# Long-term chronic kidney disease and hypertension in children previously admitted to the intensive care unit with and without acute kidney injury

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Abbreviations

AKI: acute kidney injury

GFR: glomerular filtration rate

eGFR: estimated glomerular filtration rate

CKD: chronic kidney disease

SCr: serum creatinine

CysC: cystatin C

HTN: hypertension

ICU: intensive care unit

PICU: pediatric intensive care unit

ACR: albumin to creatinine ratio

MA: microalbuminuria

**Abstract**

Acute kidney injury (AKI) is common in hospitalized children and associated with poor health outcomes. AKI leads to chronic kidney disease (CKD) and hypertension (HTN) in adults, however this association is unclear in children. CKD is associated with increased cardiovascular disease and currently, children admitted to the intensive care unit (ICU) are not being followed up for kidney function monitoring. Hypothesis: AKI during ICU stay increases long-term risk for CKD and HTN. Methods: An ongoing study of previously ICU-admitted children from Montreal and Edmonton identified by mailing and from participation in previous studies (exclusions: known pre-ICU renal disease, transplant, dialysis, geographical distance). Protocol: a study visit 6 years±6 months from ICU admission (blood, urine, physical exam, and clinical data collection). Outcomes: CKD (low estimated glomerular filtration rate [eGFR] or high urine albumin/creatinine ratio [ACR]), and pre-HTN or HTN ($\geq 90^{th}$ or $\geq 95^{th}$ age-gender-height blood pressure percentile). The primary exposure is AKI during ICU. Results: 243 children were followed up 5.8+1.1 years post-ICU. Mean age of the study population was 10.6±5.6 years at follow-up. When assuming no AKI in patients without SCr measured during ICU stay, AKI incidence was 25%. At follow-up, 15.5% and 4.6% of all patients had pre-HTN and HTN, respectively. Composite outcome of CKD or pre-HTN in No AKI/AKI Stage 1 vs. AKI Stage 2 or 3 was 34% vs. 68% ($p<0.05$). Composite CKD or HTN in No AKI/AKI Stage 1 vs. AKI Stage 2 or 3 was 22% vs. 54% ($p<0.05$). Patients with AKI Stage 2 or 3 were more likely to develop CKD or pre-HTN (adjusted OR: 3.5. 95% confidence interval: 1.0-12.0). Conclusion: CKD and HTN are common 6 years post-ICU. Patients who develop Stage 2 or 3 AKI in ICU are at higher risk for developing long-term CKD or high blood pressure. This study will help create renal function follow-up guidelines for children after ICU stay.

**Résumé**

L'insuffisance rénale aigüe (IRA) est un phénomène fréquent chez les enfants hospitaliser et qui est associé a une mauvaise santé. L'IRA amène à l'insuffisance rénale chronique (IRC) et l'hypertension artérielle chez les adultes, cependant cette association n'est pas claire chez les enfants. L'IRC est associe avec une augmentation de maladies cardiovasculaires. Actuellement les enfants en soins intensifs ne sont pas objet d'un suivi pour la surveillance de la fonction rénale. Hypothèse : L'IRA acquis pendant un séjour aux soins intensifs augmente le risque de développer l'IRC et l'hypertension artérielle. Protocole : Une étude rétrospective sur les enfants précédemment admis aux soins intensifs de Montréal et d'Edmonton. Patients sont identifiés par la poste et de la participation à des études antérieures (exclusions: maladie rénale connus avant les soins intensifs, la transplantation, la dialyse, la distance géographique). Une visite d'étude est fait à partir de 6 ans après l'admission au soins intensifs (sang, urine, examen physique, et la collection de données cliniques). Définition des résultats : IRC (un faible taux de filtration glomérulaire ou microalbuminurie) et pré-hypertension ou hypertension (≥ 90e ou ≥95e percentiles). L'exposition principale est l'IRA acquis au cours de l'admission aux soins intensifs. Résultats : 243 enfants ont été suivis 5.8±1.l ans après admission aux soins intensifs. L'âge moyen de la population était 10.6 ± 5.6 ans. Lorsqu'on suppose qu'aucun des patients sans SCr mesurées pendant le séjour aux soins intensifs n'est atteint d'IRA, l'incidence d'IRA était de 25%. Lors du suivi, 15.5% et 4.6% de la population étaient atteint de pré-HTN et HTN, respectivement. IRC ou hypertension artérielle dans non-IRA/IRA niveau 1 vs. niveau 2 ou 3 = 34% vs 68% ($p < 0.05$). L'IRC ou HTN dans les non-IRA/IRA niveau 1 vs. IRA niveau 2 ou 3 = 22% vs 54% ($p < 0.05$). Les IRA niveau 2 ou 3 étaient plus susceptibles de développer l'IRC ou la pré-HTN (OR ajusté:3.5, intervalle de confiance de 95% : 1.0 à 12.0). Conclusion: L'IRC et l'hypertension arrivent souvent six années post-soins intensifs, avec une différence significative entre les patients sans IRA/niveau 1 IRA et niveau 2 ou 3 IRA vis-à-vis incidence de, et chances de progression vers, l'IRC ou la pré-HTN. Cette étude permettra de créer des directives de suivi de fonction rénale pour les enfants après un séjour aux soins intensifs.

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**Preface & Contribution of Authors**

This thesis is original work by the author, Kelly Benisty.

The study presented is ultimately based on the methodological and theoretical designs of Dr. Michael Zappitelli and Dr. Catherine Morgan, with contribution of ideas from collaborators Dr. Ari Joffe, Dr. Daniel Garros, Dr. Reginald Sauve and Dr. Adrian Dancea.

Data collection from long-term follow-up visits was mainly performed by the author and by Julie Anne Doucet (RN), as well as Ana Palijan, research coordinator, Erin Hessey, graduate student, registered nurses Nancy Nault , Chantal Dessureault, Sandra Pepin, and Valerie Gagne, with study blood work additionally performed by Matthew Kocal.

Data collection from index PICU admission collected from hospital charts and online medical database was performed mainly by Jessica Ojiaku and Louis Huynh.

Data entry was performed by the author as well as Mike Pizzi, Merna Ghaffar, and Adrienne Kinman.

Main administrative assistance and support was given by Mike Pizzi, with contributions from Merna Ghaffar and Adrienne Kinman.

Data analysis was performed using the statistical software STATA® 12.0 (StataCorp, College Station, TX, USA) by the author with assistance from Dr. Zappitelli.

## Chapter 1*:* Introduction

### *1.1 Overview*

The primary purpose of this thesis is to characterize the long-term renal outcomes of non-cardiac surgery children admitted to the pediatric intensive care unit (PICU) and evaluate AKI as a risk factor for long-term chronic kidney disease (CKD) and hypertension (HTN). AKI is common in hospitalized children and is independently associated with poor hospital outcomes, such as mortality and longer length of stay [1,2]. Common causes of AKI are sepsis, hypotension, nephrotoxic medication use and complex cardiac repair [3]. These conditions lead to an acute reduction in glomerular filtration rate (GFR), leading to disturbances in nutritional and drug metabolism, fluid retention, electrolyte imbalances, and in the worst case, the need for dialysis. AKI is currently diagnosed by a rise in serum creatinine (SCr), which is a marker of GFR. Studies done on adults and using animal models suggest that AKI is a risk factor for long-term renal outcomes, including CKD and HTN[4-6]. CKD and HTN are important risk factors for cardiovascular disease; their pathogenesis begins early in life, underlining the importance of early detection [7,8]. Although the AKI to CKD and HTN disease progression is seen in adults after ICU admission, it is unclear whether AKI is a risk factor for long-term renal abnormalities in children. This lack of knowledge is compounded by the fact that currently, the standard of care is not to follow up children for renal function monitoring post-ICU discharge. Therefore, there is no existing data from which to study the effect of AKI on long-term outcomes. Since CKD and HTN are cardiovascular disease risk factors, it is important to understand if AKI is in fact a risk factor for these renal abnormalities such that we can intervene early and prevent disease progression. It is equally important to understand which ICU or pre-ICU factors play a role in disease progression and whether they are associated with the exposure such that we can properly control for these factors and inform clinicians on who to follow up post-ICU discharge.

1.2 *Thesis hypotheses and objectives*

The overall hypotheses for my thesis project are:

1. CKD and HTN are prevalent at 6 years after pediatric ICU admission.
2. The presence of AKI during pediatric ICU stay increases the long-term risk for CKD and HTN.

The specific objectives of my thesis project are:

1. To review the current state of knowledge surrounding AKI and renal outcomes (CKD, estimated GFR, albuminuria, and HTN) in children, as well as existing evidence on the link between AKI and renal outcomes.
2. To estimate the prevalence of CKD, pre-HTN and HTN, 6 years after pediatric ICU admission.
3. To examine the relationship between AKI during ICU stay and CKD or HTN at 6 years following ICU admission.
4. To evaluate other non-AKI factors that may be associated with AKI and/or long-term renal outcomes.

*1.3 Outline of thesis*

Chapter 2 of this thesis presents a literature review on AKI epidemiology and outcomes, and its potential relationship with renal outcomes (CKD and HTN). It also presents a review on CKD and HTN in children. Chapter 3 is a review article on measuring and estimating GFR, one of the main outcomes of the current work, in children. This article was written by the author of this thesis and published in *Current Pediatrics Report* in December 2014. In chapter 4, the methodology of the thesis work is described in detail. Chapter 5 consists of a manuscript describing the AKI to CKD and HTN relationship in children previously admitted to the ICU. Finally, chapter 6 concludes the thesis with an overall review of findings, the importance of results found, and future directions. Of note, some portions of this thesis will be repetitious to some extent. Methods described in chapter 4 will be abridged in the methods sections of the manuscript in chapter 5, and the discussion of the manuscript will be repeated and elaborated on in chapter 6, the conclusion chapter.

## Chapter 2: Literature review

### *2.1 AKI is common in the pediatric intensive care unit*

Acute kidney injury (AKI) is the clinical syndrome of an abrupt decline in renal function, which typically occurs in hospitalized patients. With AKI, GFR decreases and other functions of the kidney become impaired, the extent to which depends on the severity of the injury. AKI may be associated with reduction in urine output and resulting fluid overload (edema), HTN, electrolyte and acid-base balance disturbances, abnormal vitamin D metabolism and reduction in production of erythropoeitin. Moreover, nutrition provision of children with AKI is complicated by the inability to provide adequate fluids or certain nutrients, and dosing drugs excreted by the kidney in AKI is complicated, leading to both under- and over-dosing of drugs. In very severe cases of AKI, renal replacement therapy (or dialysis) may be required. Thus, the presence of AKI in hospitalized children substantially complicates medical management.

AKI in hospitalized children most commonly occurs in patients admitted to the intensive care unit (ICU), associated with other non-renal disease processes leading to kidney cell injury or "secondary" renal injury. Primary renal diseases (e.g., glomerulonephritis, urinary stones or hemolytic-uremic syndrome) as causes of AKI are actually quite rare[9,10]. The reported incidence of AKI in non-cardiac patients admitted to the ICU ranges widely, from 0.85% [11] to 82% in the most critically ill children [1]. This variable reported incidence is due to differences in AKI definition used, with the most conservative definitions (e.g., defined by need for dialysis) leading to the lowest reported incidence. The most common causes of AKI are conditions which lead to renal tubular cell injury or acute tubular necrosis (ATN, described in section *AKI pathophysiology* below), including sepsis, hypotension, multiple organ dysfunction syndrome, nephrotoxic medication administration, and complex cardiac repair [12,13]. AKI incidence in the ICU is strongly associated with overall illness severity, as shown by studies demonstrating that higher Pediatric Risk of Mortality (PRISM) score (an illness severity score), requiring mechanical ventilation and documented infection are strongly associated with AKI development [1,2,14] .

Children undergoing cardiac surgery represent up to 40% of patients admitted to pediatric ICU in some centres. They are at very high risk for AKI development, with reported incidence of post-operative AKI ranging from 20% to as high as 88% [15-17]. Neonates are at particularly high risk for post-operative cardiac surgery AKI [18,19]. Risk factors for developing cardiac surgery AKI include longer cardiopulmonary bypass time, longer cross-clamp times, higher inotropic score, surgery type, hypothermic circulatory arrest, lower gestational age, and preoperative ventilation[16,20,21] . As indicated above, all of these medical conditions and identified risk factors contribute to renal cell damage, via different mechanisms, which ultimately lead to ATN, GFR reduction and the clinical syndrome of AKI.

*2.2 AKI pathophysiology*

AKI typically involves structural damage to renal tissue, acute tubular necrosis (ATN), often caused by ischemia due to the conditions mentioned above. In ATN, renal blood flow is reduced primarily due to endothelial injury, which results in an imbalance of local vasoactive substances; intra-renal vasoconstriction is enhanced while vasodilation is reduced, and oxygen and substrate delivery to the tubule cells is decreased[22]. ATN also entails changes in renal tubular fluid dynamics, specifically obstruction of tubular fluid flow, movement of the glomerular filtrate back into the circulation and activation of tubuloglomerular feedback, resulting in a subsequent decrease in GFR [23]. Early after ischemic renal injury, there is a drop in intracellular ATP causing metabolic disturbances and alterations in the cytoskeleton[24]. Subsequent reperfusion induces generation of reactive oxygen species causing oxidative cell damage[22]. After ischemic injury in ATN, there is cell death, cell sloughing, dedifferentiation of tubular cells, proliferation of surviving cells and finally repair, where cells up-regulate various growth factors and re-differentiate until the epithelium is restored[22]. The processes of cell death involve two pathophysiologic mechanisms: the first being necrosis, characterized by cell membrane degradation, nuclear shrinkage, and cytoplasmic swelling and inflammation, and the second being apoptosis, described as cytoplasmic and nuclear shrinkage, DNA fragmentation and cell breakdown into

apoptotic fragments which are removed via phagocytosis[25] . The pathogenesis of ischemic ATN also involves an inflammatory response which consists of endothelial injury, recruitment of leukocytyes to the injury site, and consequent production of inflammatory mediators by tubule cells such as tumor necrosis factor-a , interleukin (IL)-6, IL-1b , and chemotactic cytokines[22]. Indeed, it has been demonstrated that inflammatory cytokine levels predict mortality in patients with AKI[26]. As described above, ischemia therefore not only causes intra-renal hemodynamic alterations, but also inflammatory and oxidative damage. Therefore, disease conditions which lead to inflammation or increase in local oxidative stress may also cause ATN even in the absence of overt renal ischemia, such as systemic inflammatory response (e.g. sepsis), nephrotoxic medication administration or rhabdomyolysis. In the pediatric ICU, multiple causes of ATN are often present (e.g., ischemia due to hypotension with administration of nephrotoxic antibiotics), sometimes complicating the ability to identify the exact etiology of AKI in a given patient. However ultimately, the final pathway is ATN, with GFR reduction and the resulting multiple management challenges.

*2.3 AKI in the pediatric ICU is associated with poor hospital outcomes*

Given the multiple complications associated with AKI described above, it is not surprising that many studies have consistently shown that AKI is independently associated with a number of hospital outcomes such as mortality, longer length of hospital and ICU stay, and need of prolonged mechanical ventilation (Thesis Table 1[2,14,16,21,27-34]). In children undergoing cardiac surgery, AKI is additionally associated with prolonged recovery after heart surgery [35]and increased occurrence of postoperative complications [36]. A recent pooled analysis of pediatric AKI studies demonstrated that regardless of the AKI definition used, the relationship between AKI and poor hospital outcomes is strong and consistent[37]. It is noteworthy that most of these studies showing the important negative impact of AKI on poor hospital outcomes have been published in the last 10 years, largely due to the fact that it is only in 2004 that international efforts were initiated to standardize AKI definition to allow for the valid study of AKI across studies and patient populations.

**Thesis Table 1. Recent studies[a] (last 5 years) showing negative health outcomes of AKI in the ICU**

| Author, Year | AKI definition | Sample size | Population type | AKI incidence | Outcomes |
|---|---|---|---|---|---|
| Miklaszewka, 2014[28] | pRIFLE | 25 | All PICU patients | 1.2% | Mortality [40%] |
| Mamikonian, 2014[16] | pRIFLE | 40 | Cardiac ICU- cardiac surgery with cardiopulmonary bypass | 88% | N/A |
| Gil-Ruiz, 2014[29] | pRIFLE | 409 | PICU- Cardiopulmonary bypass surgery | 20% | Longer PICU length of stay [12 day], need of prolonged mechanical ventilation [61%], mortality [14.6%] |
| Soler, 2013[30] | pRIFLE | 266 | PICU (excluded patients with pre-existing end-stage renal disease or renal transplantation, included admission≥24 hours and age between 1 month and 21 years) | 27.4% | Mortality[9%], longer hospital [20 days] and PICU [22 days] length of stay |
| Ricci 2013[21] | pRIFLE | 160 | Cardiac PICU- cardiopulmonary bypass, children younger than 1 year | 56% | Longer PICU length of stay [4 days] and prolonged mechanical ventilation [2 days] |
| Totapally 2013[31] | Decrease in eGFR of 50% or more from baseline | 284 | PICU- treated with vancomycin | 17.2% | Mortality [22.45%] |
| Martin, 2013[27] | pRIFLE | 1496 | PICU (excluded neonates, cardiac surgery patients, and those with pre-existing renal | 4.4% | Mortality [44%] |

| | | | | | |
|---|---|---|---|---|---|
| | | | dysfunction) | | |
| Krishnamurthy, 2013[32] | AKIN | 165 | PICU and non-critically ill children | 25% | Mortality [46.2%], partial renal recovery [17.5%] |
| Hui ,2013[33] | pRIFLE | 140 | PICU (excluded neonates) | 56% | Mortality [21%] |
| Alkandari, 2011[2] | AKIN | 2,106 | PICU (excluded cardiac surgery and renal transplant patients, included those admitted ≥ 12 hours and at least one night) | 18% | Mortality[10.3%], longer PICU length of stay [9.7 days] and required mechanical ventilation[60%] |
| Selewski 2014[34] | KDIGO | 3,009 | PICU and cardiac ICU (excluded end-stage renal disease, new renal transplant, missing PRISM III data, or no measured SCr during ICU admission) | 24.5% | Longer PICU length of stay [214 hours], prolonged mechanical ventilation [3 days] and mortality [11.3%] |
| Bresolin 2013[14] | pRIFLE | 126 | PICU and non-critically ill patients (excluded neonates, CKD and dialysis patients) | 46% | Mortality[14.3%], longer hospital and PICU length of stay[?], higher illness severity score [Pediatric Risk of Mortality index score=7.2] |

[a]*Review using Medline via Ovid search (search term: acute kidney injury/epidemiology, limited to all children and past 5 years). List of studies is non-exhaustive.* pRIFLE: pediatric Risk, Injury, Failure, End Stage Renal Disease definition (AKI defined as ≥25% reduction in estimated glomaruler filtration rate); AKIN: Acute kidney Injury Network Definition (AKI defined by ≥50% rise in Scr from baseline or a 27 umol/L increase in Scr); KDIGO: Kidney Disease Improving Global Outcomes (AKI defined by a ≥50% rise in Scr)

*2.4 AKI definition*

The first standard definition of AKI was proposed in 2004 by the Acute Dialysis Quality Initiative group, in an effort to use a consistent AKI definition when comparing findings across studies[38]. Before then, many studies used "need for dialysis" as the main

definition for AKI; that was problematic as it only identified the most severe, rare cases and did not allow for the evaluation of epidemiology and outcomes across the spectrum of severity of renal injury. Since then, there has been a concerted international effort to define AKI using SCr and urine output. This process has been iterative and progressive. Three main AKI definitions have been proposed. Each of these definitions are similar, in that they provide a graded AKI severity staging, based on either rise in SCr, decrease in estimated GFR or decrease in urine output. The first definition was an AKI classification system termed the Risk, Injury, Failure, Loss, End-Stage Kidney Disease (RIFLE) criteria [39], as follows: RIFLE R or "Risk" (SCr increase of 1.5 times baseline or 25% eGFR decrease from baseline); RIFLE I or "Injury" (SCr increase 2 times from baseline or 50% eGFR decrease from baseline); RIFLE F or "Failure" (SCr tripling from baseline or 75% eGFR decrease or SCr level ≥4 mg/dl) ; RIFLE L or "Loss": persistent loss of renal function for over four weeks; RIFLE E "End-stage renal disease". A pediatric version of the RIFLE definition was also proposed, which defined acute changes in estimated GFR, rather than acute changes in SCr [1]. However, this definition lost favour, since estimating GFR in children requires height measurement, which is often not performed or inaccurate in critically ill children. The AKIN definition [38] has the same SCr rise strata as RIFLE, except the names for the strata are stages 1, 2 and 3 instead of RIFLE R, I and F. Also, AKIN stage 1 can alternatively be defined by a ≥26.5 μmol/L rise in SCr from baseline, and those treated with dialysis are classified into stage 3 of AKIN, regardless of change in SCr. Furthermore, AKIN criteria require change in SCr to occur within 48 hours. Finally, the KDIGO (Kidney Disease: Improving Global Outcomes) AKI definition was proposed in 2012 and is now the internationally accepted AKI definition. This definition is shown in the Table (Thesis Table 2) below. The KDIGO definition is advantageous in that it incorporates features of the past RIFLE and AKIN definitions, and also has some pediatric-specific components.

**Thesis Table 2. KDIGO classification of AKI[40]**

| Stage | Creatinine criteria | Urine output criteria |
|---|---|---|
| **1** | 1.5–1.9 times baseline<br>Or ≥0.3 mg/dl (≥26.5 mol/l) increase | <0.5 ml/kg/h for 6–12 h |
| **2** | 2.0–2.9 times baseline | <0.5 ml/kg/h for ≥12 h |
| **3** | 3 times baseline or<br>≥4.0 mg/dl (≥353.6 mol/l) increase<br>Or initiation of RRT<br>Or in patients <18 years, a decrease in eGFR <35 ml/min/1.73 m2 | <0.3 ml/kg/h for ≥24 h<br>or<br>anuria ≥12 h |

*RRT*: renal replacement therapy. *eGFR*: estimated glomerular filtration rate

Each of these definitions includes similar staging for increases of SCr from 1.5 to 3-fold increases from baseline. These definitions also include changes in urine output, however there has been significant work showing that low urine output is not a sensitive parameter for AKI diagnosis[28] ; changes in urine output in addition to SCr adds little to AKI ascertainment and to the association between AKI and outcomes [1,41], thus most studies base AKI on SCr criteria. Despite these small differences in recent AKI definition methods, a meta-analysis that pooled studies using KDIGO-equivalent AKI definitions demonstrated that 1 in 3 children worldwide experience AKI during a hospitalization episode[37]. Furthermore, independent of the definition used, the relationship between AKI and poor health outcomes is always significant (Thesis Table 1).

An important challenge in reporting epidemiology of AKI relates to ascertaining of AKI status. Baseline SCr (or SCr from prior to acute illness) is needed to calculate the relative rise, however it is not always available in hospitalized patients. In order to estimate baseline SCr when it is unknown, the Acute Dialysis Quality Initiative recommended to assume normal estimated GFR and to use GFR prediction equations (which incorporate SCr) to back-calculate what normal baseline or "non-illness" SCr would be in a given patient. In children, the most widely used GFR equation is the

Schwartz equation (or the "CKiD" equation). The Schwartz GFR equation includes not only SCr, but also height. Therefore, when height of the child is also unknown, this poses a second challenge in estimating baseline SCr. Different methods have been proposed for estimating baseline SCr when both SCr *and* height are unknown, such as using normative SCr values for age and gender, and more recently, using height-independent GFR equations to estimate baseline SCr. The height-independent equation used in the analysis of the current work to estimate baseline SCr when unknown, is described in more detail in the published review article (Chapter 3). It must be acknowledged that using these surrogate measures of baseline SCr may lead to misclassification of AKI and thus improved methods of estimating baseline SCr are needed[42-44].

In summary, because of the vast amount of research performed to develop and refine AKI definition, AKI epidemiology, risk factors and hospital outcomes have been well characterized and the important association between AKI and hospital outcomes has been clearly demonstrated. However, increasing evidence is showing that AKI is not only associated with short-term outcomes, but also long-term sequelae.

*2.5 AKI may lead to chronic kidney disease (CKD) and hypertension (HTN): evidence from animal models and in adult patients.*

Although it is clear that AKI has negative short-term consequences, in animal models and adult human studies, AKI has been shown to have negative long-term consequences as well, namely progression to chronic kidney disease (CKD) and hypertension (HTN) [45,46]. Although ATN, caused by AKI, is followed by proliferation of survivor cells, recovery may be incomplete[47]. The pathogenic link between AKI and CKD as suggested by animal models may include capillary loss due to alterations in endothelial cells[48] , progressive interstitial fibrosis and damage to functioning nephrons[49,50] . Fibrosis is the formation of excess connective tissue and extracellular matrix, also known as scarring, which impairs function of the underlying organ. Ischemic AKI in rats has been shown to stimulate the regulation of fibrogenic factors such as transforming growth factor (TGF)-b and extracellular matrix genes [51-53] . Early on in renal injury, the renal interstitium recruits inflammatory leukocytes and myofibroblasts,

which are intermediary cells between a fibroblast (extracellular matrix and collagen synthesizing cell) and a smooth muscle cell. The tubules, inflammatory cells and myofibroblasts synthesize the molecules that activate the fibrogenic factors (e.g., TGF-b) [54,55]. Excess extracellular matrix production ensues, and renal matrix-degrading protease activity is impaired, enhancing matrix accumulation[56] . The fibrosis is progressive since once the scarring is deposited, it increases the distance for diffusion to the functioning renal tissue, promoting further hypoxia and fibrosis [48]. Eventually the scarring propagates enough to cause functional kidney damage, leading to CKD.

Not only is the AKI-CKD relationship well documented in animal models, but epidemiological studies done in adults also demonstrate the AKI to CKD progression. It is clear from these studies that AKI is an independent risk factor for incident CKD and progression to end-stage renal disease[57]**.** A systematic review and meta-analysis including 13 studies in adults with follow-up ranging from 6 to 75 months, showed that, after adjusting for important confounders, survivors of AKI have a greater risk for CKD, end-stage renal disease and other adverse outcomes compared with non-AKI patients[46]. Furthermore, the risk for CKD in adults with AKI was enhanced as severity of AKI increased. In adults, the relation between AKI and CKD progression was shown to be modified by the presence of pre-existing proteinuria, which is a measure of pre-AKI CKD [46]. This is important to differentiate in children, in whom underlying CKD (pre-AKI CKD) is actually very rare. Number of repeated AKI episodes has also been shown to be associated with the development of incident CKD [58,59], strengthening the human evidence that AKI causes permanent renal cell damage. Hospitalized patients are likely exposed to multiple AKI-provoking events during their illness, which may worsen AKI and lower the chance of recovery[60,61]. Finally, adults with AKI have been repeatedly shown to be at increased risk for other non-renal long-term outcomes, such as major cardiovascular events, re-hospitalization and mortality [46,57,62]. It is postulated that the association of AKI with non-renal cardiovascular outcomes may be related to the fact that CKD is a very important and acknowledged risk factor for cardiovascular disease and mortality[8,63].

*2.6 The long-term outcomes of AKI in children*

Although the progression from AKI to CKD is well characterized in adults, it is unclear whether AKI leads to CKD in children. A few studies have demonstrated that the prevalence of CKD and HTN at variable follow-up periods is high in children who have had AKI in the past (Thesis Table 3). Considering that the Canadian child population pre-HTN and HTN prevalence is 2.1% and 0.8%, respectively [64], the data shown in Table 3 below shows a clinically significant difference between the general child population and children with past AKI. Similarly, prevalence of microalbuminuria (a marker of CKD) in the general pediatric population is approximately 11.8% [65], in contrast to the higher prevalences of microalbuminuria shown below in children with past AKI. These studies contribute to the understanding of renal outcomes in children ***who have had*** AKI; however there are some methodological issues to consider before stating that AKI leads to CKD in children. The AKI definitions used were not all standardized and almost all studies have not evaluated renal function in non-AKI patients. Thus, the association between the presence of AKI and future renal outcome has essentially not been evaluated. Only Zwiers et. al. considered the non-AKI patients in order to evaluate the odds of developing CKD and HTN after an episode of AKI[66] . However due to the specific population studied (neonatal extracorporeal membrane oxygenation survivors), results cannot be extrapolated to the general PICU population. Data on the long-term renal consequences of AKI from all causes, independent of previous renal impairment, are almost non-existent in children. A longitudinal follow-up study of both AKI and non-AKI pediatric patients is needed in order to demonstrate with greater certainty that AKI independently leads to negative renal outcomes. Once this is shown, we will be able to suggest renal function follow-up guidelines and actively attempt to reduce cardiovascular risk through early CKD treatment, since such guidelines currently do not exist. Conversely, recommending follow-up of renal function after ICU admission, without having the evidence to support such a drastic change in health care, could be a burden to the health care system and to patients.

**Thesis Table 3. Representative pediatric studies providing information on long-term renal function follow-up after different types of AKI in either PICU or general hospital-admitted children.**

| Author, Year<br>Study design (N) | **Population**<br>**Follow-up time** | **AKI definition** | **Schwartz GFR<90** ml/min/1.73m$^2$ | **BP>95$^{th}$ percentile** | **Alb/ creat>30** |
|---|---|---|---|---|---|
| Slack, 2005[21]<br>Prospective (12) | Meningococcemia<br>Median=4.2 years | Dialysis need | **25%** | **25%** | N/A |
| Askenazi, 2006[67]<br>Cross-sect (45) | Hospitalized<br>3.7±0.9 years | Discharge summary GFR<75 ml/min/1.73m$^2$ | **44%** | **21%** | **28%** |
| Buysse, 2008[19]<br>Cross-sect (16) | Meningococcemia<br>Median = 9.8 years | SCr doubling | **8.3%** | **18.8%** | **18.8%** [a] |
| Sinha, 2009[22]<br>Prospective (24) | Hospitalized<br>6 months | Admission Diagnosis | N/A | **18%** | N/A |
| Viaud, 2011[68]<br>Retrospective (13) | PICU<br>Median=16 years | RRT | **61%** | **15%** | **54%** [a] |
| Mammen, 2012[20]<br>Retrospective (126) | PICU, including cardiac surgery and other<br>1-3 years | AKIN definition | **49%**[b] | **3%** | **10%** |
| Zwiers, 2014[66]<br>Cross-sect (169) | Neonatal extracorporeal membrane oxygenation survivors<br>Median= 8.2 years | RIFLE classification | **5%** | **20.8%** | **15%** [a] |

Table was derived by both Dr. Zappitelli and the author of the present work using Pubmed and Medline via Ovid searches. [a] proteinuria: protein/creatinine ratio>20 mg/mmol;; [b]GFR measurement using reference standard plasma disappearance study . *BP*: blood pressure; *Alb*: albumin; *Creat*: creatinine; *RRT*: renal replacement therapy; *Tx*: transplant. *PICU*: pediatric intensive care unit. *RIFLE*: Risk Injury Failure Loss End-stage renal disease. *AKIN*: Acute Kidney Injury Network

*2.7 CKD and HTN are associated with poor health outcomes in children*

Childhood CKD is known to be an important risk factor for cardiovascular disease and its many negative health outcomes are well established. Pediatric patients with severe CKD have a significantly greater risk of mortality, with cardiovascular disease accounting for 25–50% of deaths in children and young adults with childhood onset CKD[69-71]. Pediatric CKD is associated with a number of complications and structural changes to the heart, including left ventricular hypertrophy, subclinical diastolic

dysfunction, increased carotid intima media thickening, coronary artery calcification, HTN, acidosis, anemia, and fluid overload as well as increased cardiovascular disease events such as myocardial infarction [7,8,72,73]. Extensive work has been done on the health outcomes of children with CKD. The CKiD (Chronic Kidney Disease in Children) study, a prospective cohort study of 586 children with an estimated glomerular filtration rate (eGFR) of 30-90 mL/min/1.73m$^2$, (normal GFR being above 90 mL/min/1.73m$^2$) has helped to identify risk factors for CKD progression and outcomes in children with CKD. The CKiD study found HTN (54%) and dyslipidemia (45%) to be common in their cohort, with higher prevalence of left ventricular hypertrophy in children with HTN. Children with lower GFR were at a greater risk of dyslipidemia. Increasing levels of proteinuria (a marker of CKD) were also associated with an increased prevalence of dyslipidemia. This study also reported that children with CKD had poorer overall Health-Related Quality of Life scores and poorer physical, school, emotional, and social domain scores compared with healthy children. Indeed, growth impairment is common in children with CKD and has been documented to negatively affect psychosocial development and quality of life in adults with child-onset CKD[74]. The CKiD cohort also demonstrated poorer neurocognitive function, and scoring below normative data on measures of IQ, academic achievement, attention regulation and executive function[73]. Not only is low GFR associated with poor outcomes, but microalbuminuria, the second marker of CKD, has also been established as a risk factor for cardiovascular disease morbidity and mortality[75 76]. CKD-associated cardiovascular disease pathogenesis in children begins early in life with exposure to an atherogenic environment. This speaks to the importance of prompt identification of risk factors for CKD and early CKD detection in order to allow for monitoring and intervention.

*2.8 Measuring renal outcomes in children*

2.8.1 GFR

One of the main renal outcomes I evaluate in my work on long-term AKI outcomes is GFR. It is important to clearly delineate how GFR is measured and estimated in children in order to have as accurate an outcome measure as possible for

my research and to also understand the potential limitations of this outcome. A comprehensive published review article is included in the following chapter on GFR measurement and estimation in children, written by the author of the current work.

2.8.2 Albuminura

Plasma proteins are generally not filtered through the glomerular filtration barrier when blood flows through the glomerulus, because of their large size. However, a small amount of plasma protein does appear in the urine via filtration. This filtered protein is normally reabsorbed by the proximal tubular renal cells and broken down into component amino acids[77]. Proteinuria refers to increased concentration of proteins in the urine above the normal threshold. This condition may occur due to glomerular injury, wherein the glomerulus is permeable to large molecular weight proteins such as albumin. Additionally proteinuria may occur due to tubular injury. With tubular injury (whether acute, with ATN, or chronic tubular injury) there is incomplete tubular reabsorption of normally filtered proteins; there may also be abnormal loss of intracellular renal tubular cell proteins due to tubular damage and death. Albuminuria refers specifically to the abnormally high amount of the plasma protein albumin in the urine. Recently, the clinical and research focus on proteinuria in kidney disease, has been on albuminuria, rather than general proteinuria, because albumin is the main urinary protein excreted in excess in most kidney diseases. Moreover, recent epidemiologic data have demonstrated a strong graded relationship between the degree of albuminuria and risk of mortality, kidney outcomes and cardiovascular disease at all levels of GFR[78].

Children normally have urinary protein concentrations comparable to adult levels by about 2 to 4 years old. At younger ages, children have *higher* urinary losses of both glomerular and tubular proteins due to lack of proximal tubule and glomerular maturation[78]. Another factor specific to young patients, which complicates interpretation of urine protein concentrations, is orthostatic (or postural) proteinuria, referring to physiologic increase in urinary protein concentrations occurring with the ambulatory position and resulting physiologic increase in GFR. Orthostatic proteinuria is

reported to occur in at least 2-5% of adolescents[79] and is not thought to be pathologic. Urinary concentration, related to level of hydration, may also affect interpretation of urine protein concentration. In order to correct for level of hydration, urinary albumin concentration is usually expressed as a ratio to the urine creatinine concentration because creatinine excretion is considered to be fairly constant throughout the day[80,81]. Overt albuminuria is defined as a urine albumin to creatinine ratio (ACR) above 3 mg/mmol and is currently a definition criterion for the presence of CKD, according to the recent KDIGO evidence-based international guidelines. This definition overlaps with the term 'microalbuminuria', which refers specifically to an ACR between 3 and 30 mg/mmol. In children, albuminuria is considered a marker of chronic renal damage as well. For example, children with primary HTN have increased prevalence of micoalbuminuria compared to children with white coat HTN (which is not considered pathological)[82]. Evaluation of ACR is thus a major component of evaluating for renal damage, the presence of CKD and evaluating the consequences of conditions that lead to renal damage.

2.8.3 HTN and blood pressure (BP) measurement

Elevated BP has a strong relationship with progression of CKD and its treatment is critical in caring for children with CKD, regardless of the underlying cause of the disease[83] . Studies done in children suggest that persistent elevated BP is associated with long term sequelae, specifically left ventricular hypertrophy and increased carotid intimal-media thickness, increased risk of developing kidney failure, decreased carotid artery elasticity [84,85]. In healthy children, because there is no data linking specific BP cut-offs with adverse cardiovascular or renal events, the HTN definition is established from a population-based normative distribution of BP. The BP percentiles are gender, age and height-specific, with three main categories[86]:

1. Normal BP – Both systolic and diastolic BP <90$^{th}$ percentile.
2. Pre-HTN – Systolic and/or diastolic BP ≥90$^{th}$ percentile for age, gender, and height but <95$^{th}$ percentile or if BP exceeds 120/80 mmHg (even if <90$^{th}$ percentile for age, gender, and height).

3. HTN –defined as either systolic and/or diastolic BP ≥95$^{th}$ percentile for age, gender and height measured on three or more separate occasions. The degree of HTN is further delineated by the following two stages:
    a. Stage 1 HTN – Systolic and/or diastolic BP between the 95$^{th}$ percentile and 5 mmHg above the 99$^{th}$ percentile.
    b. Stage 2 HTN – Systolic and/or diastolic BP ≥99$^{th}$ percentile plus 5 mmHg.

Current recommended method of BP measurement includes the use of an oscillometric device. When BP exceeds 90$^{th}$ percentile, measurement should be repeated by manual auscultation. In order to ensure maximum accuracy, an appropriate cuff size should be used and high BP readings must be confirmed on repeated visits [87]. HTN in children might be masked and not identified during outpatient measurements alone, thus home BP measurements or 24 hour ambulatory blood pressure monitoring (ABPM) may be useful to identify masked HTN. In fact, it has been shown that using ABPM in patients with CKD may give a better measure of overall BP and kidney disease progression than casual office BP readings [88].

2.8.4 CKD definition

The KDIGO definition of CKD in children includes criteria for GFR and albuminuria, as well as staging classification based on cause, GFR and albuminuria (Thesis Figure 1 and Figure 2). In adults, CKD is defined as either GFR<60min/1.73 m$^2$ or ACR>3mg/mmol present for >3 months. However, normal GFR is considered to be above 90 ml/min/1.73m$^2$. In adults, the choice of cut off of 60 ml/min/1.73m$^2$ to define CKD is based on a) the high prevalence of GFR between 60 and 90 ml/min/1.73m$^2$ in older adults due to physiologic decrease in kidney function with age[89-92], and b) the demonstration that GFR <60 ml/min/1.73m$^2$ is associated with increased cardiovascular disease risk[93-95]. However, healthy children should generally have GFR above 90 ml/min/1.73m$^2$, since there should not be a physiologic decline in renal function early in life. Thus, most studies in children define CKD as GFR < 75 to 90 ml/min/1.73 m$^2$ [65-67,73]. The GFR criterion also does not apply to infants <2 years of age, because GFR physiologically increases in early life until age 2[96,97]. Thus age-normative GFR values

should ideally be used to define CKD in children less than 2 years old, though this is rarely performed in published literature[98,99]. Finally, the criteria of duration of >3 months cannot apply to newborns or infants <3 months of age. It is crucial to note that CKD is not easily detectable in its early stages, since children will remain asymptomatic until very late in the course of CKD[78]. It is for this reason that early and targeted monitoring is important in order to identify patients at risk for progression to CKD, such that intervention may occur as early as possible, potentially preventing further renal damage.

**Thesis Figure 1. GFR staging categories for CKD according to KDIGO[40]**

**GFR categories in CKD**

| GFR category | GFR (ml/min/1.73 $m^2$) | Terms |
|---|---|---|
| G1 | ≥90 | Normal or high |
| G2 | 60-89 | Mildly decreased* |
| G3a | 45-59 | Mildly to moderately decreased |
| G3b | 30-44 | Moderately to severely decreased |
| G4 | 15-29 | Severely decreased |
| G5 | <15 | Kidney failure |

Abbreviations: CKD, chronic kidney disease; GFR, glomerular filtration rate.

*GFR*: glomerular filtration rate; *CKD:* chronic kidney disease

**Thesis Figure 2. Albuminuria staging categories for CKD according to KDIGO[40]**

**Albuminuria categories in CKD**

| Category | AER (mg/24 hours) | ACR (approximate equivalent) (mg/mmol) | ACR (approximate equivalent) (mg/g) | Terms |
|---|---|---|---|---|
| A1 | <30 | <3 | <30 | Normal to mildly increased |
| A2 | 30-300 | 3-30 | 30-300 | Moderately increased* |
| A3 | >300 | >30 | >300 | Severely increased** |

Abbreviations: AER, albumin excretion rate; ACR, albumin-to-creatinine ratio; CKD, chronic kidney disease.

*AER*: albumin excretion rate; *ACR*: albumin-to-creatinine ratio; *CKD*: chronic kidney disease

2.8.5 Current standards of evaluating GFR

Cys C: a better marker of GFR than SCr

As described in the review article in the following chapter, gold standard methods for measuring GFR are time consuming, expensive and relatively invasive. GFR

is therefore estimated using serum markers of glomerular filtration. Currently, SCr is the routine diagnostic test of renal function. In order to estimate GFR from SCr, validated SCr-based prediction equations are used (hereafter referred to as SCr-eGFR)[100]. However SCr poses challenges for estimating GFR in children. This is because SCr, which is produced by the muscles, is affected by muscle mass, gender and growth [101]. Furthermore, kidney handling of SCr may be affected by diet and medication, among other factors[102] ; thus other GFR markers have been studied. Cystatin C (CysC), a small molecular weight protein excreted by glomerular filtration, has been shown to be a more accurate and sensitive marker of detecting mild renal dysfunction, than SCr is[103-105] . Therefore, GFR estimated by CysC equations (CysC-eGFR) may be more accurately related to other renal abnormalities, like microalbuminura and HTN, than SCr (SCr-eGFR) is and may be more likely associated with long term renal outcomes. Though this seems logical, this has never been shown. Recently, equations have been derived which combine both SCr and CysC [102,106]. These equations appear to be most accurate for estimating GFR. A large study done in children with CKD confirmed that incorporating SCr and CysC in eGFR equations leads to more accurate GFR estimation in children with CKD[107] . Therefore, GFR estimated by combined SCr and CysC (SCr-CysC-eGFR) may be more strongly related to other renal abnormalities, like microalbuminura and HTN, than SCr (SCr-eGFR) is and may be more likely associated with long term renal outcomes. If SCr-CysC-eGFR more strongly relates to other renal abnormalities, then it is likely a more valid measure of renal function and can be used as a screening tool for CKD when evaluating the long-term renal outcomes of AKI during ICU stay.

*2.9 Summary: main knowledge gaps*

In summary, although there is mounting literature surrounding AKI diagnosis and progression to CKD, there exist several key knowledge gaps that need to be addressed. There is no published data on the expected long-term renal outcomes of *all* children who have been admitted to ICU and it remains unclear whether AKI is a risk factor for long-term renal abnormalities in children. This lack of knowledge is compounded by the fact that currently, the standard of care is not to follow up children for renal function

monitoring post-ICU discharge. Therefore, there is no existing data from which to study the independent effect of AKI on long-term outcomes. Since CKD and HTN are cardiovascular disease risk factors, it is important to understand if AKI is in fact a risk factor for these renal abnormalities such that we can intervene early on and prevent disease progression. The following project aims to address these important knowledge gaps, provide evidence upon which to base recommendations for post-ICU renal follow-up and ultimately improve the long-term outcomes of critically ill children.

## Chapter 3: Measuring GFR in Health and Disease

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### *3.1 Preamble to this published literature review*

In order to have a comprehensive understanding of this project, it is important to amass sufficient knowledge on the main exposure and outcomes, as well as the issues surrounding their measurement. For this reason, an in-depth review was performed on measuring GFR in children. GFR is one of the main outcomes of this project and defining the best method of measuring, as well as estimating, this parameter plays a critical role in developing the methodology of the current work. One especially relevant highlight of this review article is the discussion on use of SCr for renal function measurement. SCr is used to not only define AKI, the main exposure of my hypotheses, but is also the main variable used in eGFR equations defining my outcome. The review article below is a critical analysis and narrative review of measuring renal function in children, which greatly contributed to deciding and confirming how renal outcome was defined for the current thesis project and how estimated baseline SCr was defined for AKI definition.

### *3.2 The manuscript*

#### 3.2.1 Introduction

Glomerular filtration rate (GFR) is the best overall index of kidney function. GFR assessment is the foundation for diagnosing renal disease in clinical practice. In

hospitalized children, GFR evaluation plays a crucial role in ensuring correct medication doses, monitoring for nephrotoxicity, and determining pre-hospital renal function. In children with early CKD or being screened for CKD, reduced GFR may be the only sign of kidney damage. In patients with established CKD, GFR evaluation is vital for monitoring kidney disease progression. Therefore, accurate methods to evaluate GFR in children are needed.

Direct or gold standard measurement of GFR, performed by measuring clearance of exogenously administered substances, is often infeasible in clinical settings, is costly and labor-intensive and thus only performed when precise renal function is needed. Equations or formulas that estimate GFR using endogenous renal clearance biomarkers are used most frequently in clinical practice. The most common GFR marker in these formulas is serum creatinine (SCr). However, several factors interfere with SCr measurement and interpretation, thus it is not an ideal marker. Estimating GFR in children comes with special considerations, as factors determining GFR and SCr change physiologically during early life postnatal kidney development. New GFR estimation formulas have been studied in recent years, with the goal of improving the limitations of established formulas. This article will review different methods of GFR measurement, GFR equations and issues surrounding these measures, with the goal of providing the reader with an appreciation of how to approach interpretation and evaluation of GFR.

3.2.2. What is GFR?

The kidneys perform various regulatory functions including filtration (glomerular), reabsorption and secretion (tubular), and hormonal secretion (renal cells), maintaining hemodynamic, fluid and electrolyte homeostasis, red blood cell production and healthy bone metabolism. With CKD, several metabolic and endocrine abnormalities are present (e.g., electrolyte disturbances, bone mineral disorders), each of which may potentially serve as biomarkers of abnormal renal function. However, these CKD perturbations are closely related to the most commonly used renal function parameter: GFR. Each nephron has a glomerulus, which contains capillaries. Glomerular filtration is the process of blood flowing through these capillaries, creating an

ultrafiltrate. Its rate (GFR) is determined by factors including glomerular number, renal blood flow, capillary pressure and capillary wall permeability. Total GFR reflects the total nephron mass performance (sum of each single nephron GFR). An average healthy adult GFR is about 120 ml/min/1.73m$^2$, with a wide normal range (90-149 ml/min/1.73m$^2$). GFR above this level is considered "hyperfiltration", which may be a sign of early CKD or lead to CKD. Several factors influence GFR other than the presence of kidney damage, including protein intake, pregnancy, obesity, some vasodilatory antihypertensives or hyperglycemia (tend to increase GFR), or increasing age, some anti-hypertensives and reduced intravascular volume (may decrease GFR)[1]. Therefore, ideally, GFR measurement and interpretation should be performed when patients are in a "steady state", well hydrated and using standardized measurement protocols.

3.2.3 Normal GFR in children and staging decrement in GFR

The Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guideline provides practice recommendations on several kidney disease topics, via international expert group consensus and detailed evidence review [2]. KDIGO recommends a graded staging of CKD, from normal GFR (>90 ml/min/1.73m$^2$) to end stage renal disease (GFR <15 ml/min/1.73m$^2$), shown in Table 1. These stages are used for screening and following patients with CKD, evaluating risk for abnormalities related to CKD (e.g. anemia) and assisting in decision-making on need for dialysis initiation. As stated in the KDIGO guideline, these GFR-based CKD categories may be applied to children. The exception is in children <2 years of age, before which GFR increases physiologically over the first 2 years of life; therefore the KDIGO CKD staging is not similarly applicable. For example, GFR <60 ml/min/1.73m$^2$ is normal in a 3 month old, but consistent with Grade 2-3 CKD in a 5 year old. By age 2, average normal GFR is similar to older child and adult levels.

**Manuscript 1, Table 1. Staging of CKD based on level of glomerular filtration rate, as per the KDIGO clinical practice guideline[2].**

| CKD grade | GFR (ml/min/1.73 $m^2$) | Terms |
|---|---|---|
| G1 | >90 | Normal or high |
| G2 | 60–89 | Mildly decreased* |
| G3a | 45–59 | Mildly to moderately decreased |
| G3b | 30–44 | Moderately to severely decreased |
| G4 | 15–29 | Severely decreased |
| G5 | <15 | Kidney failure |

Schwartz and Work recently reviewed in detail, studies which have measured GFR using gold standard or plasma disappearance methods in normal children, providing an estimate of normative child GFR values [3]. One work of particular interest is that of Piepsz et al (2006) [4]. They measured GFR by plasma disappearance of $^{51}$Cr-ethylenediamenetetraaceticacid (Cr-EDTA) in 623 children evaluated for potential mild urogenital abnormalities, only including patients with no significant kidney defects and with equal bilateral renal function. They confirmed that GFR rises progressively from neonatal age to 2 years old, stabilizing at a GFR of about 105 ml/min/1.73$m^2$ thereafter (Table 2). They also provided percentile values for GFR across age groups; for example GFR=95 ml/min/1.73$m^2$ for a 1.5 year old child falls approximately at the 50$^{th}$ percentile value for age. Perhaps it would be more rational to express GFR in terms of percentile values (as done with height or weight) in the <2 year old age group; however, this would require research to determine how to use such percentile values in clinical care, determine what percentile is "abnormal" and how this relates to patient outcome. At present, clinicians should at least be aware than "normal GFR" is lower in children less than 2 years of age and may use the values provided in Table 2 as a guide to determining normal *vs.* abnormal GFR.

**Manuscript 1, Table 2. Normal glomerular filtration rate from age 0.1 to 2 years old, based on Cr-EDTA plasma disappearance method[4].**

| Age group (years) | Mean (± SD) GFR (ml/min/1.73m$^2$) | 10$^{th}$, 50$^{th}$ and 90$^{th}$ percentile GFR value (ml/min/1.73m$^2$)* |
|---|---|---|
| ≤0.1 | 52.0 (9.0) | 30, 42, 54 |
| 0.10-0.30 | 61.7 (14.3) | 40, 53, 70 |
| 0.30–0.66 | 71.7 (13.9) | 52, 71, 92 |
| 0.66–1.00 | 82.6 (17.3) | 61, 84, 108 |
| 1.00–1.50 | 91.5 (17.8) | 63, 91, 118 |
| 1.50–2.00 | 94.5 (18.1) | 70, 97, 123 |
| >2.00 | 104.4 (19.9) | 70, 98, 124 |

* The 10$^{th}$, 50$^{th}$ and 90$^{th}$ percentile of GFR values shown were extrapolated visually from percentile curves published in the reference.

An important and somewhat controversial issue is the method used to standardize or scale GFR. To compare GFR among infants, children and adults, a standard reference scale reflecting patient size is required. Kidney weight would be ideal, although reliable measurement methods are not currently available. Body surface area (BSA) has been the traditional and is the current recommended method to scale GFR[2]. However, one must be cautious when scaling GFR to BSA in patients with extremes of weight, since weight is included in BSA calculation. In very obese patients, scaling to BSA leads to GFR underestimation. For example, a patient with a non-scaled GFR of 90 ml/min and BSA=2.0 has a BSA-corrected GFR = (90 ml/min X 1.73m$^2$)/ 2.0 m$^2$ = 78 ml/min/1.73m$^2$. If the patient loses weight to a BSA=1.8, BSA-corrected GFR will rise substantially, simply due to weight loss. This limitation should be considered when applying GFR values to very obese or malnourished individuals. Other body measures

have been suggested to scale GFR to, including extracellular volume, total body water or body cell mass [5-7], but these have not been studied extensively.

3.3.4 GFR measurement methods

Gold standard GFR evaluation refers to measuring renal clearance of an administered exogenous filtration marker, where clearance of a substance ($C_{[S]}$) is expressed in ml/min by the following equation:

$C_{[S]} = U_{[S]}$ X V (ml per minute)/$P_{[S]}$ ,

where $U_{[S]}$ is urine concentration of the substance, V is urine flow rate, ($P_{[S]}$) is plasma concentration of the substance. An ideal filtration or clearance marker is freely filtered at the glomerulus (thus not protein-bound), and is neither secreted nor reabsorbed by tubules (i.e., unchanged in urine). GFR measurement agents should be non-toxic and distributed extracellularly. There are generally two methods to measure clearance or GFR: evaluating substance plasma disappearance and a more cumbersome method including urinary collections. The gold standard method requires urine collections. The filtration marker is injected subcutaneously or intravenously (as a bolus or bolus plus infusion), followed by several urine collections obtained every 10 to 30 minutes. The substance's plasma disappearance rate is monitored post-injection, and the average calculated clearance (using the formula above) is calculated with each urine collection and taken as the GFR. To stimulate urine flow, fluid is administered both before and during the protocol[8].

3.3.5 Inulin

The only known ideal gold standard filtration marker is inulin (Table 3). Inulin clearance measurement involves continuous intravenous infusion and meticulous urine sampling through a urinary catheter or voluntary voiding, as described above. Although inulin has the characteristics of an ideal GFR marker, there are limitations to this clearance method[9]. Hydration is required to maintain high urine flow rate, and complete voiding is not always possible. This is especially problematic in children with urologic issues or who are incontinent. This method is time-consuming and uncomfortable, thus is not part of routine practice. Because of these and other disadvantages, alternative

clearance methods and filtration markers are used.

### 3.3.6 Radioactive markers of GFR and the plasma disappearance method of measuring GFR

Nuclear medicine techniques have been developed using radiolabeled agents (or tracers) with similar GFR marker characteristics as inulin (Table 3). Commonly used tracers include radiolabeled 99m Tc-diethylene triamine penta-acetic acid (DTPA), Cr-EDTA and iothalamate. These markers are generally used to measure GFR using the plasma disappearance method, avoiding the need for timed urine collection and continuous infusion.

### 3.3.7 Plasma disappearance method of GFR measurement

Plasma clearance of a marker is measured after a bolus intravenous injection. These methods are described in detail in several reviews and only briefly described here [3,9]. GFR is calculated using the marker concentration administered divided by the area under the curve of plasma concentration over time. A "two-compartment system" is used to mathematically model the disappearance curve, where the marker is injected into a first compartment (the intravascular space), equilibrates with the second (the extracellular space), and is subsequently excreted via glomerular filtration from the first compartment. Time must be allowed for equilibration to occur before post-injection samples are drawn, otherwise GFR is overestimated [10,11]. Drawbacks of this GFR measurement method include requirement of multiple blood samples and the time needed to characterize the disappearance curve, especially in patients with very low GFR (more time is needed to clear the substance). A simplified technique has been developed to include only a few blood samples, increasing feasibility of plasma disappearance GFR methods. There is general consensus that obtaining at least 3-4 samples for measuring the markers, up to 4 to 5 hours after injection, is adequate [3,11,12]. In children with lower GFR (<30 ml/min/1.73 $m^2$) obtaining samples as long as 12-24 hours after injection will provide more accurate results.

3.3.8 Markers used in plasma disappearance GFR measurement methods

Table 3 summarizes many of the markers used. Several studies have used the iodine isotope I-iothalamate[13-15]. However, there appears to be significant renal tubular secretion of iothalamate, causing GFR overestimation, which may at least partially explain the recent move away from iothalamate GFR measurement[16,17].
Other markers commonly used are radio-isotopes 99m Tc-DTPA[18] and 51 Cr-EDTA[4] , both of which correlate strongly with inulin clearance[19]. Cr-EDTA is primarily used in Europe, while DTPA is used more frequently in North America. These markers do have limitations (Table 3) leading to overall GFR underestimation[2]. However, DTPA plasma disappearance GFR measurement has specifically been shown to overestimate GFR at very low GFR levels, which should be kept in mind if using this method to make treatment decisions about dialysis initiation or CKD progression[20,21]. Moreover, both tracers are associated with some radiation exposure. There is likely substantial variability between clinical centre protocol GFR measurement methods using DTPA and Cr-EDTA; if these methods continue to be widely used (they are currently the most commonly used GFR measurement methods in clinical practice), their protocols should be standardized.

**Manuscript 1, Table 3. Summary of markers used to measure GFR.**

| **Marker** | **Marker information** [72] | **Method summary** | **Strengths** | **Weaknesses** |
|---|---|---|---|---|
| Inulin | 5200 Da inert fructose polymer | Gold standard method. Either bolus or bolus + continuous infusion, includes urine collection for clearance measuremen | -Gold standard marker<br>-No side effects[73] | -Urine collection challenges<br>- Assay challenges, expensive[72] |

| | | | | |
|---|---|---|---|---|
| | | ts | | |
| Iohexol | 821 Da nonradioactive contrast agent | Mainly used for plasma disappearance method. Also used for plasma/urine collection clearance method | -Low toxicity<br>-Rare adverse events<br>-Inexpensive<br>-<br>Nonradioactive<br>-Sensitive assay allows for low dose[22] | -Assay is expensive[2]<br>-Possible tubular reabsorption or protein binding<br>-Theoretical potential for nephrotoxicity, allergy with high doses [74] |
| Iothalamate | 614 Da iodine isotope | Mainly used for plasma disappearance method. Also used for plasma/urine collection clearance method | - Inexpensive<br>- Extensively studied in children | -Significant tubular secretion[17]<br>- Radioactive (non-cold form) |
| 99m Tc-DTPA | 393 Da radioactive tracer | Single dose injection, plasma disappearance method | -Short half life[75]<br>- Extensively studied in children | -Plasma protein binding [76]<br>-Radioactive |
| 51 CR-EDTA | 292 Da radioactive tracer | Single dose injection, plasma disappearance method | - Extensively studied in children | -Radioactive<br>-Possible protein binding[76]<br>-Possible tubular reabsorption[77] |
| Creatinine clearance | 113 Da, endogenous, freely filtered marker | Single plasma measurement and 24h urine collection | -Easy, inexpensive | -Tubular secretion<br>-Influenced by many factors such as muscle mass, gender, age, and nutrition |

Recently, an alternative, non-radioactive agent, iohexol, has been studied in children for measuring GFR using the plasma disappearance method. Iohexol is a low osmolarity contrast agent, used in low doses for measuring GFR[10]. Advantages include lack of radiation and favourable toxicity profile [22]. Iohexol is measured by high-performance liquid chromatography and mass spectrometry, so although the substance is inexpensive, the assay is costly. An advantage to iohexol plasma disappearance is that it has been studied and validated in detail by the Chronic Kidney Disease in Children study group (CKiD)[3,10,23] . In the original CKiD study (2009), the investigators used 4 post-injection time points to measure iohexol, up to 5 hours post-injection, consistent with recommended measurement timepoints described above[10,23] . This group also demonstrated that accurate iohexol GFR measurement may be possible using fewer blood samples [24]. Moreover, use of "dried capillary blood-spot" samples (capillary blood samples on a filter paper, later measured for ioxehol) for iohexol measurement is being investigated for GFR measurement [25,26]. If more widely validated, this method of obtaining post-iohexol injection samples would be an exciting and non-invasive alternative to the current method.

3.3.9 Endogenous creatinine clearance

Depending on centre resources, GFR measurement methods described above may be impractical for timely GFR evaluation. An alternative method is the creatinine clearance, involving urine collection over a period of time (typically, 24 hours, but shorter periods may provide similar results[27]) and obtaining a single SCr measurement at the beginning or end of the urine collection. Using the clearance formula above, urine and serum creatinine concentrations and average urine flow rate (total ml of urine collected/minutes of collection, followed by scaling to BSA of 1.73$m^2$), GFR may be estimated. This method is simple, since SCr measurement is widely available and inexpensive. Although SCr is freely filtered at the glomerulus and not protein-bound, there are several limitations to the creatinine clearance. SCr concentration is associated with gender, age, nutrition and muscle mass, independent of GFR. Urine collection may not always be complete when performed at home, leading to inaccuracy. Importantly,

creatinine is secreted by the proximal tubules, more so at lower GFR, leading GFR overestimation. Cimetidine blocks tubular creatinine secretion, which improves the accuracy of GFR measurement [28,29]. Thus, if using creatinine clearance to measure GFR, particularly in patients with low GFR or for following renal function over time, these limitations must be considered.

3.3.10 Estimating GFR using serum markers and equations

3.3.11 SCr

Despite limitations described above, SCr remains the most commonly used GFR marker, is inexpensive and child normative values have been published. Importantly, international efforts have led to promoting SCr assay measurement standardization to the gold standard isotope dilution mass spectroscopy method [30,31]. In 1976, Schwartz et al published the well-known "Schwartz formula" to estimate GFR [32]. For almost 30 years, this equation was recommended to estimate GFR in children. This formula was derived using an older SCr assay, using creatinine clearance as the reference standard and is therefore inappropriate for estimating GFR today.

In 2009, the CKiD group published several new equations, in a large CKD child cohort (mean GFR about 40 ml/min/1.73m$^2$), using iohexol clearance as the reference standard [23]. The CKiD equations include various combinations of physical and laboratory variables (including height, blood urea nitrogen concentration, age, gender, SCr), as well as what is now often referred to as the "new bedside Schwartz formula" (top of Table 4). Similar to the previous Schwartz equation, it is based on the strong linear relationship between the height to serum creatinine ratio (height/SCr) (height, a surrogate of muscle mass, important in SCr generation) and GFR. Height/SCr is multiplied by a regression-derived constant or "k". This equation is currently recommended for child GFR estimation [2]. It is simple to calculate, only requiring SCr and height. However, considerations should be made when using this equation. The equation was derived from children with substantially reduced kidney function and including few older teens (whose muscle mass is relatively higher and therefore, may require a different "k" value to relate height/SCr to GFR). Recent studies actually show

that the new Schwartz equation performs reasonably well to estimate GFR in children with higher GFR and older children[33,34], but this deserves further study. Because of problems associated with SCr concentrations and non-renal factors, estimating GFR in children at the extremes of muscle mass (particularly very low muscle mass, such as wheelchair bound children) using this equation, will be much less accurate[35] . Some authors have suggested that to achieve the best GFR estimation accuracy within a given centre, a "locally-derived" k value may be calculated. Specifically, whatever reference standard GFR method is used within a centre, may be regressed on height/SCr of children who had those GFR measures, deriving a centre-specific k value [33,35,36]. Theoretically, this k value should be the most appropriate for that centre's patient population, ethnicity profile, and GFR reference method used. However, whether this leads to differences in clinical decision-making has not been evaluated. Other methods proposed to improve SCr -based GFR equations have been to incorporate other easily measured variables (such as blood urea nitrogen[23] or body cell mass [5]), but these methods increase complexity of quick GFR estimation and have not been validated. A non-exhaustive list of SCr-based equations which have been either validated externally or incorporating novel methods of SCr-based eGFR, is provided in Table 4. Of great importance is that adult-derived equations should not be used to estimate GFR in children. Derivation of these equations included small numbers of young adults and several include variables not routinely measured in pediatric care. Moreover, adult equations have been shown to poorly estimate GFR even in adolescents and young adults.[37,38].

3.3.12 Height-Independent GFR estimation: is it possible?

Height is not always available clinically and is not available in laboratory databases, making large, population-based child GFR research impossible. Recently, Pottel et. al. developed a height-independent child GFR equation (Table 4). The equation constant is the "Q" value, which differs by age; this value represents the median SCr value from a population of healthy children (in the case of the Pottel equation, from European children[39-41]). Although height-dependent GFR estimation was

more accurate, their height-independent method performed quite well and may represent a major step forward in population-based child GFR research. In principle, Q values could be derived in different patient populations, including North America (using SCr values measured in large populations of normal children) or within a given centre. Further study of this GFR estimation method would be highly worthwhile.

3.3.13 Cystatin C

To overcome the limitations of SCr, another endogenous GFR marker, cystatin C (CysC), has been evaluated. CysC is a small, cysteine protease inhibitor protein that is freely filtered at the glomerulus and not significantly affected by age, gender or muscle mass[11] . The exception is in children less than age 1, who have higher CysC concentrations[11], potentially due to GFR being physiologically lower. CysC may be higher in patients treated with steroids and those with a renal transplant[13,42-48] and concentrations may be affected by inflammatory and thyroid disorders [44-48]. CysC has also been shown to increase as a result of certain malignancies; however it is unclear whether this is due to CysC production by tumor cells or rather impairment in renal function[45]. Nevertheless, CysC-based formulas demonstrate better performance in oncology patients compared to those based on height/SCr [49-51]. Several CysC- eGFR equations have been derived (some are summarized in Table 4). In general, CysC equations are more accurate for estimating GFR and more diagnostic of abnormal GFR, than SCr equations[52,53] . CysC can not be used to measure clearance since it reabsorbed by proximal tubular cells. It is important to note that the two published CysC assays (turbidimetric and nephelometric) lead to substantially different values[54-56]. Most recent data suggest that the nephelometric CysC assay leads to more accurate GFR estimation [52,57]. Currently, the cost of CysC measurement is approximately 8-10 times SCr measurement; however, if its use becomes more widespread, this cost may decrease. Population-based normative CysC values have been published [58,59]. Ultimately, whether using CysC as opposed to SCr in clinical care actually leads to differences in decision-making or on patient outcomes, remains unknown.

Table 4 displays several eGFR equations, including recently-derived equations

which combine both SCr and CysC [13,23,39-41,57,60-66]. These equations appear to be most accurate for estimating GFR. The CKiD group confirmed that incorporating SCr and CysC in eGFR equations leads to more accurate GFR estimation in children with CKD. Research is needed to validate this finding in different populations and determine if patient outcomes are affected by such improved GFR estimation.

**Manuscript 1, Table 4. Summary of several published GFR estimation equations, using serum creatinine, Cystatin C or both.***

| Marker | Equation | Population derived from | N | Reference standard GFR measure |
|---|---|---|---|---|
| **SCr** | | | | |
| Modified Schwartz (CKiD), 2009[23] | 0.413*height/SCr | CKD (Median GFR: 41.3 ml/min/1.73m$^2$) | 349 | Iohexol |
| Height-independent equations (Pottel, 2012, Hoste 2013)[39,40] | 107.3* SCr/Q<br>Two methods to calculate Q:<br>Q= 0.0270 *Age + 0.2329 (height [ht] independent)<br>Q=3. 94 -13. 4 * ht +17.6* ht$^2$ -9. 84 * ht$^3$ + 2.04 * ht$^4$ (height dependent) | Healthy children (Mean GFR: 87.6 ml/min/1.73m$^2$) | 353 | Cr-EDTA |
| Gao et. al., 2012[63] | Females:<br>60*Height/SCr +6.25*height/SCr$^2$ +0.48*age-21.53<br>Males:<br>60*Height/SCr+6.25*height/SCr$^2$ +0.48*age-25.68 | CKD and normal renal function (Mean GFR: 85.7 ml/min/1.73m$^2$) | 551 | Inulin |
| **CysC** | | | | |
| CKiD-CysC, 2012[57] | 40.6 (1.8/CysC)$^{0.93}$ | CKD (Median GFR: 41.3 ml/min/1.73m$^2$) | 600 | Iohexol |

| | | | | |
|---|---|---|---|---|
| Zappitelli, 2006[13] | $75.94/[CysC^{1.17}]$ If renal transplant, × 1.2 | CKD, kidney transplant, other transplant (Mean GFR:73.6 mL/min/1.73 $m^2$) | 103 | Iothalamate |
| Hoek 2003[64] | $-4.32 + 80.35(CysC)^{-1}$ | Adult- workup for living kidney donor transplant (Median GFR: 81 ml/min/1.73 $m^2$) | 93 | Iothalamate |
| Le Bricon, 2000[65] | 78/CysC + 4 | Adult-kidney transplant (Median GFR: 49 ml/min/1.73 $m^2$) | 25 | 51 Cr-EDTA |
| Rule, 2006[66] | $66.8 \times (CysC)^{-1.30}$ | Adult- CKD, kidney transplant, other transplant, workup for living donor transplant(Mean GFR: 57ml/min/1.73$m^2$) | 460 | Cold Iothalamate |
| **Combined CysC+SCr** | | | | |
| New CKiD[57] | $39.8*[ht(m)/SCr]^{0.456}[1.8/CysC]^{0.418}[30/BUN]^{0.079}1.076\ male_{[ht(meters)/1.4]}^{0.179}$ | CKD (Median GFR: 41.3 ml/min/1.73$m^2$) | 600 | Iohexol |
| Zappitelli 2006(CysCrEq)[13] | $(507.76 \times e^{0.003\times height})/(CysC^{0.635} \times SCr^{0.547}$ [µmol/L]) If renal transplant, ×1.165<br><br>If spina bifida, × $(SCr^{0.925}$[µmol/L])/40.45 | CKD, kidney transplant, other transplant (Mean GFR: 73.6mL/min/1.73 $m^2$) | 103 | Iothalamate |

| | | | | |
|---|---|---|---|---|
| Chehade et. al., 2013[62] | Females: $0.423x(Ht/SCr)-0.043x(Ht/SCr)^2-14.53xCysC+0.693x\ age+18.25$ Males: $0.423x(Ht/SCr)-0.043x(Ht/SCr)^2-14.53xCysC+0.693x\ age+21.88$ | CKD (Mean GFR: 85ml/min/1.73m$^2$) | 243 | Sinistrin~ |
| Bouvet, 2006[61] | $63.2 \times (SCr^*/96)^{-0.35} \times (Cys\ C/1.2)^{-0.56} \times (weight\ kg/45)^{0.30} \times (age/14)^{0.40}$ | Kidney transplant, drug monitoring (Mean GFR 95 ml/min/1.73$^2$) | 100 | Cr-EDTA |
| **Beta-trace protein** | | | | |
| Benlamri et. al., 2010[60] | 10^(1.9020+(0.9515xLOG(1/BTP))) | Renal Pathologies (Median GFR 105.5ml/min/1.73$^2$ ) | 387 | 99mTcDTPA |

*Markers expressed in conventional units (mg/dL for SCr or mg/l for CysC) and height in cm, unless otherwise indicated.
~Sinistrin: fructose polymer similar to inulin[62]

3.3.14 Which equation to use?

Given the large number of equations available to estimate GFR, it can become quite overwhelming to decide which equation should be used. It is likely reasonable to use the currently recommended Schwartz equation in most clinical scenarios. However, one must consider individual patient characteristics to decide when using an alternative equation may be most appropriate. When high accuracy is desired, it has been proposed to estimate GFR using both SCr and CysC equations and calculating the mean. However, in cases of reduced muscle mass such as in patients hospitalized for prolonged periods, paralysis or amputation, the SCr-based estimates will be inaccurate and a CysC-eGFR will be more accurate. In some situations, CysC-eGFR will be less accurate, such as in patients receiving high doses of glucocorticoids. Children treated for cancer are of

particular consideration since they undergo considerable changes in muscle mass over a short time period and receive many medications including steroids; thus, both SCr-eGFR and CysC eGFR equations may be inaccurate. In such cases, there may be significant discrepancy between SCr and CysC-based eGFR estimates and GFR measured by a reference procedure should be considered[67]. At our centre, we selectively measure CysC in children whom we feel SCr-eGFR is particularly inaccurate (e.g., wheelchair bound child, evaluated for CKD) and when there is great uncertainty we pursue gold standard GFR measurement.

3.3.15 Other serum markers

Other serum markers of GFR have been investigated for estimating GFR in children, including beta trace protein (BTP) and beta 2-microglobulin (B2M), both low molecular weight proteins which are freely filtered at the glomerulus [68-70]. Little research on these markers has been performed, and future studies will elucidate the extent to which they may offer more accurate GFR estimation than is currently feasible with SCr and CysC[60,71].

3.3.16 Conclusion and proposed application

Although there has been a large amount learned on how to measure GFR, estimating GFR as accurately as possible using SCr and on novel markers of GFR, there remain many knowledge gaps. For example, although iohexol GFR plasma disappearance measurement method for measuring GFR is likely the best characterized and most valid current feasible method to measure GFR, the extent to which this method is different to the most commonly used tracer methods is not known. Although we now have new candidate serum GFR markers, like CysC, more accurate for estimating GFR, whether we should actually replace SCr for CysC or in what patients to do so, is unclear. What is clear is that inaccurate GFR measurement or estimation can potentially impact negatively on patients, such as when screening for CKD (overdiagnosis or missed diagnoses), dosing life-saving medications or making critical decisions on prognosis and dialysis initiation in patients with CKD. We propose that given the current state of knowledge and available evidence, when screening for CKD, or

estimating GFR in the hospital setting, the bedside Schwartz formula should generally be adequate. However, one must always look for patient characteristics which may be associated with potential for inaccuracy of GFR estimation using this equation (e.g. very low muscle mass, very obese, limb amputation, older adolescent males), especially when critical decisions will be made based on eGFR (e.g. a patient awaiting transplant listing). In such cases, CysC measurement may be useful to provide a better estimate of GFR. Should we change to using CysC to measure renal function? It is unknown if this will lead to improved outcomes. However, until centres and individual physicians begin using this test or until research shows improvement in outcomes or resource use from using CysC, we will never know. In patients evaluated for CKD presence, one should be cautious about assuming that "borderline" eGFR is normal or abnormal (e.g eGFR between 80 and 100 ml/min/1.73m$^2$) given the poor precision (variability) of current equations and consider performing a reference standard GFR test. With regards to GFR measurement methods, we suggest that clinicians be aware of what method is being used in their centre (e.g. DTPA plasma disappearance *vs.* iothalamate) so as to be able to better interpret results with knowledge of the test potential limitations. For similar reasons, it is also worthwhile to be aware of the specific test methodology used (e.g. 2 point versus 5 point-tracer plasma measurement for DTPA GFR tests). Often, the test may be modified to fit specific patient characteristics, such as in a child presumed to have very low GFR, in whom requesting for prolonged post-injection plasma measurements would be helpful. In the research study setting, the goal should be to estimate GFR as accurately as possible, therefore whenever feasible, we propose using SCr and CysC eGFR measurement.

### 3.3.17 References


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### *3.3 Post-reflection to this published review manuscript*

GFR is a crucial renal outcome and this review played a significant role in the current work, by appreciating the limitations of the known and widely used eGFR Schwartz

equation. The review led to selecting alternative suggestions for GFR estimation that should be considered in future studies of this nature. This review highlighted the limitations in using SCr to estimate GFR and the importance of including CysC when estimating GFR. The limitation of SCr as the definition of AKI can also be understood from the above article. Of great benefit was the identification of the recently published height-independent GFR equations. The current thesis project uses these height-independent equations to define baseline SCr when it is unknown and when height is unknown, crucial to the definition of AKI. To our knowledge, we will be the first to propose and apply this height-independent GFR estimation method of defining baseline SCr for AKI definition.

## Chapter 4: Methods for the thesis project

As stated in Chapter 1, section 1.2, the overall hypotheses for my thesis project are:

3. CKD and HTN are prevalent at 6 years after pediatric ICU admission.
4. The presence of AKI during pediatric ICU stay increases the long-term risk for CKD and HTN.

The specific objectives of my thesis project are:

5. To review the current state of knowledge surrounding AKI and renal outcomes (CKD, estimated GFR, albuminuria, and HTN) in children, as well as existing evidence on the link between AKI and renal outcomes.
6. To estimate the prevalence of CKD, pre-HTN and HTN, 6 years after pediatric ICU admission.
7. To examine the relationship between AKI during ICU stay and CKD or HTN at 6 years following ICU admission.
8. To evaluate other non-AKI factors that may be associated with AKI and/or long-term renal outcomes.

### *4.1 Design, setting and patients*

I performed a longitudinal follow-up prospective cohort study performed at 6 years ± 6 months after PICU admission. This single-visit study is being conducted at both the Montreal Children's Hospital (PI: Michael Zappitelli) and the Stollery Children's

Hospital in Edmonton, Alberta (Edmonton PI: Catherine Morgan). The Research Ethics Boards of both institutions approved the study protocol and parents/legal guardians provided written informed consent. Additionally, children over 7 years of age provided written informed assent

Inclusion criteria:

1. Children admitted to the ICU at the ages of 0-18 years old.
2. ICU admission between Jan. 2005 and Jan. 2010.
3. ICU admission for ≥2 calendar days.

Exclusion Criteria:

1. Known renal disease before ICU admission (renal transplant, dialysis, eGFR<30% normal for age, tubulopathy, glomerulonephritis, nephrotic syndrome) or one of these diagnoses as the primary reason for ICU admission.
2. Unable or unwilling to provide consent or assent (if >7 years old).
3. A priori refusal of future bloodwork at the time of consent.
4. Unwilling to return for study visits AND live too far (>3.5 hours by car) from the research centre for a home visit.

A basic analysis was performed comparing patients who were included *vs.* excluded. Only age at ICU admission and length of ICU were available to compare the two groups. Of 515 patients (n included =323, n excluded=192there was a statistically significantly longer length of ICU stay ($p<0.05$) in the excluded patients, but no significant difference in age at ICU admission ($p>0.05$). More data will be collected at the end of the study to further examine the risk of selection bias.

*4.2 Study cohorts and recruitment strategies*

There are three main cohorts recruited into this study:

1. Western Canadian Complex Pediatric Therapies (WCCPT): A neonatal cardiac surgery cohort in Alberta currently participating in an ongoing prospective neurodevelopmental study. Subjects are contacted by a known research team member, clinician or contacted through the website and family newsletters. Our research team then approaches patients for invitation to participate.

2. MCH PICU study: Previously studied non-cardiac surgery MCH PICU study cohort. These children were part of a prior prospective ICU study (performed between 2007 and 2010) examining early AKI biomarkers. They had clinical data, blood and urine collected daily until ICU discharge. Subjects have agreed to being contacted from the previous study and are contacted by phone for invitation to participate in my current study.
3. PICU mailing cohort: non-previously studied PICU mailing cohort: previously admitted ICU patients are identified from clinical databases and medical records from both the Montreal and Edmonton sites, then reviewed for inclusion/exclusion criteria and mailed a letter from the ICU directors. Families are invited to return a signed form allowing us to contact them and responders are phoned for informed consent.

For the purpose of the current thesis analysis, only non-cardiac surgery patients admitted to the PICU are included. Study subjects who have undergone cardiac surgery will be studied separately in future manuscripts, once recruitment is complete (the majority of patients are being recruited and studied in Edmonton, i.e., the WCCPT cohort).

*4.3 Data collection*

4.3.1 Clinical data collection at the time of the study visit:

A single study visit is performed at 6 years ± 6 months following ICU admission at home or in centre. Blood and urine are collected, as well as height, weight and blood pressure. We are collecting clinical data on family and patient medical history, medication and hospital or emergency room visits since discharge, as well as cardiovascular risk factors, quality of life, and blood draw results since discharge. Medical records are being obtained from other institutions when necessary in order to ensure thorough data collection on any medical event or blood draw result.

Study visits, except for blood draw, are performed by research nurses, graduate students, and research assistants, all of who are trained as per the detailed and standardized Manual of Procedures of the study. Blood is drawn by the research nurses

or clinic nurses from the vein, finger pokes, or from a port when is the case. Medical records data are collected by graduate students and research assistants who are trained by the Case Report Form standardized instructions manual.

- Blood testing: 4 mls of blood is collected to measure SCr and CysC. Blood is kept on ice, centrifuged at 2000 rpm, 4°C for 15 minutes, and the plasma stored at -80°C.
- Urine collection: Urine is collected as a mid-stream sample in a sterile cup, or collected using diaper cotton balls or a urine bag in the case of incontinence. After urinalysis, urine is centrifuged for 15 minutes and supernatant stored at -80ºC until measurement of albumin and creatinine.
- Blood pressure: Blood pressure is measured with an automated blood pressure monitor. Three seated measures are taken in a quiet setting to avoid white coat hypertension. A cuff that is appropriate to the size of the child's upper right arm is used to ensure the correct measurement of blood pressure in children, as described in The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents published by the American Academy of Pediatrics[86]. The average of the 2 lowest blood pressures is calculated.
- Growth assessment: Weight and height is measured three times using calibrated scales and stadiometers and percentiles are calculated.
- Medical history: Data are collected on family and subject history of renal disease and HTN, as well as medication taken around the time of the visit, and hospital or emergency room visits since discharge.
- Data available from the parent studies: Detailed in-hospital and baseline data are available for subjects in the WCCPT and MCH PICU cohorts including demographic, renal and clinical variables (vasopressor, ventilation, fluid balance, complications) and hospital outcomes. In the PICU mailing cohort, index hospitalization data are collected from the hospital computer database OACIS and by chart review.

- Data entry: Data are entered prospectively into a Redcap database, a web-based application designed to support data capture for research studies. Urine and blood samples results are also entered into the database. Data are extracted from Redcap in order to perform analyses. Quality of entered data is assured by performing double data entry, wherein someone other than the data collector enters 10% of all charts into Redcap. Duplicated charts are then assessed for discrepancies.

4.3.2 Data collection from the index ICU admission (excluding AKI data, described below)

Data from ICU admission are currently still being collected (Thesis Table 4). These data are collected only post-follow-up study visit in order to avoid renal outcome ascertainment bias; a researcher may be influenced by knowledge of data from the index PICU admission when evaluating renal outcomes. We are approximately 40% complete with this data collection, which is mainly being collected through chart review and also through electronic hospital health records. The data being collected include pre-ICU, diagnostic, and daily clinical ICU variables, as well as various laboratory measurements. The purpose of this data collection is to be able to accurately and precisely describe our study population, as well as to be able to explore any factors which may confound, or modify the effect of, the relationship between AKI and renal outcomes and include them in our statistical models.

**Thesis Table 4. Brief summary of variables being collected from index ICU admission**

| Variable type | Pre-ICU variables | Diagnostic variables | Daily clinical ICU variables |
|---|---|---|---|
| **Categorical** | Past medical history | Primary ICU diagnosis | Cardiac arrest |
| | | Infection | Mechanical ventilation |
| | | Sepsis if infection | ECMO |
| | | Positive culture | Dialysis |
| | | Nephrology consultation | Vasopressors |
| | | | Nephrotoxins |
| **Continuous** | | | Daily fluid balance |
| | | | SCr |
| | | | PRISM variables (first 24 hours) |

*4.4 Laboratory data collection*

After a study visit, once the urine and blood samples are processed, they are aliquotted and frozen in the MCH nephrology lab freezer. The Montreal site also receives frozen, processed urine and serum samples from the Edmonton site. These samples are inventoried and stored in the MCH freezer (processing and storage as described in 4.3.1. above) until they are sent for analysis.

- SCr, urine albumin and urine creatinine: To reduce inter-laboratory variation, the samples are measured in a single laboratory at the MCH, using an enzymatic, IDMS-standardized assay. Urine creatinine is measured by modified Jaffe assay and urine albumin by nephelometry (Prospec II, Siemens).
- CysC: Blood samples are sent to the Cincinnati Children's Biomarker Laboratory, where they measure serum CysC (coefficient of variation=1.1%).. Measurement is performed by the laboratory of Dr. Devarajan, using standardized nephelometric methods (Siemens BN-II, Siemens, AG; www.Siemens.com).

Individuals performing the SCr and CysC assays were blinded to the clinical data.

*4.5 Exposure and outcomes definition*

Primary exposure: AKI during ICU using the internationally accepted criteria of the *Kidney Disease Improving Global Outcomes (KDIGO)* definition which is primarily based on SCr rise (Thesis Table 2, Chapter 2, Section 2.4). The exposure is defined either as: Any AKI *vs.* no AKI; Stage 2 or worse AKI *vs.* no AKI or Stage 1 AKI. In addition, some patients had no SCr measurement performed during PICU admission. In those patients, we assume that no AKI occurred during PICU admission for most analyses. The rationale behind assuming that patients with no SCr measured during ICU admission did not develop AKI, is that they were likely not ill enough to have warranted SCr measurement and thus are highly unlikely to have developed AKI. However, in select sensitivity analyses, we exclude these patients from analysis. These data are collected

retrospectively using the hospital laboratory databases and chart review, *after* the study visit is performed, in order to avoid bias in ascertaining the long-term renal outcomes. Baseline SCr is the lowest SCr value in the 3 months before ICU admission[40]. When baseline SCr is unknown but height at admission is known, the Schwartz eGFR equation is used to estimate baseline SCr, assuming normal renal function (GFR = 120 ml/min/1.73m$^2$) at baseline[1,40,108]. For children ≤2 years old, in whom GFR is physiologically lower than children over 2 years old, normative GFR values are used when assuming normal GFR to estimate baseline SCr[99] (Manuscript 1 Table 2, Chapter 3). When height is unknown, it is not possible to use the Schwartz equation. Therefore, a height-independent eGFR equation is used[109], again assuming normal renal function at baseline as described above.

Primary outcomes**:**

1. Presence of CKD using the KDIGO criteria (Thesis Figure 2 and Thesis Figure 3, Chapter 2, Section 2.8.4). :
    a. Abnormal GFR: Estimated GFR [eGFR] <90ml/min/1.73m$^2$
    For the primary outcome, GFR is estimated using the standard Schwartz formula[102], which includes SCr and height and also using the combined SCr-CysC Zappitelli equation[106].

    OR

    b. Albuminuria: urine albumin/creatinine (ACR) >30 mg/g (3mg/mmol).

*Because all children are over the age of 2 years old at the time of the study visit, there is no need to adjust for age when defining abnormal GFR or microalbuminuria outcomes.*

2. HTN: defined using the average of the 2 lowest systolic or diastolic blood pressures.
    a. Pre-HTN: ≥90th and <95th percentile BP for age, gender and height OR ≥120 / >80, even if less than the 90th. In patients aged 18 and 19 years old, pre-HTN was defined as SBP between 120 and 139 inclusively OR DBP between 80 and 89 inclusively. In patients ≥20 years old, pre-HTN was defined as SBP>130 to less than 140 OR DBP ≥80 and less than 89[64,110].

HTN: ≥ 95th percentile for age, gender, and height. In patients ≥18 years old, HTN was defined as SBP ≥140 and DBP ≥90We also examine systolic and diastolic blood pressure z-scores to compare BP as a continuous variable between groups.

*4.6 Statistical analysis*

The overall approach to the statistical analysis described here is based around the thesis objectives and objectives of the manuscript in the following Chapter. In summary, the goal of the analysis is to evaluate prevalence of renal outcomes during the post-PICU study visit performed and provide a detailed phenotype of renal outcomes in the whole cohort, followed by evaluating for evidence of an AKI-outcome association. For the latter, it is important to control for potential confounders and effect modifiers of the AKI-renal outcome associations. However, a large proportion of patients still have not had index PICU admission data collected. Therefore the analysis described below is focused on describing the analysis using the data currently available and providing a brief description of future planned analyses. Odd's ratios were expressed with 95% confidence intervals (CI). Analyses were performed using the statistical software STATA® 12.0 (StataCorp, College Station, TX, USA).

*General descriptive analyses*

The distribution of the main exposure and outcome variables was examined, in addition to the distribution of pre-index PICU admission variables (e.g., baseline eGFR, diagnostic data, baseline comorbidities), ICU admission variables (e.g., illness severity scores, use of vasopressors and mechanical ventilation) and post-ICU admission variables (e.g., post-PICU hospitalizations and ER visits, new comorbidities) collected. Appropriate univariate analyses will be performed to explore potential confounders. As well, variables that make biological sense as confounders will be included in the regression models. Pairwise correlations will be tested among the significant variables prior to performing multiple regression analyses to help in confounder selection (to avoid co-inclusion of highly correlated variables in regression models).

*Analyses for thesis objective 2: Estimate the prevalence of CKD (defined by low GFR or microalbuminuria), pre-HTN and HTN, 6 years after pediatric ICU admission.*

The proportion of subjects attaining the long-term renal outcomes was estimated in the whole cohort. Specifically, prevalence of eGFR <90ml/min/1.73m$^2$ (by both the SCr-based and combined SCr-CysC-based eGFR equations), ACR >30 mg/g (microalbuminuria), composite outcome of CKD (low eGFR *or* microalbuminuria), HTN, pre-HTN, CKD *or* HTN, and CKD *or* pre-HTN. Mean and standard deviation eGFR, blood pressure z-scores and ACR at the long-term follow-up study visit were calculated.

*Analyses for thesis objective 3: Examine the relationship between AKI during ICU stay and CKD or HTN at 6 years following ICU admission.*

The proportion of subjects with each of the renal outcomes was compared by patients with *vs.* without AKI (with AKI defined using each of the methods described above: e.g., AKI *vs.* no AKI; AKI Stage 2 or 3 *vs.* no AKI or Stage; including and excluding patients without known AKI status, etc), using Chi-square test or Fisher's exact test, as appropriate. T-tests or the Mann-Whitney U tests were used to compare the renal outcomes expressed as continuous variables (e.g., BP z-scores) between groups, depending on variable distribution.

Logistic regression analysis was used to evaluate the unadjusted relationship between the presence of AKI and each of the dichotomous renal outcomes (unadjusted OR, 95% CI). Linear regression analysis was used to evaluate the relationship between AKI and each of the renal outcomes expressed as continuous outcomes (e.g., BP z-scores, eGFR value).

Following this, a systematic approach was used to determine what variables should be considered for inclusion in the multiple regression models of the AKI-renal outcome relationship, as potential confounders or effect modifiers (e.g. pre-ICU variables, ICU variables, etc), keeping in mind that a more parsimonious model should be favoured. First we performed univariate analysis evaluating a) the relationship between other variables and the presence of AKI and b) the relationship between other variables and the renal outcomes was performed on several socio-demographic (e.g., gender, age, family income), pre-ICU variables (e.g. comorbidities, baseline GFR), diagnostic (e.g., ICU admission diagnosis), ICU (e.g. vasopressor use, mortality risk score)

and post-ICU (e.g., hospitalizations, new comorbidities) variables, using either t-tests, Mann-Whitney U tests, spearman correlation, Chi-square or Fisher's exact tests, depending on variable distributions. Of note, the data for the Pediatric Risk of Mortality (PRISM)[111] score during PICU admission are being collected, but have not yet been calculated. PRISM score will be included in the final manuscript analysis, but is not included here. In addition, the PRISM score was chosen as the "illness severity" score, rather than more recent illness severity scores, since PRISM does not include a renal component (to avoid correlation with the main exposure, AKI). Variables which were either thought to have a rational clinical basis to be considered as confounders, or which were significant on univariate analyses, were considered for inclusion in multivariate analyses. A priori, given the fact that much of the pre-PICU and PICU admission data have not been collected, we chose to exclude most of these variables from the current multivariate regression analysis all together. Next, we evaluated whether any of these potential other variables were highly correlated (e.g., correlation > 0.8). In these instances, only one of the highly correlated variables would be included in the multivariate analysis. Next, we grouped the other potential variables into two rational and clinically-based variable groupings, for which we had highly complete data: a) baseline or socio-demographic variables (e.g., age, gender, study site) and b) post-PICU admission variables (e.g., number of post-PICU emergency or hospital admission events). Finally, specifically pre-defined multivariate models were evaluated to ascertain the AKI-long-term renal outcome relationship: a) univariate logistic (or linear) regression as outlined above, b) a multivariate model including the selected baseline/sociodemographic group of variables and c) a multivariate model including the selected baseline/sociodemographic group of variables and the selected post-ICU variables. In logistic regression analyses (i.e., with renal outcomes defined as dichotomous outcomes), adjusted OR (aOR) and 95% CI were estimated; in linear regression analyses, adjusted beta-coefficients and 95% CI's were evaluated to determine statistical significance of the adjusted AKI-outcome relationships.

Once all of the data are collected, the approach to analysis will be similar.

However, we will additionally evaluate pre-ICU variables, and ICU diagnostic and other variables for inclusion in the multivariate analyses. We will also perform more robust model selection approach to determine how robust our models are, such as evaluating the likelihood ratio test to determine the effects of removing variables from the models. With the complete sample size available for analysis, we will also be able to perform evaluation of variables (e.g., gender, age, illness severity, baseline eGFR, etc) for evidence of effect modification (i.e., inclusion of select interaction terms in the multivariate models and evaluating their statistical significance and their effect on the AKI-outcome effect measures).

*Analyses for thesis objective 4: Evaluate other non-AKI factors which may be associated with AKI and/or long-term renal outcomes.*

It is of interest to clinicians and for future research to evaluate the relationship between ICU-related variables (e.g., sepsis, use of mechanical ventilation, illness severity) and long-term renal outcomes, in order to begin suggesting a targeted approach with which patients should be followed up for renal function monitoring. In the current analysis, we have only examined the univariate relation with such variables and the renal outcomes, due to current sample size/data availability constraint. In future analyses, we will explicitly evaluate the adjusted relationship between these variables and long-term renal outcomes, using the multivariate analysis approach described above.

**Chapter 5 Manuscript:** ***Long-term chronic kidney disease (CKD) and hypertension (HTN) in children previously admitted to the intensive care unit (ICU) with and without acute kidney injury (AKI)***

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*5.1 Pre-amble to the manuscript*

The following is a manuscript written with the aim of submission for publication by June 2016. The initial target journals will be high impact pediatric or nephrology journals, since this data will be extremely novel and potentially impact on how children with AKI are followed post ICU (e.g., Pediatrics, Journal of Pediatrics, Journal of the American Society of Nephrology, Kidney International). Of note, preliminary results of the manuscript have been presented in oral presentation and poster format at the 2014 and 2015 Canadian Society of Nephrology meetings and other local meetings[112,113]. Because there is still data to collect, the results and conclusions are not final. Recruitment is nearly complete, and study visits are currently being performed on an ongoing basis (will be completed by Winter, 2016, with about 50 study visits remaining to be performed). Similarly, data on index ICU admission is incomplete. Once all data are collected, all analyses described below will be repeated in an almost identical approach as is outlined in the manuscript and detailed above in Chapter 4. For the purposes of this thesis, the WCCPT cardiac cohort described in Chapter 4, section 4.2 and all cardiac patients are excluded from the current manuscript analysis as their recruitment is ongoing and they will be analyzed in a separate study.

**Manuscript**

*5.2 Introduction*

Acute kidney injury (AKI) is characterized by an abrupt decline in renal function and is mainly defined by a rise in serum creatinine (SCr) [40]. AKI is common in children admitted to the pediatric intensive care unit (ICU), with incidence ranging from 4.4% [27]

to 82% in the most critically ill children[1] . AKI is independently associated with poor hospital outcomes such as mortality, longer length of hospital and ICU stay, need of prolonged mechanical ventilation[14,20,29] and prolonged recovery after heart surgery in cardiac surgery patients[35] . Most AKI in hospitalized children is due to acute tubular necrosis (ATN)[114]. Although ATN is known to resolve and is followed by proliferation of survivor cells, recovery may be incomplete [47]. Indeed, animal studies and epidemiological studies done on adults demonstrate that AKI leads to chronic kidney disease (CKD) [45,46] . The pathogenic link between AKI and CKD as suggested by animal models may include capillary loss due to alterations in endothelial cells [48], progressive interstitial fibrosis and damage to functioning nephrons. This ultimately leads to CKD[56] , which is an important risk factor for cardiovascular disease progression[7]. Similarly, endothelial dysfunction in childhood CKD is associated with HTN, left ventricle hypertrophy, and increased cardiovascular disease events such as myocardial infarction [7,8]. Studies done in children with AKI have shown high prevalence of CKD or HTN at follow-up from hospital admission; however, almost no studies have included non-AKI individuals, so the independent effect of AKI on long term CKD and HTN in children is unknown[67,68,115]. This lack of knowledge is compounded by the fact that the current standard of care is not to monitor renal function post ICU discharge. There is a need to elucidate whether AKI is a risk factor for progression to CKD and HTN such that early intervention can be put in place and disease progression can be prevented. There is also a need to understand the overall renal outcome of children admitted to the ICU, in order to develop a rational approach to targeting children appropriately for long-term renal follow-up.

Evaluating long-term renal outcome in otherwise healthy children is challenging. CKD diagnosis includes evaluating GFR, microalbuminuria (urine albumin excretion) and blood pressure. SCr is the current routine diagnostic test of renal function, and is incorporated into prediction equations to estimate GFR (SCr-eGFR) [100]. However SCr poses challenges for estimating GFR in children. This is because SCr, which is produced by the muscles, is affected by muscle mass, gender and growth. Furthermore, kidney

handling of SCr may be affected by diet and medication among other factors[102]. Cystatin C (CysC), a small molecular weight protein excreted by glomerular filtration, has been shown to be a more accurate and sensitive marker of detecting mild renal dysfunction than SCr [104,105,116] . Recently, it has been shown that estimating GFR using equations which incorporate both SCr and CysC, leads to more accurate GFR estimation, than using SCr-based GFR equations. We hypothesize that 1) CKD and HTN are common in children who were previously admitted to the ICU and that 2) AKI during ICU stay increases long-term risk for CKD and HTN. We also hypothesize that the AKI-outcome relationship will be stronger when using GFR equations that incorporate both SCr and CysC, compared to using SCr-based eGFR equations alone.

*5.3 Methods*

5.3.1 Design, setting and subject selection

This is longitudinal follow-up prospective cohort study performed at 6 years ± 6 months after PICU admission. This single-visit study is being conducted at both the Montreal Children's Hospital (MCH) and the Stollery Children's Hospital in Edmonton, Alberta. Children admitted to the ICU at the ages of 0-18 years old between Jan. 2005-Dec. 2011 for ≥2 days were included in the study. Patients with known renal disease before ICU admission (renal transplant, dialysis, eGFR<30% normal for age, tubulopathy, glomerulonephritis, nephrotic syndrome), those who were unable or unwilling to provide consent or assent (if >7 years old), refused bloodwork a priori at the time of consent, and who were unwilling to return for study visits and live too far (>3.5 hours by car) from the research centre for a home visit were excluded. This study included neonates undergoing cardiac surgery. However, they are excluded from the current analysis as their recruitment is ongoing and they will be analyzed in a separate study.

The research Ethics Board of both institutions approved the study protocol and parents/legal guardians provided written informed consent. Additionally, children over 7 years of age provided written informed assent.

5.3.2 Study population

There are two main cohorts included in this study:

1. MCH PICU cohort: a previously studied non-cardiac surgery MCH PICU study cohort. These children were part of a prior prospective ICU study examining early AKI biomarkers. They had clinical data, blood and urine collected daily until ICU discharge. Subjects have agreed to being contacted from the previous study and were contacted by phone.
2. Mailing cohort: a non-previously studied PICU mailing cohort. Previously admitted ICU patients were identified from clinical databases and medical records from both the Montreal and Edmonton sites, then reviewed for inclusion/exclusion criteria and mailed a letter from the ICU directors. Families were invited to return a signed form allowing us to contact them and responders were phoned for informed consent.

5.3.3 Data collection

**Clinical data**

A single study visit was performed at 6 years ± 6 months following ICU admission at home or in centre. Blood and urine were collected, as well as height, weight and blood pressure (each expressed as percentile values and z-scores, as per published guidelines[86,117]). Blood pressure is measured with an automated blood pressure monitor. Three seated measures are taken in a quiet setting to avoid white coat hypertension. A cuff that is appropriate to the size of the child's upper right arm is used to ensure the correct measurement of blood pressure in children. The average of the 2 lowest blood pressures is taken. At study visits, clinical data on family and patient medical history, medication taken (with a focus on any blood pressure medication and commonly used nephrotoxins), as well as cardiovascular risk factors (e.g., smoking) and quality of life (evaluated using the Pediatric Quality of Life questionnaire[118]) were collected. Families were queried about any hospital or emergency room visits since PICU discharge, as well as whether or not they have had any blood tests performed since PICU discharge. If *any* of these were responded to positively, then the medical

records from any hospital, ER or laboratory test centre which the family recalled visiting were requested, received and reviewed in detail. All SCr results available from these records and details on hospitalizations or ER visit diagnoses were recorded.

Data from the index PICU admission were collected retrospectively using medical charts and hospital laboratory system databases. Briefly, index PICU admission data included: a) pre-ICU variables (baseline eGFR, pre-ICU organ comorbidities); b) ICU variables (primary PICU admission diagnosis, length of stay, infection, sepsis diagnosis, use of vasopressors, mechanical ventilation; treatment with nephrotoxins); c) post-ICU variables (smoking/alcohol use, anti-HTN medication use, system comorbidities diagnosed post-ICU, number of health care events).

When reporting results on nephrotoxins during ICU, specific nephrotoxins being referred to are: NSAIDS, aminoglycosides, acyclovir, ganciclovir, amphotericin and vancomycin

**Laboratory data collection**

Four milliliters of blood were collected to measure SCr and CysC and up to 30 mls urine was collected to measure albuminuria. Blood was kept on ice, and within 6 hours of collection, centrifuged at 2000 rpm, 4ºC for 15 minutes, and the plasma stored at -80ºC. After urinalysis, urine was centrifuged for 15 minutes and supernatant stored at -80ºC until analysis. All biospecimens were shipped to and stored at the MCH sites, every 3 to 6 months. To reduce inter-laboratory variation, the samples were measured for SCr, urine albumin and urine creatinine in a single laboratory at the MCH, using an enzymatic, IDMS-standardized assay. Creatinine was measured by modified Jaffe assay and urine albumin by nephelometry (Prospec II, Siemens). Serum Cys C was measured using a nephelometer (Siemens BN-II, Siemens, AG; www.Siemens.com) at the Cincinnati Children's Hospital Biomarker Laboratory (coefficient of variation=1.1%). Individuals performing the SCr and CysC assays were blinded to the clinical data.

5.3.4 Exposure and outcomes

Exposure: AKI during ICU using the internationally accepted criteria of the Kidney Disease Improving Global Outcomes (KDIGO)[40] definition which is primarily based on SCr

rise (≥50% rise from baseline or ≥26.5 µmol/L rise from baseline). When baseline SCr was unavailable, it was calculated using the CKiD GFR equation[102], assuming baseline normal renal function. Normal GFR was assumed to be 120 ml/min/1.73m$^2$ in children over 2 years old. In children under two years old, 50$^{th}$ percentile normal GFR was used based on normative percentile curves generated from children with normal renal function [119]. When height from ICU admission was missing, CKiD GFR was not possible to calculate. In these cases a validated height-independent age-based formula was used to estimate baseline SCr[109,120]. This formula was derived based on normalized SCr values in healthy children[109] and has been validated at our centre in a separate population for childhood GFR estimation[120]. AKI was expressed in the following ways: a) Any AKI *vs.* no AKI; b) Stage 2 or 3 AKI *vs.* no AKI or Stage 1 AKI. In addition, some patients had no SCr collected during ICU admission. In those patients, it was assumed that AKI did not occur. We also separately evaluated only patients who had *known* AKI status (excluding patients with unknown AKI status).

Outcomes: presence of CKD (estimated glomerular filtration rate [eGFR] <90ml/min/1.73m$^2$ or microalbuminuria [urine albumin/creatinine (ACR) >30 mg/g], pre-HTN (≤18 years old: ≥90th and ≤95th percentile BP for age, gender, height OR ≤120 / >80; 18-19 years old: SBP 120-139 OR DBP 80-89; ≥20 years old: SBP>130 to <140 OR DBP ≥80 and <89) and HTN (≤18 years old: ≥ 95th percentile for age, gender, height; ≥18 years old: ≥140/90). CKD was defined using both SCr-eGFR by the CKiD equation, and by an eGFR equation including both SCr and CysC (SCr-CysC-eGFR) validated at our centre[106]. The main outcome was the composite outcome of CKD *or* pre-HTN, using each of the GFR equations.

5.3.5 Statistical analysis

All point estimates of odd's ratios were expressed with 95% confidence intervals (CI). Appropriate univariate analyses were performed (t-tests, Mann-Whitney U tests, Chi-square or Fisher's exact tests) to compare variables between groups. All statistical analyses were performed using the software STATA® 12.0 (StataCorp, College Station, TX, USA). Proportions of subjects attaining the renal outcomes were estimated. Mean

and standard deviation was calculated for eGFR, blood pressure (z-score) and ACR at the study visit, in the whole group as well as in AKI subgroups. The relationship between AKI and dichotomously expressed renal outcomes (e.g. presence of pre-HTN; composite outcome of CKD *or* HTN) was evaluated first by univariate logistic regression (reporting unadjusted odds ratio's (OR)) and then by multiple logistic regression with calculation of adjusted odd's ratio's (OR), controlling for potential confounders. The association of AKI with continuous-expressed renal outcomes (e.g. BP z-scores) was evaluated using linear regression models. Potential other variables (e.g. age, gender, ICU variables, post-ICU variables) were considered for inclusion in the multiple regression models by a) examining the univariate relationships between these variables with the main exposure (AKI) and the renal outcomes, and b) by a priori decision to include in the multivariate models (specifically, age, gender and study site, were included regardless of univariate analysis results). In addition, potential other variables for inclusion in the model were grouped into specific categories of variables: pre-ICU/baseline/sociodemographic variables (e.g. age, gender, baseline eGFR); ICU-related variables (e.g., vasopressor use, illness severity, mechanical ventilation, ICU diagnosis); post-ICU variables (e.g., post-ICU hospitalizations, use of nephrotoxins, comorbidities).

*5.4 Results*

5.4.1 Study population

After application of the inclusion and exclusion criteria, 243 patients were included in analysis (Manuscript 2, Figure 1). Response rate of the mailing cohort was 15%, and consent rate was 83%. Consent rate of the previously studied MCH PICU cohort was 75%. A basic analysis was performed comparing patients who responded to the mail invitation (the PICU mailing cohort) *vs.* those who did not respond. Of 286 patients (n responders =50, n non-responders=236), looking at age at ICU admission and length of ICU stay, there was no significant difference in both age at ICU admission and length of ICU stay ($p>0.05$). More data will be collected at the end of the study to further examine the risk of selection bias.

The characteristics of the study population by AKI status and by No AKI/Stage 1 AKI *vs.* AKI stage 2 or 3, including all subjects are shown in Manuscript 2, Table 1. Mean

follow up time from index ICU admission of the entire cohort was 5.8+1.1 years. Mean age of the study cohort at follow-up was 10.6+5.6, and 58% were male. 38% of the cohort had no SCr measured during ICU. When assuming no AKI in patients without SCr measured during ICU stay, AKI occurred in 25% of patients; 11% of patients had AKI stage 2 or worse.

**Manuscript 2 Figure 1. Study flow.**

5.4.2 Findings

**ICU variables associated with AKI**

During ICU admission, sepsis diagnosis, as well as mechanical ventilation, use of vasopressors and use of nephrotoxins were significantly more common in patients with AKI, and age at ICU admission was significantly higher in AKI patients (Manuscript 2 Table 1). Patients with AKI had significantly longer hospital length of stay (Manuscript 2 Table 1). There were no differences in baseline eGFR, the frequency of abnormal baseline eGFR, primary ICU diagnosis or past medical history in those with vs. without AKI (Manuscript 2 Table 1).

**Post-ICU variables associated with AKI**

Age at follow up visit was significantly higher in patients with AKI and AKI was significantly more common at the MCH study site (Manuscript 2 Table 1). There were no significant differences in follow-up visit BMI, race/ethnicity, sex, and socioeconomic status, between patients with *vs.* without AKI or those with *vs.* without AKI stage 2 or worse. A higher proportion of patients with past AKI (≥ 12 years old) reported smoking cigarettes and drinking alcohol since their ICU admission, though the difference was not statistically significant (Manuscript 2 Table 1). Patients with past AKI also had a non-statistically significant higher number of post-ICU ER and/or hospital admission events (Manuscript 2 Table 1). Very few patients were on anti-HTN medication at follow up (Manuscript 2 Table 1).

**Manuscript 2 Table 1. Descriptive statistics of demographics, pre-ICU/baseline, diagnostic and ICU-related data and post-ICU variables, comparing patients with vs. without known AKI, and patients with vs. without known AKI stage 2 or worse at index PICU admission**

| **Variable** | **All**<br>**n=243** | **No AKI**<br>**n=182** | **AKI**<br>**n=61** | **No AKI/Stage 1**<br>**n=217** | **AKI Stage 2/3**<br>**n=26** |
|---|---|---|---|---|---|
| ***Socio demographic*** | | | | | |
| Age at visit (years) | 10.6±5.6 | 10.2±5.5 | 12.0±5.6* | 10.5±5.6 | 12.3±5.9 |

| | | | | | |
|---|---|---|---|---|---|
| BMI percentiles | 59.0±29.0 | 58.7±29.2 | 59.6±28.9 | 58.9±28.7 | 59.4±32.1 |
| Gender (%male) | 58.0% | 59.3% | 54.1% | 59.0% | 50.0% |
| Race (% white vs. other) | 82.5% | 83.8% | 78.7% | 81.8% | 88.5% |
| Lower SES | 15.4% | 14.7% | 17.4% | 15.3% | 15.8% |
| Study site (%MCH vs. SCH) | 89.7% | 87.4% | 96.7%* | 88.9 | 96.1% |
| ***Pre-ICU/diagnostic*** | | | | | |
| Baseline eGFR | 102.5±48.4 | 99.9±47.0 | 109.4±53.3 | 98.7±44.1 | 123.3±67.3 |
| % abnormal baseline eGFR | 30.8% | 34.2% | 21.4% | 31.8% | 25% |
| Primary ICU diagnosis | | | | | |
| Neurological | 27.4% | 21.6% | 42.9% | 28.3% | 20% |
| Respiratory | 31.4% | 35.1% | 21.4% | 32.6% | 20% |
| ENT | 25.5% | 29.7% | 14.3% | 26.1% | 20% |
| Diabetes | 15.7% | 13.5% | 21.4% | 13.0% | 40% |
| Number of past medical history items | 0.83±29.05 | 0.95±1.2 | 0.6±0.7 | 0.86±1.1 | 0.6±0.7 |
| % Past medical history items≥1 | 48.4% | 48.4% | 48.3% | 47.6% | 54.5% |
| ***ICU variables*** | | | | | |
| Age at ICU admission (years) | 4.9±5.5 | 4.4±5.3 | 6.4±5.7* | 4.6±5.4 | 6.6±5.8 |

| | | | | | |
|---|---|---|---|---|---|
| Hospital length of stay (days) | 18.4±45.8 | 18.6±52.4 | 17.5±17.2* | 18.2±48.4 | 19.5±15.3* |
| ICU length of stay (days) | 4.8±6.6 | 4.7±6.9 | 4.9±5.5 | 4.7±6.8 | 5.4±4.7 |
| Infection | 49.5% | 52.9% | 41.9% | 49.4% | 50% |
| Sepsis diagnosis | 29.2% | 20.0% | 53.8%* | 23.8% | 66.7%* |
| Mechanical ventilation | 44.5% | 38.6% | 57.5%* | 41.4% | 64.7% |
| Vasopressor use | 25.8% | 18.7% | 41.4%* | 23.2% | 45.4% |
| Nephrotoxin use | 26.9% | 17.2% | 48.3%* | 23.2 | 54.5%* |
| ***Post-ICU variables*** | | | | | |
| Smoking/alcohol | 48.0% | 41.2% | 61.5% | 45.3% | 61.5% |
| On anti-HTN meds at follow up | 2.06% | 1.6% | 3.3% | 2.3% | 0% |
| Number of system comorbidities diagnosed post-ICU | 0.5±0.7 | 0.5±0.8 | 0.3±0.5* | 0.5±0.7 | 0.3±0.6 |
| % with number of comorbidities ≥ 1 | 35.3% | 40.3%* | 21.3% | 36.4% | 26.9% |
| Number of health care events (ER or hospital admissions) | 6.9±6.6 | 6.5±5.9 | 8.3±8.4 | 6.5±6.0 | 10.8±9.9 |

- indicates p value < 0.05 between two groups

AKI: acute kidney injury; BMI: body mass index; SES: socioeconomic status; MCH: Montreal Children's Hospital; SCH: Stollery Children's Hospital; ICU: intensive care unit; ENT: ear nose and throat speciality; HTN: hyprtension; ER: emergency room

**Variables associated with renal outcomes**

Patients who had the composite outcome of either CKD (SCr-eGFR based equation) or pre-HTN or worse at follow-up were older at the time of their ICU admission, compared to patients without the composite outcome (statistically significant) (Manuscript 2 Table 2). Using the combined SCr and CysC-eGFR-based CKD definition, those with either CKD or pre-HTN at follow up reported significantly greater use of antihypertensive medication, and had significantly higher number of health care events (either emergency or hospital admissions since PICU discharge) (Manuscript 2 Table 2). There was a non-statistically significant higher proportion of males in the CKD or pre-HTN or worse group (Manuscript 2 Table 2). There was no difference in BMI percentile at follow-up, race, socio-economic status, study site, baseline eGFR, and frequency of abnormal baseline eGFR, or primary ICU diagnosis between composite renal outcome groups (Manuscript 2 Table 2).

**Manuscript 2 Table 2. Descriptive statistics of demographics, pre-ICU/baseline, diagnostic and ICU-related data and post-ICU variables, comparing patients with the composite outcome of CKD or HTN, using a SCr-based eGFR equation and using a combined SCr and CysC-based eGFR equation.**

| | **SCr-eGFR based equations used** | | **SCr and CysC-based eGFR equations** | |
|---|---|---|---|---|
| **Variable** | **No CKD or pre-HTN n=146** | **CKD or pre-HTN n= 72** | **No CKD or pre-HTN n=108** | **CKD or pre-HTN n=63** |
| ***Socio demographic*** | | | | |
| Age at visit (years) | 9.8±5.1 | 12.6±6.0* | 9.9±5.3 | 12.1±5.8* |
| BMI percentiles | 59.4±28.5 | 60.2±30.0 | 59.6±28.6 | 62.4±29.9 |
| Gender (%male) | 54.1% | 63.9% | 53.7% | 61.9% |
| Race (% white vs. other) | 81.8% | 83.3% | 84.9% | 85.7% |

| | | | | |
|---|---|---|---|---|
| Lower SES | 15.8% | 19.1% | 9.2% | 22.5%* |
| Study site (%MCH vs. SCH) | 90.4% | 86.1% | 87.0% | 84.1% |
| ***Pre-ICU/diagnostic*** | | | | |
| Baseline eGFR | 103.4±48.1 | 97.7±40.6 | 86.8±30.4 | 95.4±40.9 |
| % baseline eGFR abnormal | 26.7% | 31.2% | 35.0% | 33.3% |
| Primary ICU diagnosis | | | | |
| Neurological | 25.0% | 26.7% | 19.0% | 18.2% |
| Respiratory | 37.5% | 26.7% | 42.9% | 27.3% |
| ENT | 21.9% | 26.7% | 23.8% | 27.3% |
| Diabetes | 15.5% | 20% | 14.3% | 27.3% |
| Number of past medical history items | 0.7±1.1 | 1.0±1.2 | 0.7±1.2 | 0.9±1.1 |
| % Past medical history items≥1 | 40.0% | 61.5% | 39.6% | 60.0% |
| ***ICU variables*** | | | | |
| Age at ICU admission (years) | 3.9±5.0 | 6.9±5.9* | 4.1±5.2 | 6.3±5.6* |
| Hospital length of stay (days) | 19.6±57.0 | 16.0±20.3 | 21.4±66.1 | 16.7±21.6 |

| | | | | |
|---|---|---|---|---|
| PICU length of stay (days) | 4.9±7.5 | 4.1±4.4 | 4.7±7.9 | 4.3±4.6 |
| Infection | 56.2% | 37.0% | 59.2% | 38.1% |
| Sepsis diagnosis | 31.4% | 20.0% | 31.0% | 25.0% |
| Mechanical ventilation | 48.7% | 33.3% | 45.8% | 31.0% |
| Vasopressor use | 21.3% | 36.0% | 22.4% | 42.1% |
| Nephrotoxin use | 27.9% | 24.0% | 28.6% | 21.0% |
| ***Post-ICU variables*** | | | | |
| Smoking/alcohol | 42.9% | 54.3% | 44.4% | 48.3% |
| On anti-HTN meds at follow up | 0.7% | 5.6%* | 0.9% | 6.3%* |
| Number of system comorbidities diagnosed post-ICU | 0.5±0.7 | 0.4±0.6 | 0.5±0.7 | 0.5±0.8 |
| Patients with number of comorbidities ≥ 1 | 36.0% | 32.3% | 33.7% | 35.6% |
| Number of health care events (ER or hospital admissions) | 6.8±7.1 | 7.0±5.8 | 6.5±6.8 | 7.1±5.4* |

* indicates p value < 0.05 between two groups. AKI: acute kidney injury; BMI: body mass index; SES: socioeconomic status; MCH: Montreal Children's Hospital; SCH: Stollery Children's Hospital; ICU: intensive care unit; ENT: ear nose and throat speciality; HTN: hyprtension; ER: emergency room

**Univariate AKI association with renal outcomes**

Renal outcomes in all study patients are shown in Table 3. Whether only patients with known AKI status or all patients were included in the analysis, SCr-eGFR was lower in patients with past AKI ($p < 0.05$) (Manuscript 2 Table 4a). Generally, there was a non-statistically significant higher prevalence of abnormal SCr-eGFR, abnormal ACR, and HTN in AKI *vs.* non-AKI subjects (Manuscript 2 Table 4a). Although not statistically significant, pre-HTN and abnormal ACR were 1.3 and 2 times more common, respectively in patients with AKI Stage 2 or worse (Manuscript 2 Table 4b).

Patients with AKI or stage 2 or worse overall had higher prevalence of composite renal outcomes (Manuscript Figure 2a and 2b). These differences were generally more marked when the SCr-CysC-eGFR equation was used to define CKD (Figure 2b *vs.* Figure 2a). However, only the prevalence of outcomes CKD (no-AKI/Stage 1 AKI *vs.* Stage 2 or 3 AKI: 18% *vs.* 46%, respectively, $p<0.05$), pre-HTN or CKD (no-AKI/Stage 1 AKI *vs.* Stage 2 or 3 AKI: 34% *vs.* 67%, respectively, $p<0.05$), and HTN or CKD (no-AKI/Stage 1 AKI *vs.* Stage 2 or 3 AKI: 22% *vs.* 54%, respectively, $p<0.05$) were significantly higher in patients with AKI stage 2 or worse when the combined SCr-CysC eGFR was used (Figure 2a and 2b).

**Manuscript 2 Table 3. Detailed renal outcome prevalence in all PICU patients**

| **Variable** | **All subjects n=243** |
|---|---|
| SCr- eGFR (Mean ±/- SD) | 128.3±32.0 |
| SCr-eGFR (% abnormal) | 7.7% |
| Scr-CysC eGFR (Mean ±/- SD) | 144.0±40.5 |
| Scr-CysC eGFR (% abnormal) | 1.9% |
| ACR (Mean ±/- SD) | 2.9±14.6 |
| ACR (% abnormal) | 12.8% |
| SBP z-score (Mean ±/- SD) | 0.25±0.91 |

| | |
|---|---|
| DBP z-score (Mean ±/- SD) | -0.04±0.88 |
| Pre-HTN (N (%)) | 15.5% |
| HTN (N (%)) | 4.6% |

**Manuscript 2 Table 4a. Detailed renal outcome prevalence in all patients, by AKI status in patients with AKI status known and by AKI status in all patients**.

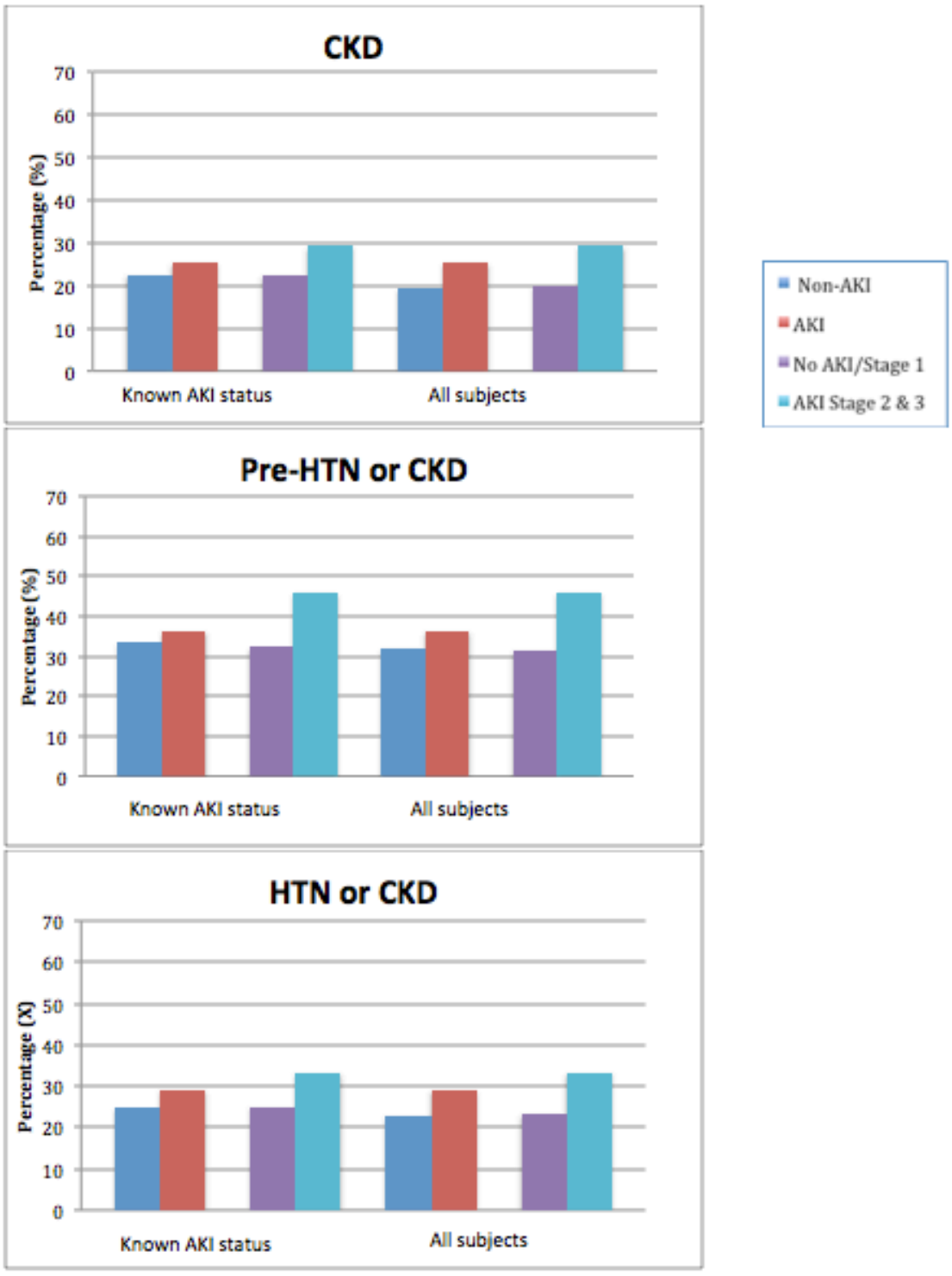
| | **Known AKI status** | | **All patients** | |
|---|---|---|---|---|
| **Variable** | **No AKI n=95** | **AKI n=61** | **No AKI n=182** | **AKI n= 61** |
| SCr- eGFR Mean ± SD | 131.7±33.9 | 121.1±29.0* | 130.8±32.7 | 121.1±29.0* |
| SCr-eGFR % abnormal | 6.9% | 12.5% | 6.1% | 12.5% |
| SCr-CysC eGFR Mean ± SD | 151.1±38.1 | 144.4±23.3 | 148.3±34.3 | 144.4±23.3 |
| SCr-CysC eGFR % abnormal | 0% | 0% | 2.5% | 0% |
| ACR Mean ± SD | 2.7±7.3 | 1.1±1.3* | 2.3±5.6 | 1.1±1.3* |
| ACR % abnormal | 11.7% | 16.1% | 13.2% | 11.7% |
| SBP z-score Mean ± SD | 0.25±1.0 | 0.16±0.9 | 0.3±0.9 | 0.16±0.9 |
| DBP z-score Mean ± SD | -0.1±1.1 | -0.05±0.7 | -0.03±0.93 | -0.05±0.7 |
| Pre-HTN N (%) | 16.1% | 15.0% | 15.6% | 15.0% |
| HTN N (%) | 3.2% | 5.0% | 4.5% | 5.0% |

* indicates p value < 0.05 between two groups

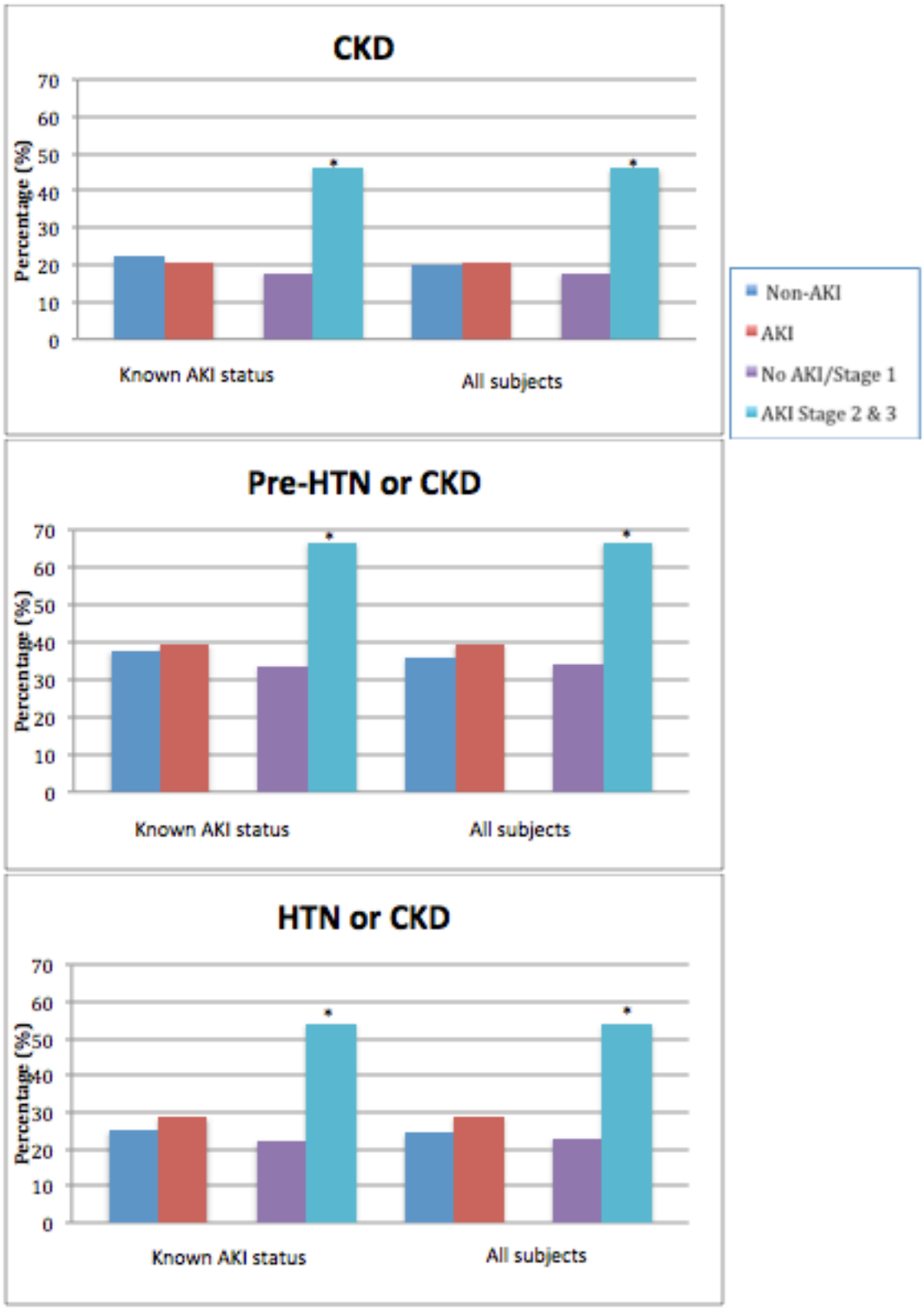
**Manuscript 2 Table 4b. Detailed renal outcome prevalence in all patients, by AKI Stage 2 or worse in patients with AKI status known and by AKI Stage 2 or worse status in all patients.**

| | Known AKI status | | All patients | |
|---|---|---|---|---|
| **Variable** | **No AKI/Stage 1 n=130** | **AKI Stage 2/3 n=26** | **No AKI/Stage 1 n=217** | **AKI Stage 2/3 n=26** |
| SCr- eGFR Mean ± SD | 127.6±32.1 | 127.1±34.1 | 128.5±31.8 | 127.1±34.1 |
| SCr-eGFR % abnormal | 10.20% | 4.00% | 8.20% | 4.00% |
| Scr-CysC eGFR Mean ± SD | 148.8±33.8 | 147.9±32.9 | 147.4±32.2 | 147.9±32.9 |
| Scr-CysC eGFR % abnormal | 0% | 0% | 2.10% | 0% |
| ACR Mean ± SD | 2.2±6.3 | 1.7±1.9 | 2.0±5.2 | 1.7±1.9 |
| ACR % abnormal | 12.30% | 24.00% | 11.40% | 24.00% |
| SBP z-score Mean ± SD | 0.2±0.9 | 0.3±0.9 | 0.2±0.9 | 0.3±0.9 |
| DBP z-score Mean ± SD | 0.88 | 0.09±0.7 | 0.85 | 0.09±0.7 |
| Pre-HTN N (%) | 14.80% | 20.00% | 14.90% | 20.00% |
| HTN N (%) | 3.90% | 4.00% | 4.70% | 4.00% |

**Manuscript 2 Figure 2a. Composite outcomes using SCr-based eGFR in AKI groups.**



**Manuscript 2 Figure 2b. Composite outcomes using combined SCr-CysC-based eGFR in AKI groups.**




* indicates p value < 0.05 between two groups.

**Multivariate relationship between AKI and renal outcomes**

When both unadjusted and adjusted for age at ICU admission, gender, study site and number of post-PICU events, patients with AKI stage 2 or worse were significantly more likely to develop CKD or pre-HTN when using SCr-CysC eGFR (crude and adjusted ORs=3.9[95%CI: 1.2-12.5] and 3.5[95% CI: 1.0-12.0], respectively) (Manuscript 2 Table 5). The odds of developing CKD only (SCr-Cysc-eGFR based) were approximately 4 times greater (unadjusted- [95% CI: 1.2-12.8]) and 3 times greater (adjusted- [95% CI: 0.8-10.5]) in AKI stage 2 or worse, statistically significantly different from no AKI/stage 1 AKI (Manuscript 2 Table 5). In adjusted analyses, the presence of any past AKI was not associated with development of renal outcomes (detailed in Manuscript 2 Table 5).

In multiple linear regression analyses, expressing the individual renal outcomes as continuous variables, there was only a statistically significant association between presence of past AKI and lower follow-up eGFR-SCr whether patients with known AKI status or all patients were included (data not shown).

**Manuscript 2 Table 5. Logistic regression analysis of AKI association with renal outcomes.**

| | CKD-Pre-HTN (SCr-eGFR) OR (95% CI) | CKD-Pre-HTN (SCr-CysC-eGFR) OR (95% CI) | CKD (SCr-eGFR) OR (95% CI) | CKD (SCr-CysC EGFR) OR (95% CI) | Pre-HTN or worse OR (95% CI) |
|---|---|---|---|---|---|
| **Known AKI status** | | | | | |
| AKI-unadjusted | 1.14 (0.6-2.3) | 1.1 (0.5-2.4) | 1.2 (0.5-2.6) | 0.9 (0.3-2.5) | 0.9 (0.4-2.2) |
| Adjusted | 0.9 (0.4-1.9) | 0.7 (0.3-1.8) | 0.8 (0.3-2.1) | 0.6(0.2-1.9) | 0.8(0.3-2.2) |
| Known AKI Stage 2 or 3 -unadjusted | 1.8(0.7-4.3) | 3.9(1.2-12.5)* | 1.4 (0.5-3.8) | 3.9(1.1-13.4)* | 1.4(0.5-4.3) |
| Known AKI | 1.8(0.6-4.9) | 3.5(1.0-12.0)* | 1.0(0.3-3.0) | 2.9(0.7-11.6) | 1.8(0.6-6.0) |

| | | | | | |
|---|---|---|---|---|---|
| Stage 2 or worse-adjusted | | | | | |
| **Unknown AKI status** | | | | | |
| Unadjusted | 1.2(0.6-2.3) | 1.1(0.5-2.4) | 1.4(0.7-2.9) | 1.0(0.4-2.7) | 0.9(0.4-2.1) |
| Adjusted | 1.0(0.5-2.0) | 0.8(0.4-1.9) | 1.1(0.5-2.3) | 0.7(0.3-2.1) | 0.9(0.4-2.2) |
| AKI Stage 2 or worse-unadjusted | 1.8(0.8-4.3) | 3.9(1.3-11.9)* | 1.7(0.6-4.3) | 4.0(1.2-12.8)* | 1.4(0.5-4.1) |
| AKI Stage 2 or 3- adjusted | 1.7(0.7-4.3) | 3.4(1.0-11.0)* | 1.1(0.4-3.3) | 3.0(0.8-10.5) | 1.7(0.6-5.3) |

* indicates p value < 0.05 between two groups. Variables adjusted for: age at PICU admission, gender, study site and number of post-PICU events. CI: confidence interval

*5.5 Discussion*

This is the first multicenter follow-up study of ICU children examining AKI as a risk factor for long-term progression to CKD and HTN. We found that patients with AKI had significantly lower SCr-eGFR at follow-up than non-AKI patients. Patients with AKI stage 2 or 3 were more likely to progress to CKD or pre-HTN when using a SCr-based eGFR (not statistically significant). This association was statistically significant and stronger when using the SCr-CysC-based eGFR equation.

We found that certain sociodemographic, ICU diagnostic, and post-ICU factors had a statistical association with AKI and with renal outcomes. Age, study site, sepsis diagnosis, mechanical ventilation, vasopressor use, and nephrotoxic medication use were all associated with AKI. The factors found to be associated with the renal outcomes were: age, lower socioeconomic status, antihypertensive medication use at follow up, and number of health care events since ICU discharge. These variables may

represent important cofounders or effect modifiers of the AKI-outcome association, which will be important to explore in future analyses with the complete dataset.

In this general non-cardiac PICU population, overall high prevalence of pre-HTN and HTN were found, 15.5% and 4.6% respectively. Considering that the Canadian child population pre-HTN and HTN prevalence are 2.1% and 0.8%, respectively [64], our data shows a clinically significant difference between the general pediatric population and children who have been previously admitted to the ICU. Similarly, prevalence of microalbuminuria in the general pediatric population is found to be approximately 11.8%[65], which is in contrast to the slightly higher prevalence of microalbuminuria found in the current study. Although we attempted to reduce the chance of capturing white coat HTN, by performing visits in a calm setting and even in homes, it is possible that that our very high prevalence reported on abnormal blood pressure may at least partially be due to white coat HTN. Future work should confirm our blood pressure findings using gold standard blood pressure measures such as 24 hour ambulatory blood pressure monitoring. Our estimation of albuminuria prevalence may have also been inflated by the presence of postural proteinuria. However, reported pediatric population prevalence of albuminuria have not accounted for this phenomenon either. Nevertheless, future studies should consider evaluating first morning urine for albuminuria, in order to reduce the potential postural effect on urinary protein excretion.

There have been several pediatric studies providing information on long-term renal function after different types of AKI in either PICU or general hospital-admitted children. More recently a retrospective study done by Mammen[115] in PICU children, including cardiac surgery patients, and *only* including patients who had AKI in PICU, showed that 49% had CKiD eGFR below 90 ml/min/1.73m$^2$, and 10% and 3% of patients had albuminuria and HTN, respectively. Another retrospective PICU study done by Viaud[68] in children with severe AKI requiring renal replacement therapy demonstrated even higher prevalence of renal outcomes, with 61%, 15% and 54% attaining CKiD eGFR below 90 ml/min/1.73m$^2$, HTN and proteinuria, respectively. However these studies did

not evaluate renal function in non-AKI patients. Only one study by Zwiers et. al.[66] considered the non-AKI patients in order to evaluate odds of developing CKD and HTN. They found that 5% of their population had SCr-eGFR below 90 ml/min/1.73m$^2$ , 21% had HTN and 15% had proteinuria. However due to the specific population studied (neonatal extracorporeal membrane oxygenation survivors), results cannot be extrapolated to the general pediatric ICU population. Data on the long-term renal consequences of AKI from various causes, independent of previous renal impairment, are limited in children.

One of the major findings of this study was that AKI stage 2 or worse was significantly associated with many of the renal outcomes: CKD and CKD or pre-HTN (logistic regression and significant difference in proportions), and CKD or HTN (significant difference in proportions). We also observed a clinically relevant increase in prevalence of pre-HTN in AKI stage 2 or worse vs. no AKI or stage 1. These findings suggest that risk stratification based on AKI stage are important in order to allow clinicians to focus on patients with more severe stages of AKI when considering which patients to follow up post-ICU. Another major finding was that results on AKI-outcome associations were stronger when the combined SCr-CysC eGFR equation was used as opposed to the SCr-eGFR formula. For example, AKI Stage 2 or 3 (adjusted) was significantly associated with CKD or pre-HTN using the combined formula (OR: 3.5 [95% CI:1.0-12.0], p-value<0.05) vs. when GFR was estimated using SCr alone (OR: 1.8 [95% CI: 0.7-4.3], p-value>0.05) (Manuscript 2 Table 5). Similarly, the rates of composite renal outcomes were significantly higher in AKI stage 2 or worse when using the combined eGFR formula (Manuscript 2 Figure 2b) as opposed to the SCr-based formula (Manuscript 2 Figure 2a). This suggests that researchers should incorporate CysC to estimate GFR alongside the SCr-based equation in future studies evaluating renal function. If a stronger association were found when using CysC, then this would provide a rationale for using CysC in clinical settings. The stronger association when adding CysC to the equation is likely due to SCr inaccurately estimating GFR, resulting in misclassification of CKD.

There are limitations to our study. The first is that there may be a selection bias in the mailing cohort associated with characteristics of patients who chose to respond. Future analyses will include a comparison of responders vs. non-responders on basic characteristics including age, gender and past AKI status. As described above, we cannot be certain that our urinary albumin results were not substantially affected by postural proteinuria. Also mentioned above, blood pressure measurements were not standardized to time of day and were only taken at one time point (though with 3 measures). Ideally, blood pressure would be evaluated during three separate visits, but feasibility in the research setting limits this procedure. Although our sample size is relatively large compared to many published pediatric studies, the current sample size was still limiting in terms of being able to perform subgroup analyses and control for a higher number of confounders. One of the most important limitations, however, was that a large proportion of our subjects had unknown AKI status, simply because SCr was not measured during PICU admission. This limitation is impossible to overcome. We assumed non-AKI status in these patients, since it is highly likely that they were simply not of high enough illness severity to warrant SCr measurement. Once the index ICU data collection is complete, we will be able to verify this hypothesis. It is likely that this assumption led to at least some misclassification, which would likely favour our results towards the null hypothesis (no association of AKI and renal outcomes).

### 5.5.1 Conclusion

Prevalence of CKD ad HTN is high in patients who have been previously admitted to the ICU. Although there is still much research needed to identify risk factors for CKD and HTN development, there appears to be a strong association with development of Stage 2 or worse AKI and later development of renal disease, confirming what animal studies and studies in adults have shown. There is a need to begin following targeted patients for renal outcomes after ICU admission, in order to detect CKD and HTN early and reduce cardiovascular risk. At the present time, it appears reasonable to focus on children with AKI Stage 2 or worse. Future research will hopefully refine the target

population for follow-up thereby considering the impact of proposing follow-up care on the patients, families and health care systems.

*5.6 Post-manuscript reflection*

This study is almost complete. What remains are approximately 50 study visits and completion of the ICU index admission data. When full data is collected, we will have a large enough sample size to be able to examine various ICU factors for confounding and effect modification, and potentially guide the development of an algorithm for post-ICU follow-up. In other words, we hope to be able to identify both AKI and non-AKI risk factors for long-term renal outcomes, so that ICU physicians may be able to identify which patients are at highest risk and should be followed up for renal function. This study may also help guide future AKI clinical trials on whether CKD and HTN should be included as outcomes. Our goal is to ultimately translate this knowledge into guidelines of post-ICU follow-up of renal function and blood pressure monitoring in children.

## Chapter 6: Conclusion

*6.1 Conclusion*

The aims of this thesis were fourfold: 1. To review the current state of knowledge surrounding AKI and renal outcomes in children; 2. To estimate the prevalence of CKD and HTN 6 years post-ICU admission; 3. To evaluate the relationship between AKI in the ICU and long-term CKD and/or HTN; 4. To evaluate other ICU/non-AKI factors which may be associated with AKI and/or long-term renal outcomes. The main knowledge gaps on this topic were described in chapter 2 (literature review), while the manuscript in chapter 5 attempted to address these gaps and describe how they can be better addressed in the future through further analyses and studies. The manuscript addresses aims 2 to 4 of this thesis, however the findings of the study have led to further questions that need answering. In order to describe these questions, it is necessary to first recapitulate the main findings of the study.

### 6.1.1 Statement of novelty and significance of findings

This is the first body of work to evaluate AKI during ICU stay as an independent risk factor for long term progression to CKD and HTN in non-cardiac PICU children. The main findings are that patients with AKI stage 2 or worse are significantly more likely to develop CKD (by SCr-CysC based eGFR) and pre-HTN approximately 6 years following ICU admission, even after adjusting for important confounders (Manuscript 2 Table 5). Patients with AKI had statistically significantly lower SCr-eGFR, and (non-significant) higher rates of abnormal SCr-eGFR, microalbuminuria and HTN (Manuscript 2 Table 4a). Although the mean eGFR for each group was still within the normal range, that of the AKI group was significantly lower than that of the non-AKI group. This suggests that eGFR may continue to decline over time and the disparity between AKI and non-AKI patients may grow. Similarly, although not statistically significant, the prevalence of eGFR below 90 was almost twice as high in the patients with AKI when status was known, suggesting that AKI might be contributing to this late CKD and HTN development.

The prevalence of pre-HTN and HTN in the entire study population was, respectively, around seven times and six times higher than what is expected in the general pediatric population; Canadian child population pre-HTN and frank HTN prevalence is 2.1% and 0.8%, respectively [64]. Higher prevalence of pre-HTN or worse was found in patients with AKI stage 2 or worse, and logistic regression showed that these patients were more likely to develop pre-HTN or worse at 6-year follow-up, even when adjusting for important confounders. Although not statistically significant, the above-mentioned findings are clinically relevant and suggest the need for more screening of HTN in this patient population and appropriate interventions (lifestyle interventions or HTN medication).

One of the major findings of this study was that AKI stage 2 or worse was significantly associated with many of the renal outcomes: CKD and CKD or pre-HTN (logistic regression and significant difference in proportions), and CKD or HTN (significant difference in proportions). There was also a clinically relevant increase in

prevalence of pre-HTN in AKI stage 2 or 3 vs. no AKI or stage 1. These findings imply an AKI-CKD/HTN relationship, and also suggest that risk stratification based on AKI stage is important in order to allow clinicians to focus on patients with more severe stages of AKI when considering who to follow up post-ICU. Another major finding was that several results were stronger when the combined SCr-CysC eGFR equation was used as opposed to the SCr-eGFR formula. For example, AKI Stage 2 or 3 (adjusted) was significantly associated with CKD or pre-HTN using the combined formula (OR: 3.5, p-value<0.05) vs. when GFR was estimated using SCr alone (OR: 1.8, p-value>0.05) (Manuscript 2 Table 5). Similarly, the rates of composite renal outcomes were significantly higher in AKI stage 2 or 3 when using the combined eGFR formula (Manuscript 2 Figure 2b) as opposed to the SCr-based formula (Manuscript 2 Figure 2a). This suggests that researchers should incorporate CysC to estimate GFR alongside the SCr-based equation in future studies evaluating renal function. If a stronger association were found when using CysC, then this would provide a rationale for using CysC in clinical settings. The stronger association when adding CysC to the equation is likely due to SCr inaccurately estimating GFR, resulting in misclassification of CKD.

6.1.2 Strengths and limitations

The strength of this study lies in the novelty of being the first study to examine AKI as an independent risk factor of CKD and HTN in non-cardiac ICU children. Because this ICU population is varied, our results will be able to be generalised to pediatric ICU populations. Also, extensive data collection at the time of the ICU admission will ensure that all relevant confounders and effect modifiers are included in the final data analysis such that our findings will be able to confidently establish whether AKI is a risk factor for long-term renal outcomes, independent from other ICU factors. Due to our incomplete data, we were unable to test for univariate associations between all potential confounders or effect modifiers and renal outcomes. However, we found that certain socio-demographic, ICU diagnostic, and post-ICU factors had a statistical association with AKI and with renal outcomes, and factors deemed most important were included in our regression models.

There are limitations to our study. The first is that there may be a selection bias in the mailing cohort associated with characteristics of patients who chose to respond. Future analyses will include a comparison of responders vs. non-responders on basic characteristics including age, gender and past AKI status. Also, as mentioned above, we cannot be certain that our urinary albumin results were not substantially affected by postural proteinuria. Furthermore, blood pressure measurements were not standardized to time of day and were only taken at one time point (though with 3 measures). Ideally, blood pressure would be evaluated during three separate visits, but feasibility in the research setting limits this procedure. Although our sample size is relatively large compared to many published pediatric studies, the current sample size was still limiting in terms of being able to perform subgroup analyses and control for a higher number of confounders. One of the most important limitations, however, was that a large proportion of our subjects had unknown AKI status, simply because SCr was not measured during PICU admission. This limitation is impossible to overcome. We assumed non-AKI status in these patients, since it is highly likely that they were simply not of high enough illness severity to warrant SCr measurement. Once the index ICU data collection is complete, we will be able to verify this hypothesis. It is likely that this assumption led to at least some misclassification, which if anything would likely favour our results towards the null hypothesis (no association of AKI and renal outcomes).

### 6.1.3 Future directions

The next step for this study is to complete data collection of both follow-up visits and index ICU admission data. When full data is collected, we will have a big enough sample size to better control for important covariates, including measures of illness severity such as Pediatric Risk of Mortality score (PRISM) and past medical history. We will be able to explore evidence of confounding and effect modification with various ICU factors. This can lead to future work on potentially developing an algorithm for post-ICU follow-up. The rationale would be that when a patient leaves the ICU, it would be beneficial for clinicians to identify who should be followed up. One approach is to see if there are other factors pre-ICU or during ICU which can help refine who should be

followed up. If everyone with a rise in SCr is followed up, they might not necessarily need it and it would be a waste of resources. Thus, we should explore what past medical history, ICU and diagnostic variables are associated with renal outcomes, with the goal of developing future prediction algorithms.

As an extension of the current study, we are currently collecting first morning urine on this population to try to correct for the potential effect of postural proteinuria. Additionally, we are performing 24-hour ambulatory blood pressure monitoring on participants to verify the true prevalence of pre-HTN and HTN in this population. Once we understand if and to what extent AKI leads to CKD and HTN in children, we can begin to study predictors of CKD after AKI and help guide future AKI clinical trials on whether CKD and HTN should be included as outcomes. Our goal is to ultimately translate this knowledge into guidelines of post-ICU follow-up of renal function and blood pressure monitoring in children, thereby improving their quality of life.

As described above, determining whether AKI leads to CKD and HTN is an important goal in understanding the natural history of AKI and whether renal function should be followed and managed after discharge from an AKI-hospitalization. Ideally, follow-up after AKI could be performed in a targeted fashion. It would be reasonable to select patients with a given AKI severity threshold (defined by SCr rise) for follow-up. However, because SCr is flawed as a marker of renal function, novel renal injury biomarkers have recently been studied as diagnostic tests of AKI to improve accuracy and timeliness of AKI diagnosis[121] . Future studies based on the current work can examine urine biomarkers' ability to predict long-term renal damage. These biomarkers are mainly urine proteins shed by renal tubular cells in response to renal injury. Two of these biomarkers are urine neutrophil gelatinase-associated lipocalin (uNGAL) and kidney injury molecule-1 (uKIM-1), both of which are strongly associated with the severity of acute renal injury, as measured by magnitude of acute SCr rise[122]. uNGAL, a small lipocalin, is upregulated in the proximal and distal tubules of the nephron after ischemic or nephrotoxic injury [123]. Its potential protective effect entails chelating iron from damaged tubules, preventing free radical formation and cell death. A study done in

ICU children demonstrated that concentrations of uNGAL on the first day of AKI evidence predicted persistent SCr elevation for >48 hours[124]. Similarly, in children undergoing cardiac surgery, the first postoperative uNGAL levels were strongly associated with severe AKI[125]. uKIM-1 is a proximal tubule transmembrane protein also highly upregulated in AKI [126]. KIM-1 promotes apoptotic and necrotic cell clearance. It has been shown that post cardiac surgery elevations of KIM-1, in combination with other biomarkers, were associated with AKI and adverse outcomes[127]. Furthermore, in hospitalized adults with AKI, KIM-1 concentrations predicted the need for dialysis [128]. If AKI leads to chronic fibrosis, and ultimately CKD, then the urine biomarker concentrations at AKI onset may predict risk for long term renal damage and CKD development. In other words, a patient with worse renal tubular injury and thus higher urine biomarker concentrations at AKI onset, may be more likely to progress to CKD in the long term. In fact, uNGAL and uKIM-1 have been shown to be elevated in children with CKD and in patients with kidney graft fibrosis[129-132] . Similarly, it has been demonstrated in adults undergoing cardiac surgery at high risk for AKI, that elevated levels of urinary kidney injury biomarkers, including uNGAL and uKIM-1, are independently associated with long-term mortality [133]. If urine kidney injury biomarker concentrations from the time of AKI insult during ICU stay can predict long-term CKD and HTN, this information would allow for the development of a diagnostic test to evaluate long-term risk and implement appropriate prevention.

Another area of future research is to examine CysC's role in predicting long-term renal outcomes after ICU admission in children. As demonstrated by our findings, SCr-CysC-eGFR is more strongly related to renal abnormalities then SCr alone. Accordingly, it is likely a more valid measure of renal function and can be used as a screening tool for CKD when evaluating the long-term renal outcomes of AKI during ICU stay.

### 6.1.4 Final Summary

High prevalence of renal outcomes is seen post-ICU in patients with AKI, and the odds of developing CKD or HTN are greater in those with more severe AKI. However, we still cannot say for certain that AKI is independently associated with these outcomes. It

is unlikely that AKI alone is associated with long-term renal outcomes; there may be other factors from the ICU that can help predict outcomes and help guide clinicians on how to follow up children after they are discharged from the ICU. The importance of focusing on patients with higher stages of AKI and also on incorporating CysC in GFR equations when evaluating renal outcomes is highlighted in the current work. Future research will hopefully refine the target population for follow-up thereby considering the impact of proposing follow-up care on the patients, families and health care systems.

**References to chapter 2, 4, 5 and 6**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