with the median age 8 months of age (IQR=0.5 -1.3 years of age). Demographic data, including underlying congenital heart lesions, are included in Table 13. No patients were diagnosed with the development of AKI while on the study, nor were any patients described by the medical team as developing low cardiac output syndrome. Finally, baseline renal function values for all 8 patients are presented in Table 14.

Table 13: Clinical Study: Demographic Information

	Median (IQR)
Subjects	8 (Gender: F=2; M=6)
Age (yrs)	0.71 (0.5-1.3)
Weight (kg)	3.9 (3.6-5.3)
Height (cm)	73 (71.75-76.5)
Congenital Heart Lesion:	Hypoplastic Left Heart (n=2) Pulmonary Artery Conduit Transposition of Great Arteries (n=2) Tetralogy of Fallot Ventricular-Septal Defect (n=2)
Development of AKI	0
Development of low cardiac output syndrome	0

Table 14: Clinical Study: Baseline Renal Function

	Median (IQR)
Plasma NGAL (ng/mL)	99 (54-140)
Urine NGAL (ng/mL)	29 (17-33)
SCr (mg/dL)	0.53 (0.45-0.62)
CrCl (mL/min per 1.73m^2)	71 (57-84)

Milrinone Therapy

A summary of milrinone therapy is presented in Table 15. None of the patients received a milrinone loading dose within the operating room, nor received a loading dose after transfer to the PICU. The median continuous infusion dose was 0.48 mcg/kg/min, with the median duration of therapy 45 hours. A total of 44 milrinone plasma concentrations were obtained from 8 patients, with the median concentration = 130 ng/mL (IQR=38-245). Twenty milrinone plasma concentrations (45%) were within the therapeutic range of 100-300 ng/mL, 17 samples (39%) were $\leq$100 ng/mL, and 7 samples (16%) were >300 ng/mL.(Figure 2). The milrinone plasma concentration range for each patient throughout their individual treatment course are presented in Figure 3. Of note, 1 patient did not achieve milrinone plasma concentrations >100 ng/mL. Finally, median milrinone plasma concentrations at specified sampling times are presented in Table 16. Milrinone plasma concentrations were highest 12-hours following initiation of therapy (261 ng/mL), with a gradual decrease in milrinone concentrations throughout the first 48-hours of therapy.

Milrinone Pharmacokinetics

All 44 milrinone concentrations were evaluated for pharmacokinetic analysis. Median volume of distribution and clearance values are presented in Table 17. A 1-compartment and 2-compartment pharmacokinetic model were initially evaluated, with the 2-compartment model ultimately chosen (Figure 4) based upon model performance utilizing the lower Akaike information criterion. The initial 2-compartment population predicted vs individual predicted model with no covariates is shown in Figure 5. The initial model demonstrates significant inter-subject variation, with population prediction model demonstrating R^2 =0.0046 while individual-based modeling demonstrated a R^2 = 0.58. Weight, age, uNGAL, and pNGAL were examined as potential significant

covariates for the pharmacokinetic variables clearance and volume of distribution (Figure 6). The population model improved with the addition of uNGAL, weight (allometrically adjusted to 0.75) and a maturation function with age. The final model was best represented by:

- $CL = CL1 \times (Wt/6)^{0.75} \times Age^{H} / (TM^{H} + Age^{H}) + CL2 \times (uNGAL/62)$
- $V = V1 \times (Wt/6)^{1}$

CL = overall clearance; CL1 = clearance term 1; Wt = weight (kg); H = Hill coefficient; TM = maturation term; CL2 = clearance term 2; V = overall volume; V1 = volume term 1.

Individually modeled milrinone dosing (actual versus predicted) using posterior distribution for all 8 patients are presented in Figure 7.

Table 15: Clinical Study: Milrinone Therapy Summary

	Median (IQR)
Milrinone Dose (mcg/kg/min)	0.48 (0.25-0.5)
Milrinone Boluses	0
Duration of Therapy (hrs)	45 (41-56)
Milrinone Plasma Concentration (ng/mL) (n=44)	130 (38-245)

Figure 2: Clinical Study: Milrinone Plasma Concentrations

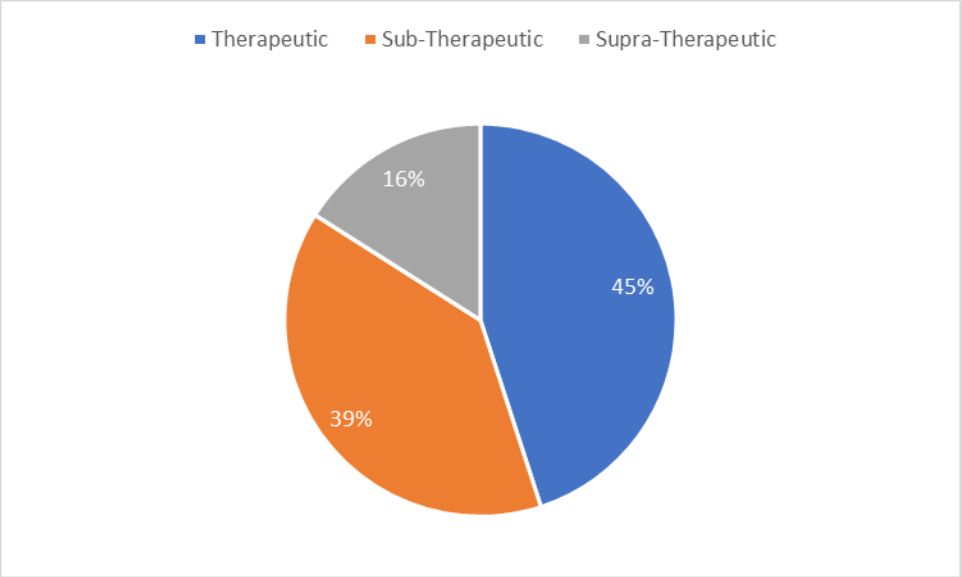
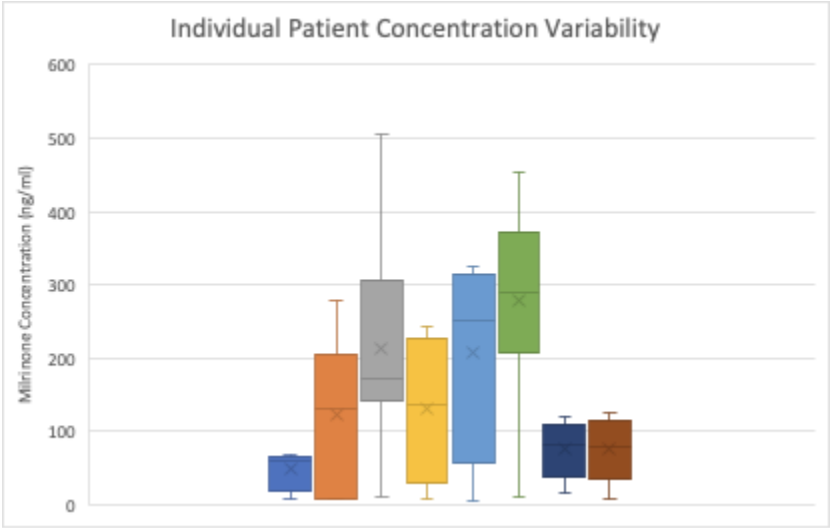


Figure 3: Clinical Study: Milrinone Plasma Concentration Range for Each Patient

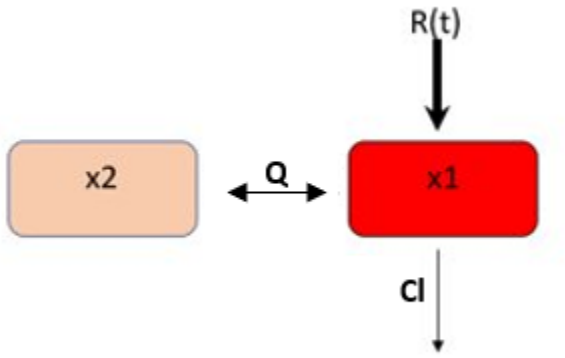


Box-plot values are presented for each of the 8 patients, with median milrinone concentrations represented by sold line, “x” represents mean milrinone concentrations, IQR represented by colored box, and “whiskers” representing minimum and maximum values for each patient.

Table 16: Clinical Study: Milrinone Plasma Concentrations per Time Interval

Milrinone Concentrations	**Median (IQR)**
12-hour (ng/mL) n=8	261 (124-357)
24-hour (ng/mL) n=8	178 (103-292)
36-hour (ng/mL) n=8	117 (81-197)
48-hour (ng/mL) n=6	94 (61-160)
Day 3 Morning (ng/mL) n=2	217 (179-255)
Day 3 Evening (ng/mL) n=2	207 (186-229)
Day 4 Morning (ng/mL) n=2	102 (57-148)
Post Therapy (ng/mL) n=8	8 (7-10)

Figure 4: Clinical Study: Base Milrinone Pharmacokinetic Model

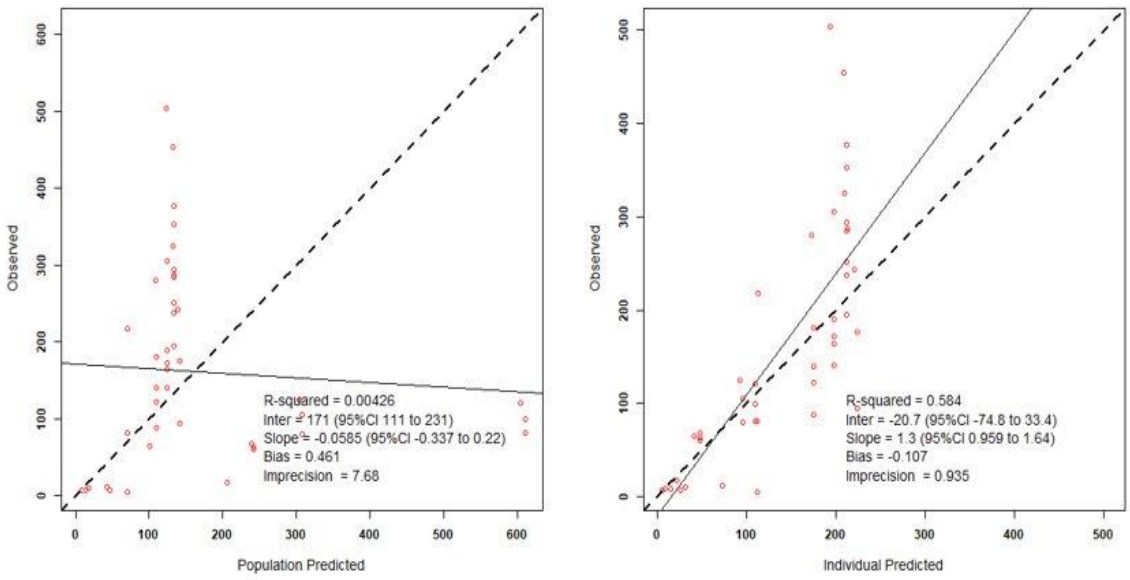


R(t) = rate of dose; CL = clearance; Q = flow; x1 = first compartment; x2 = second compartment.

Table 17: Clinical Study: Milrinone Pharmacokinetic Parameters

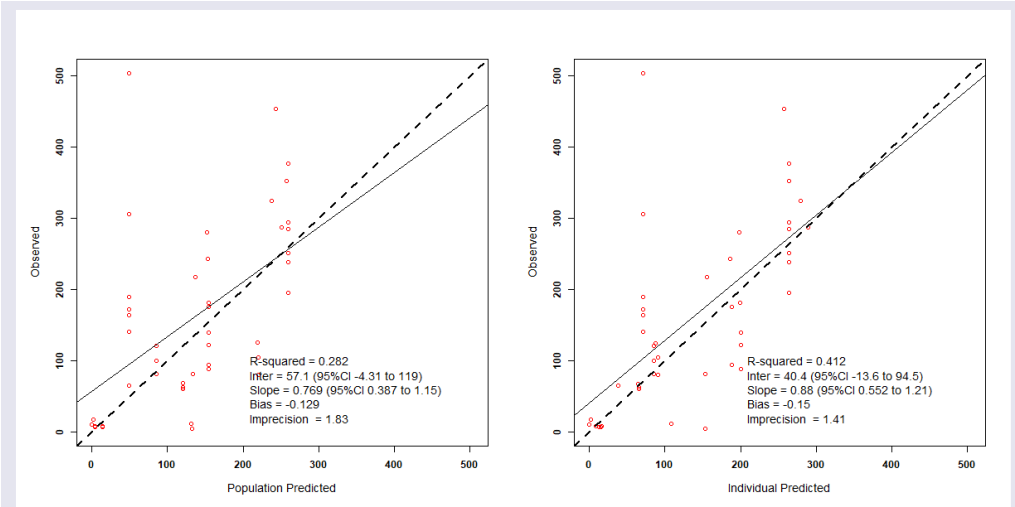
	Median (IQR)
Vd (L/kg)	0.61 (0.5-1.2)
CL (mL/kg/min)	9.8 (8.3-12.4)

Figure 5: Clinical Study: Population Predicted vs Individual Predicted Pharmacokinetic Modeling (Base Model)



Dashed line represents slope =1, solid line represents Loess smoothed line for individual data points.

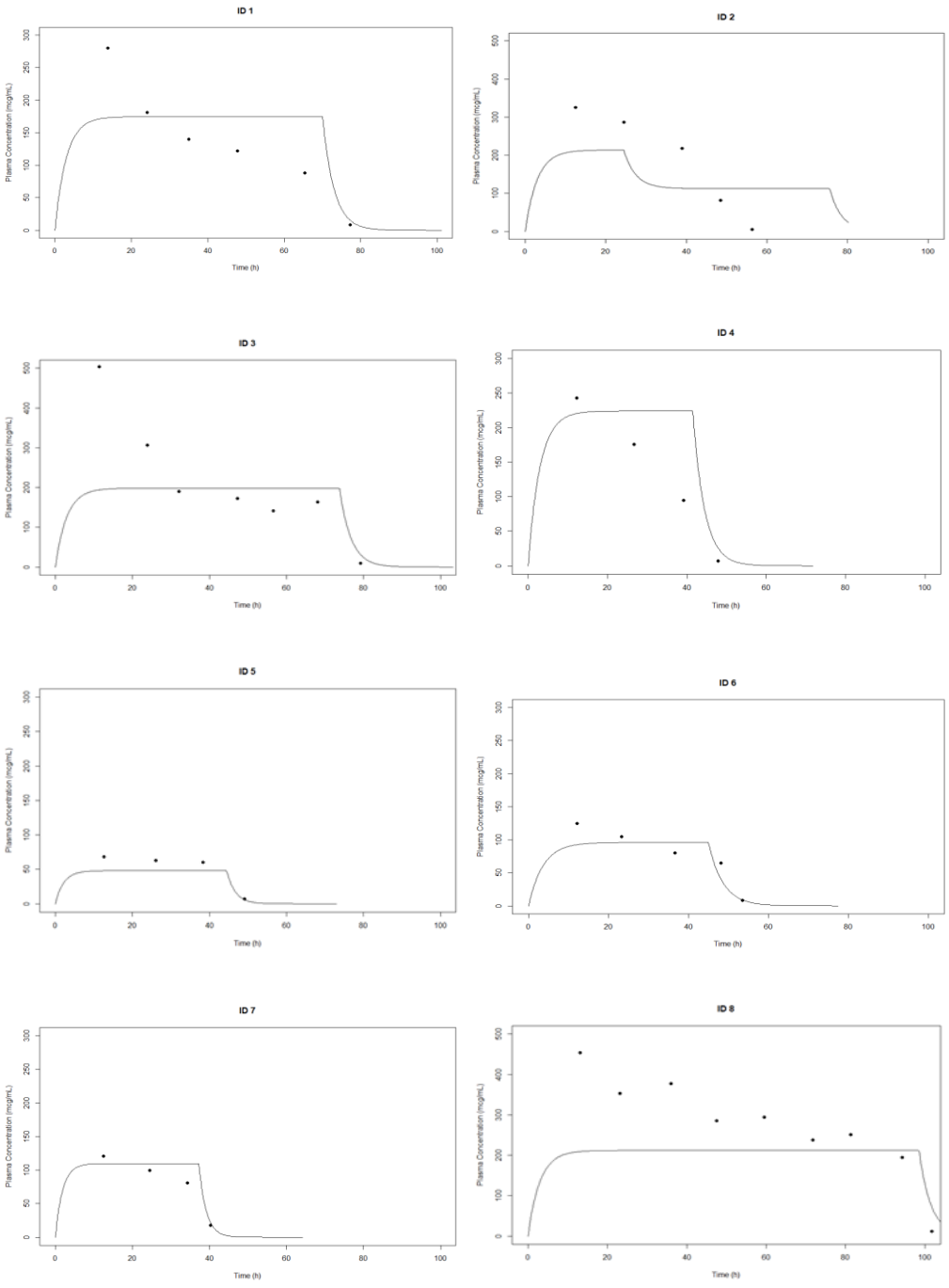
Figure 6: Clinical Study: Population Predicted vs Individual Predicted Pharmacokinetic Modeling (Final Covariate Adjusted Model)



Dashed line represents slope =1, solid line represents Loess smoothed line for individual data points.

Figure 7: Clinical Study: Bayesian Posterior Predicted Concentrations vs Individually Observed Concentrations

(Actual [dots] vs Predicted [line])



Individual patients (ID), with dots representing actual milrinone plasma concentration per time interval and solid lines representing predicted values.

Renal Biomarkers

A summary of pNGAL and uNGAL concentrations is shown in Table 18. Median pNGAL concentrations were 110 ng/mL (IQR=82-195), whereas uNGAL concentrations were 25 ng/mL (IQR=15-48). Median serum creatinine and Schwartz-derived creatinine clearance were 0.5 mg/dL (IQR=0.45-0.58) and 68 mL/min mL/min per 1.73m2 (IQR=61-92), respectively. Table 19 demonstrates pNGAL and uNGAL concentrations per time interval. Of note, both pNGAL and uNGAL concentrations were elevated at 12-hours as compared to baseline. (p-value=0.2=NS). Both pNGAL and uNGAL concentrations decreased over the 48 hours following surgery, with a return to baseline following the discontinuation of milrinone.

The correlations between creatinine clearance and pNGAL/uNGAL are shown in Figure 8. Creatinine clearance correlation to pNGAL(R^2=0.04) was lower compared to uNGAL(R^2=0.39). The correlations between serum creatinine and pNGAL and uNGAL are shown in Figure 9. Neither demonstrated significant association (R^2=0.004 and R^2=0.086, respectively).

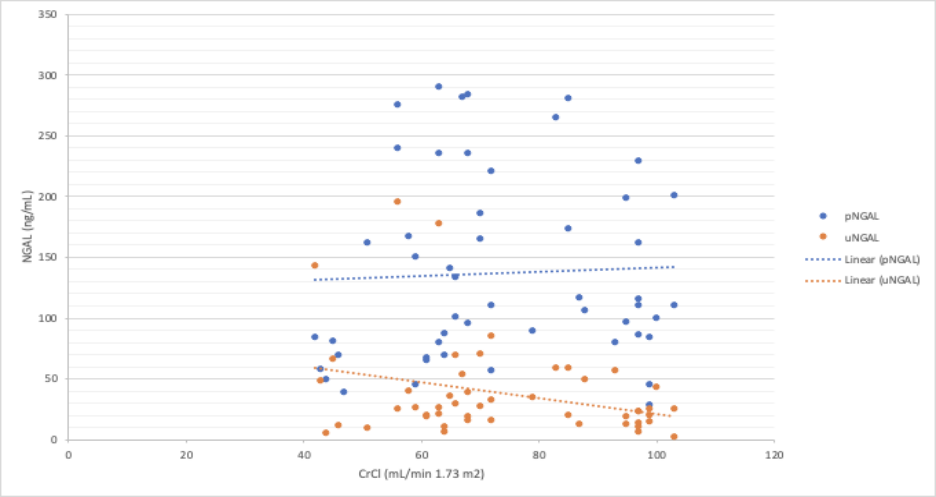
Table18: Clinical Study: Median Plasma and Urine NGAL Concentrations

	Median (IQR)
Plasma NGAL (ng/mL) (n=52)	110 (82 -195)
Urine NGAL (ng/mL) (n=50)	25 (15-48)
SCr (mg/dL) (n=52)	0.5 (0.45-0.58)
CrCl (mL/min per 1.73m^2) (n=52)	68 (61-92)

Table 19: Clinical Study: Median pNGAL and uNGAL Concentrations per Time Interval

	Median Plasma NGAL (IQR)	**Median Urine NGAL (IQR)**
Baseline (ng/mL) (n=8)	100 (54-140)	29 (17-33)
12-hour (ng/mL) (n=8)	225 (83-267)	72 (47-152)
24-hour (ng/mL) (n=8)	182 (104-249)	39 (22-60)
36-hour (ng/mL) (n=8)	150 (83-168)	21 (18-29)
48-hour (ng/mL) (n=6)	98 (74-112)	17 (13-34)
Day 3 Morning (ng/mL) (n=2)	150 (132-168)	25 (24-26)
Day 3 Evening (ng/mL) (n=2)	165 (130-200)	19 (19-19)
Day 4 Morning (ng/mL) (n=2)	107 (103-107)	16 (15-17)
Post Therapy (ng/mL) (n=8 pNGAL; n=6 uNGAL)	78 (51-111)	19 (14-25)

Figure 8: Clinical Study: Correlation Between Schwartz-derived Creatinine Clearance and NGAL Concentrations

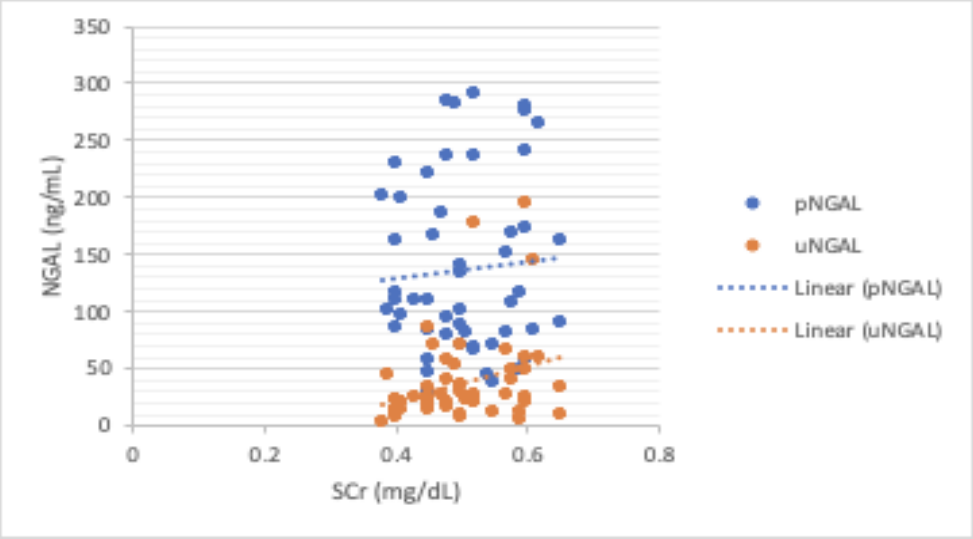


Dotted lines represent the least squares regression line for pNGAL (blue) and uNGAL (orange).

Plasma NGAL regression (R^2=0.04): y=0.1813x + 123.62

Urine NGAL regression (R^2=0.09): y= -0.653x + 86.45

Figure 9: Clinical Study: Correlation Between pNGAL and uNGAL to Serum Creatinine



Dotted lines represent the least squares regression line for pNGAL (blue) and uNGAL (orange).

Plasma NGAL regression (R^2=0.004): y=65.968x + 103.57

Urine NGAL regression (R^2=0.086): y=153.61x + 39.54

4.5 Discussion

Milrinone therapy following corrective congenital heart surgery is considered the standard of practice in most pediatric cardiac patients, despite limited prospective pharmacokinetic information. In addition to the PRIMACORP study, several smaller studies have evaluated the pharmacokinetic profiles of milrinone in pediatric patients. [2, 35- 39] In most studies mean milrinone plasma concentrations were reported independent of the percentage of patients that achieved "therapeutic" concentrations between 100-300 ng/mL. In our study, the median milrinone plasma concentration was 130 ng/mL, which would be considered within the therapeutic range. However, 55% of 44 milrinone samples in our study were outside the therapeutic range despite standardized dosing via continuous infusion. A study by Guerra et al. examined 114 plasma concentrations in 63 pediatric cardiac surgery patients receiving standard continuous infusion milrinone and demonstrated that 52% were outside the therapeutic range, with 36% supratherapeutic and 16% subtherapeutic.[2] The preliminary data from our study are consistent with these findings and highlight the variability in milrinone plasma concentrations. One patient failed to achieve plasma concentration >100 ng/mL, while 3 patients demonstrated plasma concentrations > 300 ng/mL without any obvious signs of toxicity (i.e., cardiac arrhythmias, low cardiac output).

The variability with the milrinone dose-concentration relationship is multifactorial. It would be expected that renal function would a major contributing factor to the variability, given milrinone's extensive renal elimination. Several previous studies demonstrated that creatinine clearance was considered a significant factor (along with age and weight) impacting milrinone clearance and volume of distribution.[35, 38-41]As demonstrated in Figures 6, population-based modeling improved when uNGAL, weight (allometrically adjusted to 0.75) and age were added to strengthen the predictability of clearance and volume of distribution. Although a small sample size, the potential benefit

of using uNGAL in predicting milrinone clearance and subsequent plasma concentrations warrants further study. Inter-subject variability (Figure 7) remains high and underscores the interaction of ontogeny, congenital pathophysiology, and cardiac surgery on milrinone clearance. Until a prospective, comprehensive pharmacokinetic model can be created to prospectively guide milrinone dosing, therapeutic drug monitoring will be required to consistently achieve targeted plasma concentrations between 100-300 ng/mL.

One study goal was to assess the potential use of clinical biomarkers to identify patients developing AKI and prospectively adjust milrinone dosing. The limitations with currently used diagnostic tests (serum creatinine, creatinine clearance) have been described previously and highlight the challenges in appropriately diagnosing AKI immediately following cardiac surgery. Thus, changes in renal biomarker concentrations from baseline to the immediate post-operative period may better predict patients developing AKI (compared to changes in serum creatinine/creatinine clearance). Our data demonstrate a marked increase in both pNGAL and uNGAL concentrations 12 hours following surgery, followed by a gradual decline to baseline. The trend in decreasing NGAL concentrations following surgery was consistent with previous literature.[17] Although not statistically significant, the small sample size collected to date may not be able to adequately address the changes in NGAL concentrations before and after surgery. Further collection of data is warranted to assess these changes.

Patients undergoing congenital heart repair are exposed to various insults (e.g., cardiopulmonary bypass) during surgery that may impact renal function. To date, the use of serum creatinine and/or urine output immediately post-operative has not been sensitive enough to detect renal dysfunction and the potential for AKI within the first 24-72 hours after surgery. The preliminary data from this study suggest that routine monitoring of NGAL within the first 24-72 hour of therapy may be a more sensitive

marker of potential AKI, especially uNGAL, and its potential association with altered milrinone clearance. Again, further research is warranted.

Median pNGAL and uNGAL concentrations were comparable to that demonstrated in the literature, along with serum creatinine and CrCl as calculated by the Schwartz equation.[10] However, median baseline pNGAL concentrations (99 ng/mL) were higher than mean pNGAL concentration demonstrated by Fadel and colleagues (43ng/mL). Tawfeek and collegues demonstrated that pNGAL levels were significantly elevated in pediatric patients with congestive heart failure as compared to those without congestive heart failure (290 ng/mL vs 144 ng/mL).[55] While further data collection is warranted, it is possible that the higher pNGAL concentrations in our patient population may be associated with some level of heart failure secondary to congenital heart disease prior to surgery. Moving forward, it will be important to consider underlying cardiac dysfunction and its impact on baseline AKI biomarkers as a covariable if changes in AKI markers are found to be a better indicator of developing AKI.

Finally, there appears to be a lack of association between renal biomarkers and creatinine-based renal function in our study population to date. These findings are consistent with the timing, sensitivity, and specificity of NGAL as compared to creatinine-based measures of GFR, Further, none of our patients developed AKI, which is surprising (pleasantly) with previous literature suggesting that up to 50% of pediatric cardiac surgery patients develop AKI. As subject enrollment continues, the association between serum creatinine/creatinine clearance and renal biomarkers will have to be more thoroughly assessed especially in those patients who develop AKI.

As a “proof of concept study”, the biggest limitations of this study to date are the limited number of subjects enrolled and that all patients were enrolled from a single center. Consequently, the statistical power is limited.

4.6 Conclusion

The preliminary findings from this study demonstrate that 55% of milrinone plasma concentrations were outside the therapeutic range, despite standardized milrinone dosing administered via continuous infusion. Preliminary findings suggest that uNGAL, along with age and allometrically adjusted weight, significantly impacts the milrinone population pharmacokinetic parameters clearance and volume of distribution. The inter-subject variability of the dose-concentration relationship underscores the complex interaction of ontogeny, cardiac congenital heart repair, and renal function. Thus, therapeutic drug monitoring should be considered in this patient population to guide dosing to achieve therapeutic milrinone concentrations of 100-300 ng/mL.

CHAPTER 5: Challenges and Ethics of Conducting Pediatric Clinical Research During the COVID-19 Pandemic

5.1 Introduction

The COVID-19 pandemic has impacted all aspects of healthcare. From the medication approval process to drug distribution channels to healthcare delivery models, the pandemic has impacted millions of lives. It has also highlighted challenges in our healthcare system, including those provided to pediatric patients. It is estimated that approximately one-third of today's population are children less than 18 years of age. Yet, as the SARS-CoV-2 vaccine approval process has demonstrated, clinical trials in pediatric patients continue to lag that of adults. The comments made by Dr. Shirkey 50 years ago remain true today: pediatric patients are "therapeutic orphans."[56] There are several factors impacting pharmacologic clinical trials in pediatrics, including both ethical and regulatory considerations. The complex interactions of these elements can impose challenges on the pediatric clinical trial experience. Thus, the goal of this review is to provide pharmacists and other members of the healthcare team an overview of the challenges and ethics in conducting pediatric clinical research, especially during the pandemic. It is through the understanding of these challenges that appropriate clinical research can be safely and successfully conducted in pediatric patients in the future.

5.2 Ethical Considerations

Pediatric patients are considered a "vulnerable" patient population in clinical research; thus, considerable safeguards are established to respect the rights of a pediatric patient and avoid unnecessary exploitation as subjects. These regulations were initially established in 1974 as part of the "National Research Act" and the Public Health Service's "Regulations for the Protection of Human Subjects of Biomedical and

Behavioral Research", with the later commonly referred to as the 45 Code of Federal Regulations (CFR) 46. The proceedings from the 1976 committee meeting that provided the framework for conducting clinical trials research are often referred to as the Belmont Report.[57]

The Belmont report highlights the 3 key ethical principles that should protect human subjects in clinical trials: *beneficence*, *justice*, and *respect for persons*. The principle of *beneficence* states that it is society's responsibility to do good works for its members and to prevent or do no harm. *Justice* is the principle that highlights that benefits and risks should be equally shared throughout society. *Respect for person* states that an individual has the fundamental right to make an informed decision. The Belmont report states that all three principles are weighted equally; thus, there are times where the principles may conflict with one another. One example of this conflict between principles can be seen with the SARS-CoV-2 vaccine. The principle of *justice* states that members within a society should have equal access to the potential benefits and risks of a given intervention. Given the unknown benefits and risks associated with these vaccines, the principle of *justice* states that pediatric patients should have equal access to the vaccines. However, the vaccine is not available to all age groups due to the unknown pharmacologic impact on the pediatric patients and the potential risk of harm, violating the principle of *beneficence*. Thus, there is an inherent conflict between the principles of *beneficence and justice* as to whether vaccines should be studied in children at the same time they are studied in adults.

The principle of *respect for person* states that an individual must be cognizant of risks and benefits of participating in a study to truly provide informed consent. When is a pediatric patient capable of making such a decision? The conflict arising within *respect for person* focuses upon autonomy versus paternalism. Autonomy states that a rational individual can make an informed decision, while paternalism states that an individual

must use a surrogate (e.g., parent, guardian) to make such a decision. How does autonomy versus paternalism relate to pediatric patients, particularly adolescents? States utilize “the age of majority” to define when a subject is legally capable of making an informed decision. In most states, 18 years of age is considered an adult, while several states use the age of 19. However, is there a marked change in autonomy in a 16-year-old as compared to an 18-year-old? Again, the SARS-CoV-2 vaccination program illustrates this dilemma. At the time of this writing, the Pfizer© vaccine is indicated for patients 16 years and older, while the Moderna© vaccine is indicated for patients 18 years of age and older. An 18-year-old, in most states, can provide consent to receive either vaccine, while a 16-year-old (who is eligible to receive the Pfizer© product) is not able to. To ensure that the rights of the pediatric patient are considered during clinical research process, the regulations within 45 CFR 46 state that informed consent and assent must be obtained.

5.3 Informed Consent and Assent

Research subjects “shall be given the opportunity to choose what shall or shall not happen to them. This opportunity is provided when adequate standards for informed consent are satisfied.”[57] Thus, the purpose of the informed consent process is to ensure that the potential subjects are provided unbiased information, the information should be understandable (generally written at an 8th grade level), and the consent is voluntary and the subject can withdrawal at any time. Informed consent is the legal document that certifies a subject willingly participates in the research study. Adults/guardians who serve as a child’s legal guardian must sign the informed consent, and this step cannot be delegated to others unless legally certified. In addition to informed consent, per 45 CFR 46, children should also voluntarily agree (assent) to participate in the clinical research. Informed assent is like informed consent and validates that the child willingly participates

in as a research subject. Assent is required for children ≥ 7 years of age. Youth assent (ages 7-12 years) and adolescent assent (ages >13 years) should provide an overview of the study and their participatory role within the study. The adolescent assent contains more details than the youth assent, but both assent forms should be brief and age appropriate. Patients <7 years of age should be given simple verbal explanation of the study and what they can expect as a volunteer in the study. It is important to document this conversation either on the parental permission form or in the study records for all communications.

Participation in clinical research studies requires that the subject is aware of the potential benefits and risk of participating in the trial. Risks, however, are quite varied depending on the type of research that is being conducted. The regulations in 45 CFR 46 provide categorical assessment of risk as defined in sections 45 CFR 46.404, 46.405, 46.406, and 46.407 of Subpart D of the Health and Human Services regulations.[58] Minimal risk is defined as discomfort or harm (physical and/or psychological) that one would expect during a normal day. Any risks above minimal risks are further assessed in relation to potential benefit of study subject, with greater levels of risk associated with more safeguards to protect the child. As defined in 45 CFR 46.404, research that is considered no greater than minimal risk (e.g., venipuncture, chest radiograph) requires 1 parent to sign informed consent in addition to participant assent. The same requirements are warranted when the research involves greater than minimal risk but provides the prospect of direct subject benefit (45 CFR 46.405). Research greater than minimal risk and no prospect for direct patient benefit warrants additional safeguards to protect the child; thus, both parents must sign the informed consent along with the pediatric assent (45 CF46.406). Examples in this category include skin or bone marrow biopsy, radiocontrast with sedation, and invasive urine catheterization. Finally, research otherwise not approvable (45 CF46.407) that is focused on the understanding or

preventing of a serious problem warrants both parents' signature, pediatric assent, and approval by the Health and Human Services panel of experts. Research involving de-identification of subjects and is considered below minimum risk can waive obtaining informed consent and assent per 45 CFR 46.116(d).[58]

5.4 Pharmaceutical Regulations

Historically, therapeutic misadventures in pediatrics such as sulfanilamide-induced mortality along with the chloramphenicol related "Gray-Baby Syndrome" contributed to the Federal pharmaceutical regulations throughout the 20th century. In 1979, the FDA specifically addressed that pediatric labeling required "substantial evidence derived from adequate and well-controlled studies in the pediatric population".[59] Although initially instituted to address pediatric indications and guide for use, many products instead listed "Safety and efficacy in children has not been established", a problem which continues today. In 1995, the Food and Drug Administration revised regulations for labeling in pediatric patients stating that the pharmaceutical industry "must reexamine existing data to determine whether the pediatric use subsection of the labeling can be modified based on adequate and well-controlled studies in adults, and other information supporting pediatric use".[60-63] In 1996, the National Institute of Health (NIH) and the American Academy of Pediatrics jointly reviewed reports, background papers, and a sample of NIH-sponsored clinical research abstracts and determined that 10-20% inappropriately excluded children. In response, the National Institute of Health's report "Additional Protections for Children as Research Subjects" was published in 1998 and stated that children must be included in all human research studies unless there are scientific and ethical reasons not to include them.[62]

In 2014, the FDA published "General Clinical Pharmacology Considerations for Pediatric Studies for Drugs and Biological Products".[62] This guidance document provided

an algorithm for the need of pediatric studies by extrapolating therapeutic need, pediatric pharmacology, and ontogeny. Extrapolation is staged, ranging from “no extrapolation” to “partial extrapolation” and “full extrapolation”. The initial decision point in the algorithm asks: “Is it reasonable to assume that children, when compared to adults, have a similar: 1) disease progression and 2) response to intervention”. Based upon the answer, the algorithm continues to explore pharmacokinetic and pharmacodynamic studies, leading to dose-ranging studies, safety trials, and/or pharmacokinetic studies. The guidance provided by this document, along with the July 2020 FDA publication “Pediatric Study Plans: Content of and Process for Submitting Initial Pediatric Study Plans and Amended Initial Pediatric Study Plan” provides a roadmap to efficiently and scientifically execute pediatric clinical research.[63]

The algorithm presented by the FDA assists investigators as to the appropriate extrapolation and scientific necessity to conduct pediatric clinical research. It does not, however, address all of challenges in conducting pediatric clinical studies. Pediatric clinical studies have higher costs than comparable studies in adults. One aspect contributing to the higher costs are the alterations associated with drug formulation. Age-specific formulations (e.g., suspensions, chewable tablets, intranasal) add considerable costs to the pediatric research process. The use of pharmaceutical products outside the indicated use may require an Investigational New Product (IND) application, with appropriate USP testing and validation. Pharmacokinetic sampling strategies attempt to limit the volume associated with blood concentrations; therefore, assay development requires appropriate sensitivity to use lower blood volumes. Finally, concerns about long term risks associated with novel therapeutic agents may dampen industry’s interest in pursuing a pediatric indication.

5.5 COVID-19 Challenges in Pediatric Clinical Research

The ethical and regulatory challenges associated with pediatric clinical research have been exacerbated during the COVID-19 pandemic. As the outbreak unfolded, clinical research protocols were suspended (including the proof-of-concept study presented in Chapter 4) and Institutional Review Boards (IRB) established regulatory procedures for COVID-related research. Clinical research studies focused on COVID-19 treatments (e.g., remdesivir) were deemed the highest priority. In addition, studies that offered the potential of benefit (e.g. Children's Oncology Group) were continued, but with significant restrictions. Ongoing observational clinical studies were suspended, along with new protocols; many of these studies remain suspended as of the writing of this paper. Studies that were allowed to proceed forward had to establish new screening and enrollment protocols which required approval by the local IRB. The variability of resuming "normal" healthcare activities from state-to-state have significantly impacted national pediatric research networks, with uneven enrollment significantly impacting large, multi-institutional research studies. The COVID-19 pandemic also impacted epidemiologic studies. Many pediatric patients fell behind in routine vaccines due to limited office visits. The peak rates of *Haemophilus influenza* have decreased throughout the pandemic (26% in 2019 vs 3% in 2021) [64]; thus, clinical research protocols examining treatment strategies have observed marked decreases in enrollment. Although not an exhaustive list, the examples provided underscore the disruption to pediatric clinical research protocols.

The suspension of all research during the COVID-19 pandemic highlights the inherent ethical conflicts associated with clinical research, especially in pediatric patients. The principle of *beneficence* states that society should prevent or do no harm. Is it more harmful to potentially expose patients and parents/guardians to a healthcare system during an infectious disease pandemic, or is it more harmful to suspend existing

or new protocols of potentially novel therapeutic interventions? Should pediatric patients contribute to the principle of *justice* as it relates to the SARS-CoV-2 vaccines, where everyone in a society should contribute to the inherent benefits or risks of a new therapeutic modality? Does an adolescent have the autonomy to participate in COVID-related research protocols? Based upon the principle of *respect for person*, can a parent prevent/require their child to participate in a novel clinical treatment regimen based upon social and/or political beliefs? While these are complex questions, it is important for pharmacists and other members of the healthcare team to consider the fundamental underpinnings of the conflict to begin a thoughtful resolution process.

5.6 Conclusion

The need for pediatric clinical research studies have never been higher, especially during the COVID-19 pandemic. Despite this need, there remain ethical, regulatory, and logistical challenges in conducting pediatric clinical research. It is important that pharmacists and other healthcare providers are aware of these issues to best facilitate pediatric clinical research opportunities.

CHAPTER 6: FUTURE ASSESSMENT OF RENAL FUNCTION IN PEDIATRIC PHARMACOLOGY

6.1 Introduction

It is estimated that over 80% of medications used in pediatric patients have not been scientifically evaluated in the pediatric patient population.[59] Thus, dosing recommendations frequently are derived from adult pharmacokinetic data or smaller, pediatric clinical trials. Guidance regarding the appropriate extrapolation of adult pharmacokinetic data to the pediatric patient population was provided by the FDA's "General Clinical Pharmacology Considerations for Pediatric Studies for Drugs and Biological Products".[62] However, the primary focus of these pediatric studies has been to establish the appropriate dosages, administration intervals, and formulations unique to the pediatric population. While renal morphogenesis and maturation are often considered in pediatric dosing intervals, hemodynamic and renal functional adaptation as well as glomerular and tubular maturation throughout childhood are often overlooked. This is especially true when considering acute changes in renal function. Dosing alterations are frequently provided for adult dosing based upon estimates of creatinine clearance, commonly calculated by the Cockcroft-Gault calculation. Unfortunately, the incorporation of creatinine clearance calculators in pediatric pharmacology is inconsistent, in both clinical research and bedside practice.

As clinical research continues to evaluate the appropriate use of medications in pediatric patients, it is important to recognize the current knowledge of pediatric renal function as well as the knowledge deficits. Understanding the current knowledge gaps related to pediatric renal function can lead to the development of more specific diagnostic tools, a more targeted application of drug development, and a more scientific approach regarding medication regimen adjustments to improve clinical care. Thus, the

purpose of this chapter is to provide an overview of the current limitations in assessing renal function during renal maturation and morphogenesis, the potential role of pharmacogenomics in assessing renal disorders, and to discuss the potential role of an AKI acuity index with the potential incorporation of biomarkers into such an index that could guide therapeutic drug monitoring.

6.2 Methods

A literature search was performed in the following databases: Medline (Pubmed), SCOPUS (Elsevier), Embase (OVID), and Cochrane Central Register of Controlled Trials (CENTRAL), from January 1990 to present. An additional search was carried out using Google Scholar. Databases were searched using the following key-words: “pediatrics”, “renal function”, “diagnostic testing”, “pharmacogenomics”, “renal development”, “pharmacokinetics”, “tubular assessment”, “acute kidney injury”, “drug development”, “renal biomarkers”, and “predictive model”. Articles were limited to English-language studies published in peer reviewed journals, with additional publication identified from review articles published.

6.3 Assessment of Renal Maturation and Morphogenesis

Exogenous and endogenous markers have historically been used to assess renal function.[65-73] Inulin is the most frequently used exogenous biomarker and is considered the gold standard in measuring GFR.[67-68] Inulin is a naturally occurring polysaccharide that is freely filtered by the glomeruli and is not metabolized nor secreted by the renal tubules. Clinical studies administered inulin via continuous infusion or as a single dose. Leake demonstrated that inulin clearance was directly proportional to gestational age.[68] Guignard demonstrated that mean GFR by inulin clearance was 10.8 ± 1 mL/min/m^2 in full term neonates during the first several days of life, which doubled during the first 2 weeks

of life .[69] However, the use of inulin for clinical research has largely been replaced with equations that estimate GFR due to the limited availability of inulin. While understood that inulin is considered the “gold standard”, the lack of readily available product and assumption that GFR equations estimate GFR do not adequately address rapid changes in renal function. Contrast agents have been exogenously administered to assess pediatric GFR. Iothalamate and iohexol are the most commonly used agents for measuring GFR, with plasma clearance of the contrast agent serving as a surrogate for GFR.[69] A study by Berg and colleagues demonstrated a correlation between renal inulin clearance and plasma iohexol clearance.[70] In addition to contrast agents, radiolabeled isotopes have been administered to measure GFR. Cr-EDTA clearance is considered accurate and reproducible for measuring GFR in children and adults, utilizing a single blood sample at 2 hours following administration.[71] However, Cr-EDTA is not available within the United States. 99mTc-DTPA renography has been utilized to assess GFR and while it offers the advantage of differential kidney assessment, does not require blood samples, and exhibits a high correlation with Cr-EDTA, there remains concerns regarding its reliability in relation in kidney/skin relationship.[71-72]

In addition to the endogenous biomarkers previously mentioned in this dissertation (e.g., creatinine, cystatin C), beta-trace protein has been investigated as an endogenous marker of GFR.[73] Beta-trace protein is a low-molecular-weight protein that is primarily expressed in the choroid plexus and the human heart.[73] Beta-trace protein concentrations are independent of height, sex, age, and muscle mass. However, beta-trace protein is not considered an “acute phase” biomarker and is does not timely reflect acute changes in GFR. Insulin-like growth factor binding protein-7 (IGFBP-2) and tissue metalloproteinase-2 (TIMP-2) protein markers have been combined to assess renal function. As markers of cell-cycle arrest, the diagnostic test of these 2 combined products appears to identify renal injury that occurred within the previous 12 hours.[65]

Although a commercially available test is available, the routine use of this diagnostic test has not been widely accepted nor studied in the pediatric patient population.

Finally, the adoption of renal maturation and morphogenesis covariables into pharmacokinetic modeling programs has examined the impact that these variables play in pediatric pharmacology. As mentioned previously, Hornik and colleagues have incorporated clinical markers of renal function into pharmacokinetic modeling evaluations.[40] The creation of pediatric bedside modeling programs has grown in popularity (e.g. DoseMeRx™), especially with vancomycin-targeted dosing to achieve an AUC to MIC ratio >400.[74] These “bedside” modeling programs provide dosing recommendations for agents that require therapeutic drug monitoring to achieve targeted concentrations, accounting for such variables as age, weight, serum creatinine, and other cofactors that may alter pharmacokinetic parameters. Thus, it would be expected that continued growth in the utilization of “bedside” pharmacokinetic modeling programs will incorporate physiologic markers (e.g., NGAL) in future pediatric dosing regimens.

6.4 Pharmacogenomics and Renal Function

The emergence of pharmacogenomic testing over the past decade offers the potential to identify polymorphisms that may impact pediatric therapeutic drug monitoring, especially as it relates to hepatic and renal function. Most commercially available pharmacogenomic products offer pharmacokinetic and pharmacodynamic genotyping, with clinically actionable guidelines provided by US and European agencies.[75] While human genotypes do not change, the impact of ontogeny on phenotypic expression does. Kearns and colleagues described the impact of developmental pharmacology on dose and concentration response in pediatric patients, with the maturation of cytochrome P-450 isoenzymes occurring throughout the first 5 years of life.[76] Thus, while a pediatric patient may have the genotype to be an ultra-metabolizer,

the phenotypic expression of the isoenzyme requires developmental maturity to completely express this genotype. For example, tacrolimus is extensively metabolized by the CYP3A4/3A5 isoenzymes. Younger solid transplant patients who receive tacrolimus may have immature enzymatic activity independent of genotype and demonstrate an increased risk of developing tacrolimus-induced renal dysfunction.[74] Thus, use of pharmacogenomic data can not only assist in predicting patients who may develop nephrotoxicity due to medication adverse events, but can also assist in the application of genotyping to metabolic and excretion pathways, co-transport proteins (e.g., organic anion transporter), and pharmacodynamic receptors.

6.5 Renal Function Acuity Index

The future role of renal biomarkers in pediatric pharmacology may employ multiple clinical markers to identify the risk of developing AKI. The establishment of an AKI acuity index utilizes biomarkers and clinical findings to more accurately predict and diagnose AKI. Goldstein and colleagues describe an “AKI Acuity Index” that incorporates a renal angina index in combination with uNGAL measurements to assess potential AKI development.[77] Initial findings from this study demonstrated that a renal angina index composite score >8 and uNGAL concentrations >150 ng/mL accurately identified patients at risk for developing AKI and >10% fluid overload, requiring renal replacement therapy.[77] The creation of multifactorial scoring systems (e.g. acuity indices) can standardized relative risk of developing AKI, leading to prospective medications dosing adjustments as compared to a reactive dosage alterations based upon creatinine clearance. While considerable validation would be required, these acuity indices could identify high-risk patients populations earlier, facilitating dosage adjustments and/or therapeutic drug monitoring that would otherwise be missed.

6.6 Conclusions

As mentioned previously, the ultimate goal of utilizing AKI biomarkers in TDM would be the creation of a “creatinine clearance” equivalent of GFR. Renally-eliminated medications are frequently adjusted based upon creatinine clearance (Schwartz equation) but suffer from the limitations as described previously. The creation of a “biomarker-based GFR calculator” could assist clinicians in the management of pharmacologic alterations during the major stages of renal injury: initiation of the injury, extension of the injury, maintenance of the ongoing injury, and resolution. The **proof-of-concept** associated with this dissertation has demonstrated that the current method for assessing AKI in pediatric patients does not appropriately meet the needs for TDM in critically ill children. There continues to be emerging evidence that renal biomarkers (e.g., uNGAL, pNGAL) can more accurately predict AKI in pediatric patients than creatinine-based equations and that incorporating these diagnostic markers into the routine care delivery model can be translated into better medication use. The interim analysis of our clinical study demonstrates significant inter-subject variability related to the milrinone dose-concentration relationship and underscores the interaction of renal ontogeny, pathophysiology, and cardiac surgery on milrinone clearance. Finally, future bedside pharmacokinetic modeling programs that incorporates covariables known to impact clearance and volume will guide clinicians as to appropriate medication dosing, especially medications requiring TDM. While considerable work remains, there are tremendous opportunities to use renal biomarkers in critically ill pediatric patients for therapeutic drug monitoring.

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