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NGAL as a Novel Acute Kidney Injury Biomarker: A Narrative Review

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Abstract

Acute kidney injury (AKI) is common in children, particularly in the setting of pediatric intensive care. Independently of the underlying etiology, AKI has been associated with progression to chronic kidney disease. The current definition used for AKI diagnosis is based on serum creatinine elevation and decreased urinary output, which are delayed and unreliable phenomena. This prevents a rapid recognition of AKI and an appropriate management.

This thesis focuses on Neutrophil-Gelatinase Associated Lipocalin (NGAL) as a marker of AKI in children. First studied in mice models, it was discovered to be a part of innate immune antibacterial mechanisms and involved in nephrogenesis and organ regeneration. Animal studies also reported that NGAL is upregulated on the kidney after an ischemic or nephrotoxic insult.

Later, pediatric studies were performed with populations with different ages and diseases to determine the clinical value of urinary NGAL for the early detection of AKI. It was possible to detect the rise of urinary NGAL significantly before serum creatinine elevation, encouraging its use as a biomarker for early diagnosis of AKI.

Currently, NGAL is already being used in the clinical setting as a marker of AKI. This biomarker is a valuable tool to help the physician on the decision of starting renal replacement therapy.

In the future, the use of NGAL in combination with other biomarkers will allow not only an early recognition of AKI, but also the possibility of distinguishing the injured cell types, the underlying mechanisms and, consequently, allocate the most adequate treatment. This will be a game changer in the way we treat AKI and hopefully it will translate in a better long-term prognosis.

The main aim of this thesis is to review the clinical utility of NGAL as a biomarker of AKI in children.

Keywords: Acute Kidney Injury, NGAL, biomarkers.

Resumo

A Lesão Renal Aguda (LRA) é comum em pediatria, particularmente em crianças gravemente doentes internadas em unidades de cuidados intensivos pediátricos. Independentemente da etiologia subjacente, a LRA tem sido associada a progressão para Doença Renal Crónica. As definições atuais têm como base o aumento da creatinina sérica e a diminuição do débito urinário que, sendo marcadores tardios e pouco fiáveis, impedem o rápido reconhecimento desta doença e, subsequentemente, o início precoce de tratamentos apropriados.

Esta tese analisa a evidência científica sobre o papel do NGAL (*Neutrophil-Gelatinase Associated Lipocalin)* na LRA pediátrica. O NGAL foi primeiramente descrito em murinos, identificando-se a sua participação no sistema imunitário inato, na nefrogénese e na regeneração/reparação do tecido renal lesado. Os estudos animais identificaram a sua sobre-expressão no rim após um dano isquémico ou nefrotóxico. Posteriormente, foram elaborados estudos em crianças com diferentes idades e patologias, para determinar o valor clínico do NGAL urinário (uNGAL) para a deteção precoce de LRA. Foi possível identificar uma elevação do uNGAL significativamente antes da elevação da creatinina, o que encoraja a sua utilização como um biomarcador para a deteção precoce de LRA.

Atualmente, o uNGAL é já utilizado na prática clínica diária nalguns centros como marcador de LRA. Este biomarcador é uma ferramenta muito útil para a decisão de iniciar uma técnica de substituição da função renal.

No futuro, a utilização do uNGAL em combinação com outros biomarcadores permitirá não só o reconhecimento precoce da LRA, mas também a possibilidade de distinguir quais os tipos de células lesadas, os mecanismos subjacentes e, consequentemente, a melhor forma de tratamento. Esta análise permitirá revolucionar a forma como tratamos a LRA e melhorará o prognóstico a longo prazo destes doentes.

Esta tese tem como objetivo rever a utilidade clínica do NGAL como marcador de LRA em idade pediátrica.

Palavras-chave: Lesão Renal Aguda, NGAL, biomarcadores.

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Abbreviatures

AKI – Acute Kidney Injury
BW – Birth Weight
CKD – Chronic Kidney Disease
CPB – Cardiopulmonary Bypass
CysC – Cystatin C
GFR – Glomerular Filtration Rate
IGFBP-7 – Insulin Growth Factor Binding Protein 7
IL-18 – Interleukin 18
KIM-1 – Kidney Injury Molecule 1
L-FABP – Liver-Fatty Acid Binding Protein
NGAL – Neutrophil-Gelatinase Associated Lipocalin
NPV – Negative Predictive Value
NR – Not Reported
PICU – Pediatric Intensive Care Unit
PPV – Positive Predictive Value
RAI – Renal Angina Index
ROC-AUC – Receiver Operating Characteristic - Area Under the Curve
RRT – Renal Replacement Therapy
sCr – Serum Creatinine
TIMP-2 – Tissue Inhibitor Metalloproteinase 2
UO – Urinary Output
WOL – Weeks of Life

I. Introduction

Acute Kidney Injury (AKI), an abrupt renal dysfunction, is a common condition, particularly in critically ill children. It occurs in approximately one third of the children admitted to a Pediatric Intensive Care Unit (PICU), with 11 to 12% having severe AKI[1,2]. Regarding neonates admitted to a Neonatal Intensive Care Unit (NICU), the overall incidence of AKI is 30%, but it differed according to the gestational age (GA) (22 to 29 weeks – 48%; 29 to 36 weeks – 18%; >36 weeks – 37%).[3] In children submitted to cardiac surgery, AKI is a major complication, occurring in 40 to 60% of patients.[4,5] Outside the intensive care setting, AKI is also common. In children admitted to a Pediatric Emergency Department approximately 1.5 to 10.4% have AKI.[6,7]

AKI results more often from systemic diseases or its treatments than from a primary renal disease.[8,9] An insult to the kidney leads to acute renal damage, with decrease in glomerular filtration rate (GFR) and, consequently, hydroelectrolytic disturbances, retention of waste products, nutritional deficits and changes on medications' metabolism. One of those insults is the use of drugs with nephrotoxic effects, like antibiotics and nonsteroidal anti-inflammatory drugs, which are commonly used in clinical practice. A study showed that 86% of children admitted to a non-intensive care unit were exposed to, at least, one nephrotoxic medication.[10] Moreover, the children who developed AKI had exposure to a higher number of nephrotoxic drugs.[10] It was demonstrated that the use of more than three nephrotoxic drugs was associated with the development of AKI.[10] The NINJA (Nephrotoxic Injury Negated by Just-in-time Action) Program, developed at the Cincinnati Children's Hospital, shows the importance of nephrotoxicity in the pathogenesis of AKI. This Program is based on the use of informatic tools for the identification of children who are treated with aminoglycoside antibiotics for three or more days or who are exposed to three or more nephrotoxic drugs. If a child has one of these risk factors, their doctors receive an automatic alarm message and creatinine is evaluated daily. Three years after the implementation of this program there was a decrease of 38% in the use of nephrotoxic drugs and, most importantly, a 64% decrease in the rate of AKI.[11] This program was expanded to nine pediatric institutions. Five centers were able to reduce their AKI rates, achieving levels below 1.3 episodes per 1 000 patient per day, supporting the use of this method to avoid preventable nephrotoxic burden.[12]

Regardless of the etiology, AKI is a risk factor for several adverse outcomes, including prolonged hospital stay, prolonged mechanical ventilation, increased risk of renal replacement therapy (RRT) and death.[1,3,4,13] AKI is also associated with increased risk for developing hypertension, proteinuria and CKD, including CKD stage 5.[14–16] Severe AKI and the necessity of RRT are associated with a higher mortality rate,[1,3,13] which can reach 43% at three to five years after an AKI episode, including initial hospitalization mortality.[14] Considering long-term outcomes, a retrospective single-center cohort study in Cincinnati, with 100 children with nephrotoxic-associated AKI, reported that 34% had CKD and more 36% were at risk of developing it, in a six month follow-up.[16] In a Canadian cohort of 126 patients followed for a period of one to three years after the AKI episode, 10% of the patients had CKD and 47% were at risk of developing it.[15] In addition, a cross sectional study reported that 17 of the 29 patients with AKI (59%), at a 3 to 5 years follow-up, had signs of renal injury, with only six of them being followed by a nephrologist.[14] Whereas the Cincinnati retrospective study described renal injury signs in 70% of the children, with only 19% of them having follow-up with a pediatric nephrologist.[16]

Several definitions and classification systems have been developed for AKI, such as the RIFLE Criteria (Risk, Injury, Failure, Loss, End-Stage Renal Disease), in 2004, where the first three classes correspond to a progressive increase of severity and the last two to outcomes, defined by the length of kidney function loss.[17] Furthermore, this definition was later modified to better suit the pediatric population – pRIFLE – which was shown to predict mortality, length of PICU stay and RRT requirement in critically ill children.[18] Later, AKIN Classification (Acute Kidney Injury Network) was created, classifying AKI in three stages of increasing severity.[19] In 2012, the KDIGO Guideline (Kidney Disease Improving Global Outcomes) was an attempt to combine AKIN and pRIFLE to better define AKI in adults and children.[20] (Figure 1) This is, nowadays, the most frequently used definition. AKI is defined as any of the following:

- Increase in SCr by ≥ 0.3 mg/dl (≥ 26.5 μmol/l) within 48 hours; or

- Increase in SCr to ≥ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; or
- Urine volume <0.5 ml/kg/h for 6 hours

This definition has been validated for adults and children, except for neonates where defining AKI is more unclear.

The diagnosis of AKI in children by comparing pRIFLE, AKIN and KDIGO reported an inter-definitions agreement between 85% and 97%, as well as an AKI stage agreement between 77% and 93%. The pRIFLE had higher sensitivity, while the AKIN was more specific.[13]

The factors used to define AKI are common throughout the definitions above – serum creatinine (sCr) and urinary output (UO). GFR is widely accepted as the most useful index of kidney function. Nonetheless, it is difficult to measure directly and it is estimated through sCr, according to Schwartz Equation.[20] AKI is still difficult to identify, with 37% of AKI patients underdiagnosed.[6] This is likely because most children do not have a baseline sCr and UO is infrequently assessed. Moreover, sCr alone failed to diagnose AKI in 2/3 of patients with low urinary output.[1,3]

Creatinine is a delayed and imprecise biomarker, that marks glomerular function instead of glomerular damage. It rises 48 to 72 hours after the initial insult and only when about 50% of nephrons are not functioning properly. When AKI occurs, creatinine is not only filtered, but also secreted by the tubules. In this setting creatinine is not an accurate marker of the GFR and it is going to underestimate the severity of the lesion. Moreover, its concentration is influenced by non-renal factors, such as age, race, gender, muscle mass and hydration status.[21] Besides this, since it crosses the placental barrier, there is a maternal-fetal transfer that justifies the high sCr levels in the first days of life of newborns that progressively decline as GFR concomitantly increases over the first year of life. Additionally, since nephrogenesis continues until 34 gestational weeks (GW), premature neonates can present further discrepant GFR so that changes in sCr can be even more challenging to interpret.[22,23]

Defining AKI through UO also has disadvantages since severe AKI can happen even with normal UO, particularly in neonates and associated with nephrotoxicity.[16,21,23] The disadvantages mentioned above magnify the challenge of accurately diagnosing AKI, especially in the neonate population.

In conclusion, AKI has a high incidence in the severely ill children, although it is still underdiagnosed and only few of the affected children are followed by a pediatric nephrologist. Additionally, the disadvantages of the currently used AKI definitions and biomarkers make it urgent to identify novel biomarkers able to identify AKI earlier. Several kidney proteins, with low serum concentration in healthy state, have been studied as potential early AKI biomarkers and predictors of short and long-term outcomes.

The ideal biomarker should rise early in the course of the disease, be sensitive and specific, have established cut-off ranges, be minimally invasive, and of low-cost. It is also desirable that this novel biomarker allows for risk stratification, perception of damage degree and its results predict clinical outcomes and response to therapy.[24]

It is important to study AKI and these novel biomarkers in children apart from the adults, because of differences in epidemiology and comorbidities that can stand as additional risk factors for AKI and confounders in biomarkers performance.

Since there are innumerous underlying pathways resulting in AKI, according to the etiology, it is possible that there is also a myriad of biomarkers increased after a kidney damage, which can rise at different time points reflecting their involvement in the pathophysiology. It is crucial to recognize the state of the art on this matter in order to encourage more research and widespread clinical assessment of these novel biomarkers, contributing to improve AKI diagnosis and pediatric outcomes.

II. Methods

The research was performed using MEDLINE®/PubMed® from January 2000 to December 2020, using the following keywords: “NGAL”, “Neutrophil gelatinase-associated lipocalin”, “acute kidney injury”, “AKI”, “child*”, “pediatric*”, “adolescent”, “biomarkers”, “diagnosis*” and “prognosis*”. The research was limited to studies published in English. Clinical studies, as well as systematic reviews that assessed NGAL as a biomarker of AKI in children were included. Studies were excluded if the focus was not on AKI. The results obtained were screened through the title and abstract to identify if the inclusion criteria were fulfilled.

Tables regarding both urinary and plasmatic NGAL characteristics were elaborated taking in consideration the studies analyzed, including ideal cut-off, area under the Receiver Operating Characteristic curve (ROC-AUC), sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

III. NGAL as a Novel Biomarker

NGAL (Neutrophil gelatinase-associated lipocalin) is a protein of 25-kDa, protease resistant and a member of the lipocalin family of transport proteins. It was first described in 1993 in activated human neutrophils, associated to gelatinase B in secondary granules.[25] It is also synthesized in other tissues in limited quantities, such as salivary glands, stomach, colon, trachea and lungs.[26] It appears to be a part of the innate immune system iron-depletion antibacterial strategy by sequestering bacterial ferric siderophores.[27]

This protein also has a role in kidney development. It induces the formation of epithelium from the metanephric mesenchyme by transporting iron to regulate gene expression. Additionally, it decreases hepatocyte growth factor downstream signaling, which results in reduced cell migration and branching, and correct tubulogenesis.[28,29] These developmental mechanisms might also be activated as a response to kidney injury, to reconstitute previous normal morphology.[30]

1. Studies in animal models

In 2003, NGAL was identified as one of the earliest proteins expressed in mouse kidneys after ischemia. To determine if NGAL protein was overexpressed in the post-ischemic kidney, as well as present in urine, several experiments were performed in different models.[30]

Mice were submitted to unilateral (left renal pedicle clamped for 45 minutes), bilateral (both kidneys occluded for 30 minutes) and mild subclinical (bilateral kidney clamping for 5, 10 or 20 minutes) renal ischemia. NGAL was considerably overexpressed in the kidneys within three hours (h) after insult, in both unilateral and bilateral renal ischemia experiments. However, NGAL expression was quantitively higher in the bilateral ischemia model in comparison to the unilateral. The bilateral ischemia model showed that NGAL peaked at 24h and returned to normal levels by 72h.[30]

In the mild subclinical renal model, urinary NGAL (uNGAL) was also detected, even though the timing of its presence was correlated with the duration of renal ischemia (10- and 20-min ischemia - after four hours; 5-min - after six hours). NGAL was markedly expressed in the cytoplasm of proximal tubular cells, without any evidence of

NGAL-expressing neutrophiles. Finally, it was also detected as co-located with proliferating cell nuclear antigen, suggesting that the damaged cells were proliferating and regenerating at 48h post-ischemia.[30]

Mouse models were also used to study NGAL presence after nephrotoxic injury. Daily urine collections after cisplatin (Cis)-induced nephrotoxicity reported the presence of uNGAL since day 1 after insult.[30] In another study, NGAL kidney expression and uNGAL were induced three hours after Cis high dose administration (20 mg/kg), peaking at 48h and remaining high until post-96h. Whereas, sCr was only significantly increased after 96h. Similarly, NGAL was only expressed in the proximal tubules.[31]

In a subclinical nephrotoxic injury model submitted to a low dose of Cis (5 mg/kg instead of 20 mg/kg), uNGAL was present within three hours, as well. However, it appeared to be in quantitatively lower levels, suggesting a Cis dose-dependent response.[31]

Additionally, NGAL was studied in cultured human proximal tubule cells incubated with an adenosine triphosphate (ATP) reductor, simulating ischemia *in vitro*. Prior to ischemia, NGAL was already present in resting cells, but not in the medium. After ATP depletion, protein expression increased within 1h post-ischemia, both in cell pellets and in the medium. These results confirmed that human proximal tubular cells increase NGAL synthesis and secretion after ischemia, similar to what was observed in mouse models.[30]

In 2011, a study tried to better understand NGAL gene and protein expression at real time in mouse models. A double-fusion reporter gene encoding Luciferase-2 (Luc2) and mCherry (mC) was knocked in the NGAL locus. It allowed the identification of damaged sites *in vivo* and in real-time through bioluminescence and fluorescence, respectively. In addition, they reported that neutrophil deletion did not affect NGAL expression in any model.[32]

Heterozygous NGAL-Luc2-mC mice submitted to unilateral renal ischemia (15 or 30 min) showed an increased response within 3 to 6h after the insult. This response peaked at 12h, with a 25 to 80 times higher intensity. Kidney NGAL-Luc2-mC response was dose-dependent, with higher intensity levels for 30 min of ischemia. Similar NGAL results were observed after 15 min bilateral ischemia and after Cis exposure. NGAL-Luc2-mC signaling was detected in the thick ascending loop of Henle and intercalated cells of the collecting ducts. This response and uNGAL concentrations were correlated, suggesting that the protein was originated from the kidney. These results suggest that NGAL kidney expression and uNGAL can early detect AKI.[32]

To confirm that uNGAL was synthetized in the kidney, cross-transplants were performed between NGAL knockout (NGAL-/-) and wild-type (NGAL+/+) mice. NGAL mRNA and protein expression were augmented in NGAL+/+ kidneys regardless of the host type, confirming uNGAL origin in the kidney epithelia. There was only a mild rise in NGAL+/+ hosts with NGAL-/- kidneys. The authors suggest that plasma NGAL (pNGAL) from other organs could be responsible for these low uNGAL levels detected.[32]

2. Studies in children submitted to cardiac surgery with cardiopulmonary bypass

The first pediatric studies about NGAL were in children submitted to cardiac surgery with cardiopulmonary bypass (CPB). Renal ischemia-reperfusion injury occurs frequently after this procedure. Therefore, AKI cause and time of insult is known in a subpopulation with few confounders.[33]

Most studies reported significantly higher uNGAL and pNGAL levels within 2h after cardiac surgery in children who developed AKI. NGAL levels remained high until different timepoints according to the study, ranging between 15h to 5 days post-CPB. In opposition, sCr increased only 24 to 72h after surgery.[4,33–40] Some studies also observed higher NGAL levels in the first hours after CPB in children without AKI. However, these values were markedly lower than in the AKI group and returned to baseline levels 2 to 12h post-CPB.[34,35,41,42]

In a prospective single-center study from 2005, 71 children submitted to CPB were analyzed. In the AKI group, uNGAL levels peaked (mean 147 microg/L) within 2h after CPB and remained persistently high until 5 days post-insult. pNGAL performed similarly to uNGAL, despite achieving quantitatively lower peak levels (mean 61 microg/L). The amount of uNGAL at 2 h after cardiopulmonary bypass was the most powerful independent predictor of AKI. For NGAL urinary concentration at 2 h, the area under the receiver-operating characteristic curve was 0.998, sensitivity was 1.00, and specificity was 0.98, for a cutoff value of 50 microg/L.[33]

A single-center study with 196 children revealed high uNGAL levels until 48h after CPB in the patients with AKI. At the 6h timepoint, uNGAL concentration increased 26-fold (mean >1000 ng/mL) from baseline and was the best independent predictor of AKI (AUC of 0.98).[41]

A prospective multicenter study of 311 children showed a lower discriminative ability of NGAL. uNGAL had an AUC of 0.71 for predicting severe AKI and 0.67 for mild AKI. Despite this, an uNGAL value above 72 ng/mL in the first 6h post-op was associated with a fivefold increased probability of pRIFLE injury (pRIFLE-I) AKI in comparison with the lowest quintile (< 3 ng/mL). There was no association of pNGAL with severe AKI and only a weak association with mild AKI.[34] Similarly, a single-center study with 112 patients reported that pNGAL change after surgery was not statistically significant in the AKI group.[43] The neonates included had statistically significant uNGAL levels only after 6h post-surgery, instead of after 2h like in older children. uNGAL was a predictor of AKI at all timepoints, including pre-surgery, as children who developed severe AKI had significantly higher pre-operatory levels.[43] On the other hand, more recent single-center studies reported a good discriminative ability of pNGAL to predict AKI. In a cohort of 120 subjects, Dent *et al.* showed significantly high pNGAL levels in children with AKI until 24h after surgery. The biomarker had good predictive ability with an AUC of 0.96 at 2h post-CPB.[35]

Krawczeki *et al.* compared pNGAL and uNGAL in 35 neonates and 338 non-neonates (between 1 month to 18 years of age). Both markers were substantially increased in children with AKI in the two groups until 48h after CPB. In the non-neonate group, uNGAL concentrations increased with worsening severity. The discriminative ability was powerful for both biomarkers in the two groups. At the 2h timepoint, for every increase of 10 ng/mL in pNGAL there was 47% higher odds of developing AKI, whereas the same increment in uNGAL levels corresponded to the odds increasing by 32%.[36]

Reiter *et al.* showed contradictory results in neonates and infants after cardiac surgery. There was no significant difference in pNGAL or uNGAL between the AKI group and those without it. Despite that, after CPB uNGAL and pNGAL had a significant rise from baseline levels across the 59 subjects. The authors suggested that these higher levels reflected inflammation from CPB, especially because pNGAL levels were correlated with interleukin-6, a marker of inflammation. They also report shorter CPB times which can have an impact on AKI severity phenotype and, consequently, on NGAL levels.[44]

In a cohort of 106 children, Alcaraz *et al.* described the earliest increase in uNGAL levels after CPB. Levels were statistically significant from 1h post-insult in patients who had AKI and started to decrease at 15h. The biomarker had good predictive ability for AKI development at all post-op timepoints. Moreover, it was analyzed the impact of adding uNGAL to a clinical model, which included age, CPB time, total circulatory arrest use and Risk Adjustment for Congenital Heart Surgery. This combination increased the model diagnostic ability.[42]

In a study of 220 children, increasing concentrations of uNGAL were associated with higher pRIFLE stages. uNGAL also showed good diagnostic performance at all timepoints. The addition of other novel biomarkers to uNGAL increased the predictive ability, except for the 2h timepoint where uNGAL alone was the best predictor. The best performances were at 6h post-CPB for the combination of NGAL+Interleukin-18 (IL-18) and at 12h and 24h for the combination of NGAL+IL-18+Liver Fatty Acid-Binding Protein (L-FABP)+Kidney Injury Molecule 1 (KIM-1).[45]

The impact on AKI diagnostic accuracy of combining uNGAL with other novel biomarkers was evaluated in 150 children. There was an improvement at 12h post-CPB when combining NGAL+IL-18 or NGAL+IL-18+Tissue Inhibitor Metalloproteinase 2 (TIMP-2) (AUC increased from 0.938 to 0.973). At 24h post-CPB, diagnostic accuracy improved with NGAL+IL-18 or NGAL+IL-18+KIM-1 combinations (AUC increased from 0.927 to 0.957 or 0.966, respectively). The addition of more than three biomarkers did not bring any improvement in early AKI diagnosis. Children with severe AKI had higher peak levels than the ones with mild disease.[37]

AKI progression is defined as worsening of AKI stage. NGAL ability to predict AKI progression was studied in a cohort of 176 children with AKI after cardiac surgery. The patients with AKI progression presented significantly higher uNGAL levels than the group without it, while pNGAL showed no statistically significant change. The accuracy for predicting AKI progression was poor with an adjusted optimism-corrected AUC of 0.66 for uNGAL and 0.62 for pNGAL.[46]

In a study of 103 patients, uNGAL levels were significantly higher since post-op admission to PICU until 24h after the procedure in children with AKI. The AUC of this biomarker was 0.900, with an estimated cut-off of 40 ng/mL on PICU admission (sensibility 87%, specificity 73%).[38]

Since sCr is not the ideal AKI diagnostic biomarker, NGAL performance evaluation by comparison with sCr could not represent its full potential value. Therefore, Zappitelli *et al.* studied the differences of AKI determined by Cystatin C (CysC) vs sCr in a 3-center prospective cohort study of 287 children. They identified that NGAL was higher in patients sCr+/CysC+ and its association with AKI was similar, independently of the AKI definition used.[47]

2. Studies related to nephrotoxicity

As mentioned before, nephrotoxicity is a common AKI etiology and therefore another research focus of NGAL potential as a biomarker.

In a study of 26 children, uNGAL concentrations after use of contrast in cardiac catheterization yielded no significative change in comparison to baseline values.[48]

A single-center study with 30 patients under chemotherapy showed that uNGAL was significantly higher in children who developed AKI at all timepoints (baseline pre-exposure, one-week post-start of treatment and end of treatment protocol). On the other hand, sCr percentage of change was only statistically significant at the end of the treatment, with a 54% increase. This resulted in uNGAL diagnosing 33% of AKI patients one week after the start of chemotherapy, while sCr diagnosed none. uNGAL demonstrated a good discriminative ability for predicting AKI with an AUC of 0.847.[49]

Similarly, Sterling *et al*. performed a two-centers study about chemotherapy, with 30 children treated with Cis and carboplatin (Car) and 27 with ifosfamide (Ifos). After Cis-Carb-infusion, uNGAL was never statistically significantly different than pre-infusion. Despite this, increase in Cis dose administered was correlated with higher post-Cis uNGAL levels. The authors suggested, therefore, that a severe renal injury phenotype was required to observe a substantial rise in uNGAL levels. uNGAL post-Ifos-infusion was significantly higher than pre-infusion in both AKI and non-AKI groups; however, uNGAL increase was observed earlier and with higher levels in AKI episodes. uNGAL increase outside AKI episodes might point to a subclinical renal injury. Immediately after Ifos-infusion, uNGAL had a good discriminative ability for predicting nephrotoxic-AKI with an AUC of 0.82, after adjustment for age. [50]

Research was also performed on indomethacin, a nonsteroidal anti-inflammatory drug historically administered for closure of patent ductus arteriosus (PDA) in neonates. It augmented the risk of renal injury, especially in very-low birthweight (VLBW) newborns. A German study observed neonates treated with indomethacin from 2012 to 2015. During its administration, uNGAL was about two-fold higher in treated neonates compared to non-treated controls (children with closed or <1.5 mm PDA without hemodynamic significance), regardless of AKI development. In addition, uNGAL concentrations in treated children without AKI were significantly different from controls even before indomethacin treatment. For the authors, these results suggested not only subclinical AKI detection, but also a possible hemodynamic effect of PDA on the kidney and uNGAL baseline levels.[51]

Methotrexate is another nephrotoxic drug which is used in the treatment of multiple conditions, including pediatric acute lymphoblastic leukemia (ALL). Li *et al.* studied if pNGAL was able to early diagnose AKI in children with moderate- and high-risk ALL submitted to high dose methotrexate (HDMTX) (dose over 3 g/m^2). In a cohort of 62 patients, they found that pNGAL concentrations 24h after HDMTX initiation were significantly higher in those who developed AKI. When analyzing the predictive ability, the biomarker had a PPV of 47% and NPV of 96%. Its AUC was larger than 24h sCr/baseline sCr ratio one (0.851 vs 0.786, respectively). However, the conjugation of both biomarkers (24h pNGAL + 24h sCr/baseline sCr ratio) increased the diagnostic performance with an AUC of 0.883.[52]

3. Studies in neonates

AKI can be a complication of perinatal asphyxia (PA). Studies showed that pNGAL and uNGAL levels were higher in those who developed AKI from the 1st to 10th day of life.[53–55] However, all children with PA showed significantly higher pNGAL and uNGAL levels than healthy controls in a study with 35 newborns. This finding might suggest the occurrence of tubular damage after PA irrespective of AKI development based on sCr.[53] Essajee *et al.* study with 108 neonates with PA from two centers described a moderate ability of uNGAL to predict early AKI (AUC of 0.724), with positive and negative likelihood ratios of 2.0 and 0.2, respectively.[54]

Premature and/or low birthweight (BW) neonates were also analyzed to understand the possible role of NGAL in this subpopulation. Premature neonates with AKI had significantly higher uNGAL levels in comparison with non-AKI ones from birth until the 7th day of life.[56–58] In Tabel *et al.*, uNGAL values decreased from the 1st to 7th day of life in AKI and non-AKI premature neonates, which can reflect an increase in maturity and function. This single center study analyzed 50 patients, finding that GA, BW and gender were not significantly correlated with uNGAL concentrations. Consequently, these factors were not confounders of uNGAL results.[58] Parravicini *et al.* analyzed 91 neonates from two centers, finding uNGAL elevations above the reference range used (50 ng/mL) associated with severe respiratory distress syndrome, sepsis and surgical closure of PDA, which may reflect subclinical AKI.[59]

Askenazi *et al.* studied 113 premature neonates from a single center. 78% of those with AKI presented maximum uNGAL values on the day before or on the day of diagnosis.[57] Nevertheless, in a case-control study, Sarafidis *et al*. found that uNGAL was only able to detect renal injury in the same day of sCr increase. The authors suggested that this could be explained by most of their AKI cases being of mild severity and their limited number of neonates analyzed (11 with AKI and 11 controls).[60]

4. Studies in pediatric critical care units

NGAL was also studied as a potential AKI biomarker in children admitted to PICUs. This is a heterogenous pediatric cohort where it is unknown the exact timing of insult. In addition, they may present other medical conditions that can influence and confound the biomarkers results.

Single-center studies found significantly increased uNGAL concentrations at time of PICU admission in children with or that developed AKI.[61–66] Some studies reported statistically significant higher levels with worsening AKI stages.[61,63] However, Dobilienė *et al.* did not find any significant association between uNGAL and AKI severity in 107 children.[65]

Zappitelli *et al.,* with a cohort of 140 patients, reported that uNGAL ability to predict AKI development and its severity within the prior 48h of diagnosis was good with AUCs of 0.78 and 0.79, respectively. When urine samples were collected later, at the day of diagnosis or the next day, uNGAL capacity to predict AKI diminished to an AUC of 0.63.[61]

Kari *et al*. found in 40 patients that children with high uNGAL levels at admission had a 2-fold increase in the risk of AKI. Using the optimal cut-off determined of 223 ng/mL, this biomarker was able to predict 80% of AKI cases.[62]

In another study, ROC analysis was only performed in the 25 patients of the population without sepsis or pneumonia. pNGAL was one of the best biomarkers to diagnose AKI in that population, with an AUC of 0.72 to predict pRIFLE-I or worse and 0.60 to predict pRIFLE Risk (pRIFLE-R) or worse. In the children that had AKI at time of admission and in the ones who developed it during PICU stay, pNGAL and CysC combination improved the test specificity to detect AKI to 93%. Despite that, the improvement in specificity of this combination came with a decrease in sensitivity to only 20%. This was probably caused by the earlier peak of pNGAL in comparison to CysC observed in three of the four patients who developed AKI after admission. As for uNGAL, it showed poor predictive accuracy of pRIFLE-R or worse with an AUC of 0.54, which improved for predicting pRIFLE-I or worse (AUC of 0.63).[67]

In a study of 100 critically ill infants who required intubation and mechanical ventilation, 24 of those children fulfilled pRIFLE criteria at PICU admission and 19 met them within 72 hours. Considering the entire population, uNGAL levels remained significantly high in the AKI group until 48 to 72h after PICU admission. The maximum discriminative ability was at timepoint 0 to 6h, with an AUC of 0.815 and a cut-off level of 126 ng/mL (sensitivity 76%, specificity 84%). uNGAL predicted AKI in 84% of children before a rise in sCr was detected. Maximum uNGAL levels were present about 22h before AKI pRIFLE-R or worse development. Using the same cut-off (126 ng/mL), uNGAL had a PPV of 70% and NPV of 93%.[63]

In 86 children with peripheral circulatory collapse admitted to a PICU, it was reported increased uNGAL values until the 6th day post-admission. In addition, the authors found a moderate ability of uNGAL to predict ischemic AKI with an AUC of 0.77.[64] In critical neonates were observed similar results with high uNGAL levels until day 3, reaching maximum levels of 1350 ng/mL. In turn sCr increased only at day 3, while on the day of admission it was significantly lower than uNGAL.[66]

5. Studies in emergency departments

Du *et al.* studied NGAL levels in 252 children who presented to a pediatric emergency department. They found that uNGAL was significantly higher in children with pRIFLE-I than children with pRIFLE-R or without AKI. Similarly, the predictive ability was also better for pRIFLE-I with AUCs of 0.78-0.80 (analyzed independently in two centers).[68]

6. Studies combining damage and functional biomarkers

Biomarkers can reflect changes in renal function (e.g. sCr, UO, CysC) or represent kidney damage itself, such as NGAL. In the 10th Acute Disease Quality Initiative (ADQI) consensus, it was proposed that the simultaneous use of biomarkers from each category could improve AKI diagnosis by analyzing the whole spectrum of the disease.[69]

Stanski *et al.* designed a prospective study taking in consideration the 10th ADQI consensus panel directives. Four biomarker-based phenotypes were defined at admission for the 178 patients: tubular damage with loss of function (uNGAL+/sCr+), tubular damage without loss of function (uNGAL+/sCr-), loss of function without tubular damage (uNGAL-/sCr+) and without tubular damage or loss of function (uNGAL-/sCr-).[70] Patients classified as uNGAL+/sCr+ had higher probability of having any stage-AKI and severe-AKI at day 3 than those with uNGAL-/sCr+. The latter phenotype showed a NPV of 85% for development of any stage-AKI and 95% for severe AKI at day 3. As for uNGAL+/sCr- patients, they presented higher odds of any stage-AKI at day 3, as well as poorer secondary outcomes. The authors suggested the phenotype loss of function without tubular damage might define a group of patients with reversible AKI, due to the overall better outcomes they presented. Whereas, the positive tubular damage without sCr rise possibly describe subclinical AKI, with results closer to the uNGAL+/sCr+ AKI ones.[70]

Basu *et al.* also analyzed the combination of tubular damage biomarkers (uNGAL) with functional damage ones (CysC) in 345 children, to better characterize and understand

AKI phenotypes. Double positivity of these biomarkers yielded greater predictive performance for severe and persistent AKI than change in sCr alone. uNGAL+/CysC- composite biomarker also trended in the direction of better ability to predict severe and persistent AKI.[71]

Considering these results, the 23rd ADQI meeting panel of experts developed several consensuses about AKI. It was suggested that the combination of damage (e.g. NGAL) and functional (e.g. sCr) biomarkers should augment the ability to diagnose AKI and its severity. Furthermore, it was proposed a modification of KDIGO staging, considering the result of novel biomarkers (Figure 2).[72]

7. The renal angina index

Although most studies have shown considerable benefits of NGAL to predict AKI, its performance in heterogenous populations is more susceptible to statistical bias and more variable.[73] Goldstein *et al.* suggested that it was crucial to create a methodology which would allow the identification and stratification of children at-risk of AKI. The implementation of this method would additionally improve the performance of novel biomarkers.[74]

Goldstein *et al.* created the renal angina concept which combines AKI risk factors with clinical evidence of acute kidney dysfunction, like cardiac angina.[74] Basu *et al.* defined the following risk factors for the pediatric context: PICU admission, acute decompensated heart failure, stem cell transplant recipient, invasive mechanical ventilation, or treatment with vasoactive medications.[73] Therefore, as AKI risk increases, less clinical data are required to meet the threshold for renal angina. When the criteria are fulfilled, the doctor must exclude AKI through appropriately allocating biomarkers' assessment. This concept is useful because it is based on the NPV, which means that if the criteria are not met, biomarkers should not be evaluated because of the low risk of AKI development.[74,73] The Renal Angina Index (RAI) also includes changes in estimated creatinine clearance (eCCl) and the percentage of fluid overload (%FO). [75] (Figure 3)

Later, Basu *et al.* put this concept into practice in a cohort of patients admitted to the Cincinnati Children's Hospital Medical Center PICU with sepsis or septic shock.[75]

Posteriorly, it was validated in different cohorts, including a retrospective study with data from 17 different American centers.[75]

Basu *et al.* defined the cut-off for RAI as equal or above 8 as positive for renal angina. It was found that children with renal angina had higher AKI rates at day 3. These patients additionally had increased PICU stay, RRT requirement rate and mortality. RAI at day 0 was also able to predict day 3-AKI in all the cohorts with a discriminative ability between 0.74 and 0.81 and NPV between 92 and 99%. The latter results indicate that if a child has a RAI below 8 there is a very low likelihood of having clinical signs of renal dysfunction at day 3, thus biomarker assessment will not confer more benefits. Moreover, this ability to predict day 3 AKI was higher than having KDIGO stage 1 injury at day 0, with RAI having better results in terms of PPV and NPV.[75]

A retrospective study with 214 children from 17 centers confirmed day 0-RAI as a predictor of day 3-AKI in pediatric population. Additionally, the index discriminative ability (AUC of 0.80) to predict AKI was improved when combined with the evaluation of plasma biomarkers, including pNGAL. RAI added to pNGAL conferred an increase of 0.05 in the AUC. These results demonstrate that in RAI positive children, a positive biomarker increases the predictive accuracy.[76]

Finally, in the 23rd ADQI meeting, it was suggested that both functional and/or damage biomarkers should only be tested in patients at higher risk of disease, according to the Kidney Health Assessment results (patients' comorbidities and previous AKI episodes).[72]

8. Impact of underlying etiology on NGAL levels

Since NGAL is synthetized by different cells in the human body, it is crucial to understand the impact of other diseases on NGAL levels and AKI diagnosis.

Mishra *et al.* analyzed 46 children with different underlying etiologies of AKI, including acute tubular necrosis, sepsis, severe malaria, hemolytic uremic syndrome and postinfectious glomerulonephritis. Median uNGAL levels were similar between them, with no statistically significant difference according to the etiology. The authors hypothesized that this could be due to the common renal damage caused by the different diseases.[77]

On the other hand, Westhoff *et al.* performed a similar evaluation in 55 AKI patients and 86 controls. uNGAL was significantly higher in AKI due to septic shock, perinatal asphyxia, hemolytic uremic syndrome, hemodynamic instability, and hypovolemia/dehydration in comparison to controls. When the underlying cause of AKI was interstitial nephritis, vasculitis, nephrotoxic insult or renal vein thrombosis the difference was not statistically significant.[78]

Çelik *et al.* study with 30 children focused on dehydration associated AKI after acute gastroenteritis by rotavirus. uNGAL concentrations were significantly higher in the group who developed AKI, especially in the moderately dehydrated when compared with mild degree. This suggests that the dehydration severity has an impact on the level of kidney dysfunction. As for pNGAL, there was also an increase in AKI group, although not statistically significant.[79]

In another study children with sepsis had the highest uNGAL levels in comparison with children with other diagnoses (cardiac or respiratory failure, congenital diaphragmatic hernia, bronchiolitis), regardless of AKI development.[63] In a very small study four children with sepsis and AKI had significantly higher uNGAL levels than the seven septic patients without AKI. Conversely, pNGAL was higher in both groups, showing no statistically significant difference. When comparing the children with sepsis without AKI and the control group (10 healthy children), uNGAL was similar in both groups, while pNGAL was significantly increased in the former than in controls. Despite the small sample size, these results point that pNGAL is unable to accurately reflect renal impairment and, consequently, predict AKI in children with sepsis.[80] McCaffrey *et al*. reinforced the latter results. The 24 children admitted to PICU with sepsis or pneumonia showed no significant difference of pNGAL levels between AKI or no-AKI periods. Moreover, pNGAL discriminative ability for AKI was poor with an AUC of 0.54.[67]

In Wai *et al.,* 39 children admitted to a PICU with septic shock or needing extracorporeal membrane oxygenation (ECMO) had higher uNGAL levels than the 21 healthy controls, regardless of having AKI. However, the ones who developed AKI (19 out of the 39) had markedly higher levels at all timepoints (admission, peak of illness and illness resolution) in comparison to children without AKI. These results suggest that, although with lower specificity, uNGAL is a sensitive biomarker for AKI detection even in critically ill children with sepsis. In addition, the combination with Fibroblast Growth Factor-2 (FGF-2) and Epidermal Growth Factor (EGF) augmented the specificity of the diagnosis and its predictive ability, with an AUC of 0.94.[81]

9. NGAL as a predictor of outcome

It is important to understand if NGAL can also be helpful in predicting AKI outcomes, either morbidity or mortality. This would be useful for guiding treatment.

Zappitelli *et al.* studied children with 1 month to 21 years of age admitted to PICU. There was no statistically significant change in uNGAL levels between survivors and non-survivors at 30 days post-admission, regardless of the pRIFLE stage.[61] However, further studies with children ranging from newborns to 18 years of age reported significantly higher uNGAL levels in the AKI patients who did not survive.[54,63,66,77,81]

In Mishra *et al.,* uNGAL presented a moderate ability for predicting intra-hospital mortality with an AUC of 0.750 in children with AKI (50 patients with AKI and 30 controls).[77] Similarly, Westhoff *et al.* reported good ability of uNGAL to predict 30-day and 3-month mortality in the AKI group (55 children) with AUCs of 0.79 and of 0.81, respectively. For the former, uNGAL cut-off was 229 ng/mL (sensitivity 100%, specificity 50%) with PPV of 23% and NPV of 100%, whereas for 3-month mortality the cut-off was 941 ng/mL (sensitivity 75%, specificity 79%) with PPV of 38% and NPV of 95%.[78]

Although in Mishra *et al.* uNGAL values were significantly higher in the AKI group that needed peritoneal dialysis,[77] Westhoff *et al.* revealed poor accuracy of this biomarker to predict RRT despite the high levels.[78] The authors suggested this could be due to the absence of well-defined criteria for RRT initiation.[78] Moreover, it was also found that uNGAL was a significant risk factor for longer length of hospital stay.[77]

In children submitted to cardiac surgery with CPB, single-center studies observed that increased levels of pNGAL were correlated to higher morbimortality, including death within 30 days after surgery.[35,41,82,83] Additionally, one multicenter and other single-center studies showed that patients with high pNGAL or uNGAL levels until 24h post-CPB had significantly prolonged PICU length of stay, longer mechanical ventilation period and AKI duration.[34–36,38,39,41,82] uNGALs' good ability to predict poor outcomes increased when the biomarker was combined with IL-18, in a single-center study.[83]

Stanski *et al.* studied in 178 children the combination of functional and damage biomarkers to predict the following outcomes: PICU and hospital length of stay, RRT requirement and mortality. uNGAL+/sCr- patients had poorer outcomes with extended PICU and length of hospital stay compared to uNGAL-/sCr- children. When comparing the four groups, uNGAL+/sCr+ was the only group who needed RRT and had the highest incidence of all outcomes, followed by uNGAL+/sCr- patients.[70]

10. Systematic Reviews on the clinical utility of NGAL

The systematic review and meta-analysis from Haase and collaborators analyzed studies with 1842 adults and 663 children. It revealed that pNGAL and uNGAL were early biomarkers of AKI across different clinical contexts: after cardiac surgery and use of contrast, in the Intensive Care Unit (ICU) and Emergency Department. Surprisingly, the ability of NGAL to predict AKI was markedly better in children than in adults (ROC-AUC 0.930 vs 0.782; cut-off 100-135 ng/mL vs 175 ng/mL, respectively). Concerning outcomes prediction, the overall results reported a good prognostic value especially for RRT requirement (ROC-AUC 0.782) and, yet inferior, for mortality (ROC-AUC 0.706).[84]

Filho *et al.* systematic review included only pediatric studies with a total of 1629 children. It was described for uNGAL a combined AUC of 0.94 (sensitivity 76%, specificity 93%) and for pNGAL of 0.90 (sensitivity 80%, specificity 87%) for AKI prediction in children. [85]

11. Reference Ranges for NGAL

In the study of new biomarkers are required various steps, including their evaluation in healthy children. This is important to establish reference ranges and determine factors that can influence them.

Bennett *et al.* found that uNGAL was significantly increased in healthy females compared with males and it changed with age. It was higher in the 10-15 and 15-18 years of age groups. Including the complete cohort, the overall cut-off was 57.6 ng/mL.

Nonetheless, the cut-offs for males and females in the different age groups differed. No correlation with anthropometric parameters, such as height, weight and body mass index, were observed.[86]

In neonates, both full- and pre-term, uNGAL levels were also significantly higher in girls than in boys.[87–90] Both pNGAL and uNGAL had no correlation with GA (between 37 and 42 weeks) or weight percentile.[87]

On the other hand, in 25 premature newborns it was identified a negative correlation of uNGAL with BW and a positive correlation with procalcitonin (PCT) at 1st and 2nd weeks of life (WOL). At 2nd WOL, uNGAL also had a negative correlation with GA and a positive one with C-reactive protein (CRP). pNGAL was positively correlated with CRP and PCT at both weeks. There was no correlation between uNGAL or pNGAL levels and inflammatory markers after the 1st month of life. This suggests that immaturity and inflammatory status are restricting factors of NGAL for diagnosing AKI in the first WOL in this subpopulation.[91]

Single-center evaluations of premature and/or low BW neonates demonstrated that uNGAL was correlated with GA, postnatal age, and BW. There was higher uNGAL levels when that parameters were lower.[88–90] Suchojad *et al.* suggested that the association of uNGAL with GA possibly reflected the timing of renal tubular cells maturity.[91]

According to Bennett *et al.*, in healthy children with three to 18 years uNGAL concentrations range from 10.9 to 139.5 ng/mL.[86] In Haase *et al.* systematic review and meta-analysis, pNGAL and uNGAL concentrations between 100 and 135 ng/mL were defined as the optimal cut-off to predict AKI in children.[84] In premature newborns it is harder to establish reference ranges due to different confounders, such as inflammatory status, postnatal age and GA.[88–91] The NGAL tests already approved for AKI clinical diagnosis in Europe report their reference ranges – uNGAL below 131.7 ng/mL ("ARCHITECT Platform")[92] and both pNGAL and uNGAL below 250 ng/mL ("THE NGAL TEST")[93]. The latter value is higher to avoid a high proportion of false positives, according to the company.[93] However, these values are not differentiated according to age or gender.[92,93]

IV. Other Novel Biomarkers of AKI

Preclinical studies have identified several other urinary biomarkers that are being studied in pediatric population to improve AKI diagnosis. Below, it is described some research findings of the most studied molecules besides NGAL. Further studies are still needed to conclusively demonstrate their benefits for early AKI diagnosis.

<u>a) Kidney Injury Molecule-1 (KIM-1)</u>

KIM-1 is a type-1 transmembrane glycoprotein with an Ig-like domain and a mucin domain in its extracellular portion. After kidney damage, it is upregulated in the regenerating proximal tubules.[94] When this happens, its extracellular dominium is removed from the surface of the cell and shed into urine, conditioning an augment in urinary KIM-1 (uKIM-1) concentrations.[95]

uKIM-1 has been a research target for about 20 years, however its results in children are ambiguous. Some single-center studies reported significantly higher levels in children with AKI in comparison to non-AKI and healthy groups throughout different clinical contexts.[37,45,63,64,68,78] However, in a two-centers and others single-center studies, uKIM-1 had no statistically significant change between AKI and no-AKI children.[43,51,53,56,67] When there was a significant increase, it occurred at least 12h after the insult, either CPB initiation or PICU admission.[45,37,63] Additionally, discriminative ability was variable between poor to moderate performance (AUC 0.51-0.88). (Table 6)

When accounting for outcomes prediction, the results were also ambiguous. Some studies reported poor prognostic accuracy for predicting mortality, length of ICU stay and RRT requirement,[78,83] whereas in another study non-surviving AKI children had significantly higher KIM-1 concentrations.[56]

<u>b) Interleukin-18</u>

IL-18 is a pro-inflammatory cytokine, which was detected in animal models after ischemic AKI, possibly mediating tubular inflammatory injury.[96] In different single-center and one multicenter studies, IL-18 showed significantly increased levels in children with AKI from at least 4h after renal insult.[34,37,40,45,64,77] Nonetheless, others single and two-centers studies reported no association with AKI development after PICU admission, in neonates and in contrast-induced AKI.[48,56,65]

Sterling *et al*. study about nephrotoxic associated-AKI showed significantly higher IL-18 levels during AKI episodes after Ifos treatment, while post-Cis-Carb levels never significantly increased during AKI.[50] Excluding post-Cis-Carb IL-18 results, the studies reported an ACU between 0.60 and 0.886 for predicting AKI development. (Table 7)

Considering IL-18 relationship with AKI outcomes, a two-centers and a single-center studies described a poor ability to predict mortality.[56,77] On the other hand, single and multicenter studies observed good predictive ability for poor outcomes within 12h post-op, such as mortality, length of PICU stay, RRT requirement and prolonged mechanical ventilation.[34,83]

c) Liver Fatty Acid Binding Protein (L-FABP)

L-FABP is a protein expressed in several tissues, including the proximal tubules of human kidneys, associated with free fatty acid metabolism.[96] In single-center studies about AKI after cardiac surgery, urinary L-FABP (uL-FABP) was significantly higher in AKI children within 2 to 6h after CPB.[37,38,45,97] Peco-Antić *et al.* reported statistically higher uL-FABP concentrations even pre-op in patients who developed severe AKI.[43] Plasmatic L-FABP (pL-FABP) only rose at 12h post-CPB, suggesting that uL-FABP is due to shedding from the renal tubules instead of increased filtration of high pL-FABP concentrations.[97]

This biomarker was shown to be associated with AKI severity, length of hospital stay and of mechanical ventilation.[38,45,97] In AKI induced by contrast after cardiac catheterization, uL-FABP significantly increased 6h post-procedure, later decreasing until 48h. These results point it as a useful biomarker for contrast-induced AKI.[48] L-FABP showed good discriminative ability for predicting AKI, with ROC-AUCs varying between 0.78 and 0.89 (Table 8).

d) Tissue Inhibitor Metalloproteinase 2 (TIMP-2) and Insulin-like Growth Factor Binding Protein 7 (IGFBP-7)

TIMP-2 and insulin-like growth factor binding protein 7 (IGFBP-7) are related to the cell cycle and they are upregulated in the proximal tubules after kidney damage to control injured cells proliferation.[98] Their product – TIMP-2*IGFBP-7 – divided by 1000 has been studied for risk evaluation of AKI in adults. Few pediatric studies have been performed, although the ones that exist (both single and multicenter) reported positive results. Urinary TIMP-2*IGFBP-7 was significantly higher in children with AKI at 12h post-renal injury. It showed good discriminative ability to predict AKI ranging between 0.71 and 1.00.[37,51,99] (Table 9)

In a study with indomethacin-treated VLBW infants, significantly higher levels remained until 84h post treatment.[51]

The size of the cohort, type of renal insult and timepoints assessed are some factors that can influence the results above and justify the variability observed for KIM-1 and IL-18 results. This reinforces the need for further multicenter larger prospective cohort studies involving not only these molecules, but also other potential candidates. These include calprotectin, osteopontin, uromodulin, epidermal growth factor, fibroblast growth factor-2, fibroblast growth factor 23 and N-acetyl-beta-D-glucosaminidase. It will allow to better understand their role and kinetics during AKI, as well as their potential as predictors of AKI and its outcomes.

V. The Present and the Future of NGAL

NGAL has shown consistent strong results in pediatric patients and, currently, it is already being used in the clinical setting in several centers.

"TRIAGE NGAL TEST" is a point-of-care immunoassay from Alere® (Biosite Inc., San Diego, CA, USA) which analyzes both plasma and urine. "ARCHITECT Platform" from Abbott® (Abbott Diagnostics, Abbott Park, IL, USA) is an immunoassay only for urine samples. "THE NGAL TEST" from BioPorto® (BioPorto Diagnostics, Hellerup, Denmark) evaluates both plasma and urine. These three NGAL tests have already been "Conformité Européenne" (CE) marked and, therefore, are available for clinical diagnostic in Europe. As for the USA, NGAL is still not approved for diagnostic use by the Food and Drug Administration.[100]

Although much progress has been made in the comprehension of AKI pathophysiology to improve diagnostic and treatment approaches, there are still many things to discover and fully understand. Several cells that take part on nephron repair and regenerative responses after kidney injury have been already identified. Its underlying mechanisms, including attainment of pro-regenerative replicative phenotype, partial transition from epithelium to mesenchyme and cells arrested in cell-cycle in the proximal tubule, have also been described. Despite this, the genetic and molecular pathways remain to be thoroughly understood.[101]

Noncoding RNAs are genomic transcripts with functions at RNA level and are classified in microRNA (miRNA), long noncoding RNA (LncRNA) and circular RNA (circRNA). miRNAs inhibit protein translation or degrade mRNA, consequently regulating gene expression. They are part of several physiological and pathophysiological processes including AKI. Several miRNAs have been shown to be upregulated or downregulated during AKI in mice models or human patients, exhibiting roles in renal inflammation, apoptosis, or cell proliferation. Few studies also explored miRNAs potential therapeutic role, obtaining encouraging results in animal models, even though extensive studies are still required. Moreover, miRNA modulation can represent in the future a therapeutic approach to limit or reverse acute kidney damage. LncRNAs and circRNAs also have diverse biological functions in the organism, including in the kidney. This makes them promising mediators in AKI, however its role is still not fully understood.[102]

It was described that intrinsic (iAKI) and prerenal/volume depletion AKI (vAKI) activate different and non-overlapping genes. iAKI presented more significantly induced gene sets than vAKI. These included injury/repair and inflammatory pathways, whereas vAKI upregulated more metabolic, transport and osmo-regulatory gene sets. As for location, the differentially expressed genes in iAKI were on the outer medulla, while in vAKI were in the cortex. In conclusion, iAKI and vAKI models showed different clinical phenotypes, transcription patterns and activation of distinct genetic programs and specific pathways.[103] This enlightens the requirement of novel biomarkers specific for each type of renal injury. This is especially important because the markers discussed above, such as NGAL and KIM-1, are a response of tubular damage and not volume depletion.[104]

The optimal approach for disease treatment stands in personalized medicine with its ability to diagnose on a patient specific-level and implement damage-specific therapies. A single change in sCr cannot distinguish the myriad of etiologies and subtypes of injury in AKI, nor identify the specific injured cell type or predict effective target-therapeutics.

Kiryluk *et al.* suggested the construction of a genetic expression map of the normal kidney to better understand the molecular responses to insults.[104] Single-cell or single-nuclei transcriptomics have been recently developed and allow the recognition of expression of multiple genes in thousands of individual cells at the same time. Thus, these techniques allow the mapping of cell-specific responses in AKI. In the kidney of adult mice, single-cell RNA was able to quantify and classify cells in major cell clusters.[105] These approaches in humans would permit the identification of new cell types, quantification of injured cells and its localization in the nephron. Additionally, we would be able to identify the best insult-specific or nephron segment-specific transcript proteins as potential biomarkers. Ultimately, these tools will lead to the development of novel diagnostic tests, revolutionizing AKI diagnosis.[104,105]

VI. Discussion

In the last years, it has been recognized the burden of AKI and its clinical importance, particularly in pediatrics due to the possibility of CKD development later in life. Despite this, few breakthroughs have been implemented for AKI diagnosis, prevention, or treatment. Current gold standard definition of AKI is still based on sCr and/or UO, in spite of these methods of diagnosis presenting several disadvantages.[21] Therefore, AKI is diagnosed long after the insult and the consequent renal damage. This delay urged the need for novel biomarkers which could detect AKI earlier than sCr and predict its outcomes.

One of the most studied molecules is NGAL, a lipocalin with the ability to transport iron. Its functions range from antibacterial effects to regulation of gene expression, both in organogenesis and regeneration after injury.[27–29] Its role in AKI was first studied in mice models, increasing after ischemic and nephrotoxic insults.[30,31] Additionally, in real-time mice models it was confirmed the correlation between urinary levels and prior activation of NGAL gene expression in the kidney after an insult. The location of uNGAL synthesis was determined to be at the thick ascending limb of loop of Henle and the collecting ducts. It also showed the independence of uNGAL levels from serum values and neutrophil presence. This further supported uNGAL as an AKI biomarker that reflected actual renal tubular damage.[32]

Subsequently, several studies have been performed in humans, throughout distinct clinical contexts. AKI in children admitted due to sepsis was a particularly difficult scenario. Sepsis could have an impact on both urinary and plasmatic NGAL concentrations. Despite this, uNGAL was significantly higher in children who developed AKI, persisting as a sensitive biomarker for AKI even though less specific.[81,80] pNGAL results were disappointing since its levels had no significant change between AKI and no-AKI children.[67,80] These results demonstrate pNGAL inability to accurately predict AKI in septic pediatric patients.

NGAL levels in premature neonates were associated with CRP and PCT in the first weeks of life, also reflecting the effect of inflammatory conditions on this biomarker. As well, children without AKI after CPB had increased NGAL levels from baseline, which could possibly reflect inflammation secondary to the procedure.[34,35,41,42] Thus, it is crucial to carefully evaluate NGAL results in inflammatory contexts and in premature neonates.

Overall, uNGAL was significantly higher few hours after insult with its levels remaining persistently high until different timepoints, from about half a day until 5 days post-insult. This disparity in the uNGAL timing of decline was mostly due to the different timepoints assessed in the studies. The last timepoint assessed for most studies were 24 or 48h post-insult.[35–37,41,45] Incidentally, in most studies, uNGAL levels increased in a stepwise way with worse AKI severity. This possibly reflected the degree of renal damage.

After cardiac surgery with CPB, uNGAL results were transversal for a good diagnostic performance with AUCs between 0.71 and 1.00 and NPVs of 75 to 100% (Table 1). Although these results reflect the more homogenous cohort and known insult, they also encourage the use of this biomarker in this population, specially to rule out AKI. The 20th International Consensus Conference of the ADQI Group in 2017 even suggested the measure of AKI biomarkers, as NGAL, in clinical practice in patients at high-risk of AKI associated with cardiac surgery.[106]

As for the neonate's population, the ones with AKI had increased uNGAL values since the first day of life with a discriminative performance between 0.61 and 0.865 (Table

3).[53–58] However, in Parravicini *et al*. uNGAL levels were above their established cut-off in several occasions outside AKI episodes and associated with other diseases.[59] Inflammatory and infectious conditions have been shown to cause mild increases in uNGAL. Nonetheless, AKI severity, prematurity and, in consequence, state of renal maturity, can also justify the lower specificity observed in the latter study.

Finally, even in PICU population, uNGAL concentrations were significantly increased. This levels were observed in most cases since admission in the ones with AKI or that developed it during PICU stay.[61–66] The diagnostic ability was moderate with AUCs between 0.760 and 0.82 (Table 4), except for McCaffrey *et al.* that reported an AUC of 0.54.[67] This value probably reflected the late assessment of uNGAL in the course of the disease since it seems to increase before a rise in sCr is detectable. In addition, most patients already had AKI at PICU admission.[67]

In nephrotoxic-associated AKI, uNGAL discriminative ability ranged between 0.39 to 0.847 and for pNGAL the AUC was 0.851 (Table 2) in the only study about it.[52] Additionally, not all nephrotoxic drugs resulted in the increase of the biomarker. Cardiac catheterization contrast and Cis-Carb treatments were not associated with higher uNGAL levels.[48,50] It seems that not all nephrotoxic medications administered have the same mechanism or site of injury, limiting uNGAL use according to the nephrotoxin. It might also suggest that increased drug concentrations and, consequently, a severe phenotype are required for uNGAL increase.

pNGAL studies were fewer and the results were more ambiguous. In AKI associated with CPB, Parikh *et al.* multicenter study described no association with severe AKI.[34] However, in single-center studies the predictive performance was good, with AUCs between 0.906-0.95 (Table 1). As for PICU, McCaffrey *et al.* showed that pNGAL in children without inflammatory/infectious confounders was increased since admission and was better at predicting severe AKI than mild one.[67] Although pNGAL has shown promising results, it is still needed further multicenter studies in the different clinical contexts before its clinical implementation.

It is important to highlight that biomarkers performance improved when tested in at risk population. Therefore, Renal Angina Index is an important tool which analyzes risk factors and kidney dysfunction evidence to identify the children at high risk of having AKI. When the cut-off for RAI is hit, the clinician has to evaluate renal biomarkers to rule out AKI.[73,74] Further studies with this method revealed good predictive performance of RAI that improved when combined with novel biomarkers. This important prior assessment, already recognized in the 23rd ADQI meeting, provides an adequate direction for NGAL testing. It prevents its evaluation for any condition or patient, as it is already known the influence of inflammation and sepsis as NGAL confounders. It allows the improvement of diagnostic performance which, consequently, can contribute to its general clinical implementation around the world.[72,75,76]

The combination of NGAL, as a damage biomarker, with functional biomarkers also revealed improved accuracy in distinguishing renal injury phenotypes and subsequent outcomes. It allowed the identification of increased odds of severe and persistent AKI (damage+/functional+ or damage+/functional-) and of reversible AKI (damage-/functional+).[70,71] The promising results of some of the biomarkers also in research, along with the improved AUCs when combined with each other, support the possibility of a biomarkers' panel future construction. This would allow to identify the type of injured cells and the underlying mechanism, favoring the development of target therapies.

Furthermore, while more research is required to clarify some pNGAL ambiguous results, uNGAL results demonstrate an overall good accuracy for predicting AKI and its outcomes in children. The high NPV of uNGAL observed across studies further supports its clinical use in AKI exclusion. The 23rd ADQI consensus previously suggested, with a grade B of recommendation, the clinical use of damage and functional biomarkers combined to improve diagnostic accuracy.[72] Moreover, some NGAL tests are already CE marked and available for diagnostic use in Europe.[100]

While it is important to establish gender-appropriate cut-offs to define AKI and validate them in end-point studies, it is also time for a more widespread clinical implementation. Portugal medical centers can begin to implement uNGAL diagnostic testing especially in high-risk homogenous populations, like children submitted to CPB, in whom there are more consistent clinical evidence. For PICU patients with sepsis or infectious diseases and premature neonates, uNGAL testing should be approached more carefully.

VII. Conclusion

In this thesis, it was reviewed the increased incidence of AKI in the pediatric population along with the long-term outcomes associated. This represents an increased burden of disease as well as higher health costs. The additional disadvantages of the current gold standard methods to diagnose AKI earlier in the course of disease support the need of novel biomarkers.

NGAL has shown powerful results throughout the pediatric clinical contexts. Its urinary concentrations presented good diagnostic and prognostic ability for AKI, especially in combination with other biomarkers. This supports its role as a sensible and specific early biomarker of AKI. The results were also magnified when NGAL was assessed in patients at high risk of AKI, after a Kidney Health Assessment of possible risk factors. The widespread assessment of NGAL in children with higher probability of AKI could improve its diagnosis and more precocious management.

Finally, the future promises better knowledge of injured cells and pathophysiological processes occurring in AKI through tools like genomics and proteomics. This increases the hopes in patient specific-diagnosis and damage-specific therapies, revolutionizing the way AKI is diagnosed and managed.

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Supplementary Material

Definition and Criteria for AKI Stages	Modifications
pRIFLE	
Stage 1 (Risk): eGFR decreased by 25%	
Stage 2 (Injury): eGFR decreased by 50%	
Stage 3 (Failure): eGFR decrease by 75% or eGFR <35 ml/min per 1.73 m^2	
AKIN	
Stage 1: Increase in creatinine of ≥50% or Absolute increase in creatinine of 0.3 mg/dl	0.3-mg/dl increase added to stage 1 AKI diagnosed over 48-hr period
Stage 2: Increase in creatinine of ≥100%	
Stage 3: Increase in creatinine of ≥200%	
KDIGO	
Stage 1: Increase in creatinine of ≥50% or Absolute increase in creatinine of 0.3 mg/dl	eGFR threshold from pRIFLE added to stage 3 Creatinine changes (except absolute 0.3-mg/dl increase) required to occur within a 7-d time frame
Stage 2: Increase in creatinine of ≥100%	
Stage 3: Increase in creatinine of ≥200% or eGFR ≤35 ml/min per 1.73 m^2 (if age <18 yr)	

eGFR was estimated using the Schwartz method. pRIFLE, pediatric RIFLE; AKIN, Acute Kidney Injury Network; KDIGO, Kidney Diseases Improving Global Outcomes.

Figure 1. **pRIFLE, AKIN and KDIGO criteria for AKI definition and operational modifications to each definition.**
From Sutherland, S. M., Byrnes, J. J., Kothari, M., Longhurst, C. A., Dutta, S., Garcia, P., & Goldstein, S. L. (2015). AKI in hospitalized children: Comparing the pRIFLE, AKIN, and KDIGO definitions. *Clinical Journal of the American Society of Nephrology*, *10*(4), 554–561

Functional criteria	Stage	Damage criteria
No change or sCr level increase <0.3 mg/dL and no UO criteria	1S	Biomarker positive
Increase of sCr level by ≥0.3 mg/dL for ≤48 h or ≥150% for ≤7 days and/or UO <0.5 mL/kg/h for >6 h	1A	Biomarker negative
	1B	Biomarker positive
Increase of sCr level by >200% and/or UO <0.5 mL/kg/h for >12 h	2A	Biomarker negative
	2B	Biomarker positive
Increase of sCr level by >300% (≥4.0 mg/dL with an acute increase of ≥0.5 mg/dL) and/or UO <0.3 mL/kg/h for >24 h or anuria for >12 h and/or acute KRT	3A	Biomarker negative
	3B	Biomarker positive

Figure 2. **Proposed new definition of Acute Kidney Injury.** Modification of KDIGO definition including the results from damage biomarkers assessment.
From Ostermann, M., Zarbock, A., Goldstein, S., Kashani, K., Macedo, E., Murugan, R., Bell, M., Forni, L., Guzzi, L., Joannidis, M., Kane-Gill, S. L., Legrand, M., Mehta, R., Murray, P. T., Pickkers, P., Plebani, M., Prowle, J., Ricci, Z., Rimmelé, T., … Ronco, C. (2020). Recommendations on Acute Kidney Injury Biomarkers From the Acute Disease Quality Initiative Consensus Conference: A Consensus Statement. *JAMA Network Open*, *3*(10), e2019209.

Renal angina = risk of AKI * signs of injury

Risk

Study	Patients	AKI incidence
Schneider *et al.*	PICU admissions	4.5–10%
Michael *et al.*	Stem cell transplantation	11–21%
Akcan-Arikan *et al.*	Ventilation and inotropy	> 50%

→

Risk	Score
Moderate	1
High	3
Very high	5

X

Injury

Study	Outcomes
Foland	1.78 OR death for each 10% FO
Goldstein (ppCRRT)	Survival: <20% = 58%, >20% = 40%
Sutherland (ppCRRT)	OR death 8.5 for > 20% FO

→

↓eCCl	% FO	Score
No change	< 5%	1
↓0–25%	⩾ 5 %	2
↓25–50%	⩾ 10%	4
↓⩾50%	⩾15%	8

= Renal angina index (1– 40)

Figure 3. **The renal angina index.**
Basu, R. K., Zappitelli, M., Brunner, L., Wang, Y., Wong, H. R., Chawla, L. S., Wheeler, D. S., & Goldstein, S. L. (2014). Derivation and validation of the renal angina index to improve the prediction of acute kidney injury in critically ill children. *Kidney International*, 85(3), 659–667.

Cardiac surgery with cardiopulmonary bypass											
uNGAL											
Title	**Authors**	**Year**	**Cohort (n)**	**Age**	**AKI Outcome**	**Cut-off**	**Sensitivity (%)**	**Specificity (%)**	**ROC-AUC**	**PPV (%)**	**NPV (%)**
Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery	Mishra J, Dent C, Tarabishi R *et al.*	2005	Single-center (71)	Children	AKI development TP: 2h post-CPB	25 and 50 ng/mL	100	98	0.998	95	100
					AKI development TP: 4h post-CPB	25 ng/mL	100	96	1.00	91	100
Urine NGAL predicts severity of acute kidney injury after cardiac surgery: A prospective study	Bennett M, Dent C, Ma Q *et al.*	2008	Single-center (196)	Children	AKI development TP: 2h post-CPB	100 ng/mL	82	90	0.93	NR	NR
					AKI development TP: 4h post-CPB		91	91	0.96	NR	NR
					AKI development TP: 6h post-CPB		89	95	0.98	NR	NR
Postoperative biomarkers predict acute kidney injury and poor outcomes after pediatric cardiac surgery	Parikh C, Devarajan P, Zappitelli M *et al.*	2011	Multicenter (311)	1 month - 18 years	Development of severe AKI TP: first post-op measure	17 ng/mL	75	65	0.71	30	93
Temporal relationship and predictive value of urinary acute kidney injury biomarkers after pediatric cardiopulmonary bypass	Krawczeski C, Goldstein S, Woo J *et al.*	2011	Single-center (220)	< 18 years	AKI development TP: 2h post-CPB	NR	NR	NR	0.90	NR	NR
					AKI development TP: 6h post-CPB	NR	NR	NR	0.91	NR	NR
Neutrophil gelatinase-associated lipocalin concentrations predict development of acute kidney injury in neonates and children after cardiopulmonary bypass	Krawczeski C, Woo J, Wang Y *et al.*	2011	Single-center (373)	Neonates	AKI development TP: 2h post-CPB	185 ng/mL	100	93	0.95	80	100
				Non-neonates (> 30 days - 18 years)		48 ng/mL	85	86	0.92	73	93

The combined use of neutrophil gelatinase-associated lipocalin and brain natriuretic peptide improves risk stratification in pediatric cardiac surgery	Cantinotti M, Storti S, Lorenzoni V *et al.*	2012	Single-center (135)	Children	AKI development	49.95 ng/mL	78.4	81.5	0.85	NR	NR
Comparison of urinary biomarkers for early detection of acute kidney injury after cardiopulmonary bypass surgery in infants and young children	Zheng J, Xiao Y, Yao Y *et al.*	2013	Single-center (58)	< 3 years	AKI development TP: 4h post-CPB	54 ng/mL	79.3	89.7	0.857	88.5	81.2
Biomarkers of acute kidney injury in pediatric cardiac surgery	Peco-Antić A, Ivanišević I, Vulićević I *et al.*	2013	Single-center (112)	< 18 years	AKI development TP: 24h post-CPB	NR	NR	NR	0.93	NR	NR
Urinary Biomarkers and Renal Near-Infrared Spectroscopy Predict ICU Outcomes Following Cardiac Surgery in Infants Under 6 Months of Age	Hazle M, Gajarski R, Aiyagari R *et al.*	2014	Single-center (49)	< 6 months	Poor Outcome: death within 30d post-CPB, need for RRT, prolonged time for 1st extubation or ICU stay	76 ng/mL	64	87	0.79	72	83
Predictive performance of urine neutrophil gelatinase- associated lipocalin for dialysis requirement and death following cardiac surgery in neonates and infants	Bojan M, Vicca S, Lopez-Lopez V *et al.*	2014	Single-center (200)	< 1 year	Hard Clinical Outcomes: RRT requirement and mortality	950 ng/ mmol Cr	87	61	0.818	NR	NR
Postoperative neutrophil gelatinase-associated lipocalin predicts acute kidney injury after pediatric cardiac surgery	Alcaraz A, Gil-Ruiz M, Castillo A *et al.*	2014	Single-center (106)	15 days - 16 years	AKI development TP: 3h post-CPB	75 ng/mL	79	83	0.86	68	90
						280 mcg/g Cr	86	79	0.88	66	93
Combining functional and tubular damage biomarkers improves diagnostic precision for acute	Basu R, Wong H, Krawczeski C *et al.*	2014	Single-center (345)	< 18 years	Severe AKI development by uNGAL+/pCysC-	200 ng/mg Cr (0.8 mg/L for pCysC)	24	98	NR	75	75

kidney injury after cardiac surgery					Severe AKI development by uNGAL+/pCysC+		48	99	NR	88	90
					Persistent AKI development by uNGAL+/pCysC-		24	96	NR	35	93
					Persistent AKI development by uNGAL+/pCysC+		69	96	NR	59	97
Urinary biomarkers of cell cycle arrest are delayed predictors of acute kidney injury after pediatric cardiopulmonary bypass	Dong L, Ma Q, Bennett M *et al.*	2017	Single-center (150)	< 18 years	All forms of AKI development TP: 6h post-CPB	NR	NR	NR	0.965	NR	NR
Novel Urinary Biomarkers for Acute Kidney Injury and Prediction of Clinical Outcomes After Pediatric Cardiac Surgery	Yoneyama F, Okamura T, Tlragiku K *et al.*	2019	Single-center (103)	< 18 years	AKI development TP: ICU admission	40 ng/mL	87	73	0.900	NR	NR

pNGAL

Title	Authors	Year	Cohort (n)	Age	AKI Outcome	Cut-off	Sensitivity (%)	Specificity (%)	ROC-AUC	PPV (%)	NPV (%)
Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery	Mishra J, Dent C, Tarabishi R *et al.*	2005	Single-center (71)	Children	AKI development TP: 2h post-CPB	25 ng/mL	70	94	0.906	82	89
Plasma neutrophil gelatinase-associated lipocalin predicts acute kidney injury, morbidity and mortality after pediatric cardiac surgery: A prospective uncontrolled cohort study	Dent C, Ma Q, Dastrala S *et al.*	2007	Single-center (120)	Children	AKI development TP: 2h post-CPB	150 ng/mL	84	94	0.96	84	93

Postoperative biomarkers predict acute kidney injury and poor outcomes after pediatric cardiac surgery	Parikh C, Devarajan P, Zappitelli M *et al.*	2011	Multicenter (311)	1 month - 18 years	Severe AKI development TP: first post-op measure	154 ng/mL	59	54	0.56	20	86
Neutrophil gelatinase-associated lipocalin concentrations predict development of acute kidney injury in neonates and children after cardiopulmonary bypass.	Krawczeski C, Woo J, Wang Y *et al.*	2011	Single-center (373)	Neonates	AKI development TP: 2h post-CPB	95 ng/mL	88	93	0.95	78	96
				Non-neonates (> 30 days)		45 ng/mL	90	88	0.94	77	95
Plasma neutrophil gelatinase-associated lipocalin measured in consecutive patients after congenital heart surgery using point-of-care technology	Koch A, Dittrich S, Cesnjevar R *et al.*	2011	Single-center (218)	Children	pRIFLE-R or worse by a single whole point of care measurement*	NR	NR	NR	0.57	NR	NR
Whole blood assessment of neutrophil gelatinase-associated lipocalin versus pediatricRIFLE for acute kidney injury diagnosis and prognosis after pediatric cardiac surgery: Cross-sectional study	Ricci Z, Netto R, Garisto C *et al.*	2012	Single-center (160)	< 1 year	AKI development by a single whole point of care measurement*	150 ng/mL	13	91	NR	66	45
					RRT requirement by a single point of care measurement*		30	90	NR	22	93

Table 1. Urinary and Plasma NGAL results in cardiac surgery with CPB-associated AKI pediatric studies.

Nefrotoxicity												
uNGAL												
Title	**Authors**	**Year**	**Cohort (n)**	**Age**	**AKI Outcome**	**Nephrotoxic**	**Cut-off**	**Sensitivity (%)**	**Specificity (%)**	**ROC-AUC**	**PPV (%)**	**NPV (%)**
Detection of early renal injury in children with solid tumors undergoing chemotherapy by urinary neutrophil gelatinase-associated lipocalin	Almalky M, Hasan S, Hassan T *et al.*	2015	Single-center (30)	1 month - 18 years	AKI development	Chemotherapy	25 ng/mL	NR	NR	0.847	NR	NR
Urine biomarkers of acute kidney injury in noncritically ill, hospitalized children treated with chemotherapy	Sterling M, Al-Ismaili Z, McMahon K *et al.*	2017	Two-centers (30)	3 months - 18 years	Post-Cis-Carb infusion (day 1) AKI	Cis-Carb	NR	NR	NR	0.40	NR	NR
			Two-centers (27)		Post-Ifos infusion (day 1) AKI	Ifosfamide	NR	NR	NR	0.80	NR	NR
Urinary acute kidney injury biomarkers in very low-birth-weight infants on indomethacin for patent ductus arteriosus	Waldherr S, Fichtner A, Beedgen B *et al.*	2019	Single-center (32)	Preterm neonates	KDIGO AKI at 12h post-indomethacin initiation	Indomethacine	NR	NR	NR	0.39	NR	NR

pNGAL												
Title	**Authors**	**Year**	**Cohort (n)**	**Age**	**AKI Outcome**	**Nephrotoxic**	**Cutoff**	**Sensitivity (%)**	**Specificity (%)**	**ROC-AUC**	**PPV (%)**	**NPV (%)**
Serum neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for predicting high dose methotrexate associated acute kidney injury in children with acute lymphoblastic leukemia	Li H, Xu Q, Wang Y *et al.*	2020	Single-center (62)	1 - 14 years	AKI development at 24h after initiation of HDMTX	MTX	295 ng/mL	68	90	0.851	46.9	95.7

Table 2. Urinary and Plasma NGAL results in nephrotoxic-associated AKI pediatric studies.

Neonates											
uNGAL											
Title	**Authors**	**Year**	**Cohort (n)**	**Age**	**AKI Outcome**	**Cut-off**	**Sensitivity (%)**	**Specificity (%)**	**ROC-AUC**	**PPV (%)**	**NPV (%)**
Urine biomarkers predict acute kidney injury and mortality in very low birth weight infants.	Askenazi D, Montesanti A, Hundley H *et al.*	2011	Two-centers (30)	Preterm neonates	AKI development	NR	NR	NR	0.80	NR	NR
			Two-centers (123)		Mortality	NR	NR	NR	0.61	NR	NR
Serum and urine acute kidney injury biomarkers in asphyxiated neonates	Sarafidis K, Tsepkentzi E, Agakidou E *et al.*	2012	Single-center (35)	Term neonates (> 36 GW)	AKI development within 24h	18.61 ng/mL	100	83.3	0.865	NR	NR
Urine neutrophil gelatinase-associated lipocalin to predict acute kidney injury in preterm neonates. A pilot study	Sarafidis K, Tsepkentzi E, Diamanti E *et al.*	2014	Single-center (22)	Preterm neonates (27 - 32 GW)	AKI development TP: day 0	19.39 ng/mL	54.5	90.9	0.744	NR	NR
Urinary neutrophil gelatinase-associated lipocalin as an early biomarker for prediction of acute kidney injury in preterm infants	Tabel Y, Elmas A, Ipek S *et al.*	2014	Single-center (50)	Preterm neonates (28 - 34 GW)	AKI development within 24h	38.3 ng/mL	66.7	95.5	0.85	NR	NR
					AKI development on DOL 7	30.6 ng/mL	50	91	0.78	NR	NR
Urine neutrophil gelatinase-associated lipocalin in asphyxiated neonates: a prospective cohort study	Essajee F, Were F, Admani B	2015	Two-centers (108)	Term neonates	AKI development on DOL 3 TP: DOL 1	250 ng/mL	88	56	0.724	31	95
Renal function and novel urinary biomarkers in infants with neonatal encephalopathy	Sweetman D, Onwuneme C, Watson W *et al.*	2016	Single-center (95)	Term neonates	AKI development TP: DOL 3	1700 ng/mL	NR	NR	0.86	NT	NR

Is urinary neutrophil gelatinase-associated lipocalin able to predict acute kidney injury episodes in very low birth weight infants in clinical settings?	Parravicini E, Locatelli C, Lorenz J *et al.*	2016	Two-centers (91)	VLBW neonates	AKI development	NR	61	63	0.62	5	98
Acute kidney injury urine biomarkers in very low-birth-weight infants	Askenazi D, Koralkar R, Patil N *et al.*	2016	Single-center (113)	Preterm neonates (< 31 GW)	AKI development	450 ng/mL	NR	NR	0.63	NR	NR
pNGAL											
Title	**Authors**	**Year**	**Cohort (n)**	**Age**	**AKI Outcome**	**Cut-off**	**Sensitivity (%)**	**Specificity (%)**	**ROC-AUC**	**PPV (%)**	**NPV (%)**
Serum and urine acute kidney injury biomarkers in asphyxiated neonates	Sarafidis K, Tsepkentzi E, Agakidou E *et al.*	2012	Single-center (35)	Neonates > 36 GW	AKI development within 24h	89.6 ng/mL	100	92.3	0.942	NR	NR

Table 3. Urinary and Plasma NGAL results in studies of AKI in neonates.

Pediatric Critical Care Units											
uNGAL											
Title	**Authors**	**Year**	**Cohort (n)**	**Age**	**AKI Outcome**	**Cut-off**	**Sensitivity (%)**	**Specificity (%)**	**ROC-AUC**	**PPV (%)**	**NPV (%)**
Urine neutrophil gelatinase-associated lipocalin is an early marker of acute kidney injury in critically ill children: A prospective cohort study	Zappitelli M, Washburn K, Arikan A *et al.*	2007	Single-center (140)	1 month - 21 years	AKI development within 48h of first urine collection	0.2 ng/mg Cr	77	72	0.78	NR	NR
					Persistent AKI within 48h of first urine collection		78	67	0.79	NR	NR

A pilot study of urinary fibroblast growth factor-2 and epithelial growth factor as potential biomarkers of acute kidney injury in critically ill children	Wai K, Soler-García Á, Perazzo S *et al.*	2013	Single-center (60)	1 week - 21 years	AKI development TP: ICU admission	1544 ng/mg uCr	84	80	0.82	NR	NR
					AKI development - uNGAL+FGF-2+EGF TP: ICU admission	uNGAL: 548 ng/mg FGF-2: 15.55 pg/mg EGF: 31.958 pg/mg	89	89	0.94	85	93
					Mortality	6808 ng/mg uCr	80	78.6	0.80	48	94
Towards a biomarker panel for the assessment of AKI in children receiving intensive care	McCaffrey J, Coupes B, Chaloner C *et al.*	2015	Single-center (49)	< 16 years	pRIFLE-R or worse	NR	NR	NR	0.54	NR	NR
					pRIFLE-I or worse	NR	NR	NR	0.63	NR	NR
Urinary neutrophil gelatinase-associated lipocalin identifies critically ill young children with acute kidney injury following intensive care admission: A prospective cohort study	Zwiers A, de Wildt S, van Rosmalen J *et al.*	2015	Single-center (100)	1 day - 1 year	AKI development TP: 0-6h post-intubation	126 ng/mL	76	84	0.815	70	93
Urinary neutrophil gelatinase-associated lipocalin (NGAL) and serum cystatin C measurements for early diagnosis of acute kidney injury in children admitted to PICU	Kari J, Shalaby M, Sofyani K *et al.*	2018	Single-center (40)	1 month - 14 years	AKI development	223 ng/mL	72.7	89.9	0.760	NR	NR
Urine KIM-1 as a Potential Biomarker of Acute Renal Injury after Circulatory Collapse in Children	Assadi F, Sharbaf F	2019	Single-center (86)	Children	Ischemic AKI development after circulatory collapse	NR	NR	NR	0.77	NR	NR

pNGAL											
Title	**Authors**	**Year**	**Cohort (n)**	**Age**	**AKI Outcome**	**Cut-off**	**Sensitivity (%)**	**Specificity (%)**	**ROC-AUC**	**PPV (%)**	**NPV (%)**
Towards a biomarker panel for the assessment of AKI in children receiving intensive care	McCaffrey J, Coupes B, Chaloner C *et al.*	2015	Single-center (49)	< 16 years	pRIFLE-R or worse	258 ng/mL	88	62	0.60	NR	NR
					pRIFLE-I or worse	NR	NR	NR	0.72	NR	NR

Table 4. Urinary and Plasma NGAL results in studies of AKI in pediatric critical care units.

Emergency Department											
uNGAL											
Title	**Authors**	**Date**	**Cohort (n)**	**Age**	**AKI Outcome**	**Cut-off**	**Sensitivity (%)**	**Specificity (%)**	**ROC-AUC**	**PPV**	**NPV**
Urinary biomarkers to detect acute kidney injury in the pediatric emergency center	Du Y, Zappitelli M, Mian A *et al.*	2011	Single-center (252)	Children	AKI development	NR	NR	NR	0.66-0.67*	NR	NR
					RIFLE-I development	NR	NR	NR	0.78-0.80*	NR	NR

Table 5. Urinary NGAL results in studies of AKI in the pediatric emergency department.

KIM-1											
Title	**Authors**	**Date**	**Cohort (n)**	**Age**	**AKI Outcome**	**Cut-off**	**Sensitivity (%)**	**Specificity (%)**	**ROC-AUC**	**PPV**	**NPV**
Temporal relationship and predictive value of urinary acute kidney injury biomarkers after pediatric cardiopulmonary bypass	Krawczeski C, Goldstein S, Woo J *et al.*	2011	Single-center (220)	< 18 years	AKI development TP: 24h post-CPB	NR	NR	NR	0.80	NR	NR
Urine biomarkers predict acute kidney injury and mortality in very low birth weight infants.	Askenazi D, Montesanti A, Hundley H *et al.*	2011	Two-centers (30)	Preterm infants	AKI development	NR	NR	NR	0.50	NR	NR
			Two-centers (123)		Mortality	NR	NR	NR	0.65	NR	NR
Serum and urine acute kidney injury biomarkers in asphyxiated neonates	Sarafidis K, Tsepkentzi E, Agakidou E *et al.*	2012	Single-center (35)	Neonates > 36 GW	AKI development within 24h	569.8 pg/mL	40	86	0.583	NR	NR
Biomarkers of acute kidney injury in pediatric cardiac surgery	Peco-Antić A, Ivanišević I, Vulićević I *et al.*	2013	Single-center (112)	< 18 years	AKI development TP: 2h post-CPB	NR	NR	NR	0.69	NR	NR
Towards a biomarker panel for the assessment of AKI in children receiving intensive care	McCaffrey J, Coupes B, Chaloner C *et al.*	2015	Single-center (49)	< 16 years	pRIFLE R or worse during PICU admission (within maximum of 7 days)	NR	NR	NR	0.51	NR	NR
Urinary neutrophil gelatinase-associated lipocalin identifies critically ill young children with acute kidney injury following intensive care admission: A prospective cohort study	Zwiers A, de Wildt S, van Rosmalen J *et al.*	2015	Single-center (100)	1 day - 1 year	AKI occurance within 48h following PICU admission TP: 12-24h	190 pg/mL	72	67	0.737	NR	NR
Urinary calprotectin, kidney injury molecule-1, and neutrophil gelatinase-associated lipocalin for the prediction of adverse outcome in pediatric acute kidney injury	Westhoff J, Seibert F, Waldherr S *et al.*	2017	Single-center (72)	0 - 18 years	30-day mortality	4520 pg/mL	57	67	0.63	20	91
					3-month mortality	4520 pg/mL	63	68	0.68	25	91
					RRT-requirement	5064 pg/mL	67	84	0.76	67	84

Urinary biomarkers of cell cycle arrest are delayed predictors of acute kidney injury after pediatric cardiopulmonary bypass	Dong L, Ma Q, Bennett M *et al.*	2017	Single-center (150)	< 18 years	All forms of AKI TP: 24h post-CPB inititation	NR	NR	NR	~0.87	NR	NR
Urine KIM-1 as a Potential Biomarker of Acute Renal Injury after Circulatory Collapse in Children	Assadi F, Sharbaf F	2019	Single-center (86)	7 months-14 years	AKI at day-6 post-PICU admission	0.8 ng/mg Cr	91	81	0.81	NR	NR
Urinary acute kidney injury biomarkers in very low-birth-weight infants on indomethacin for patent ductus arteriosus	Waldherr S, Fichtner A, Beedgen B *et al.*	2019	Single-center (32)	Preterm neonates	KDIGO AKI at 12h post-indomethacin initiation	NR	NR	NR	0.70	NR	NR

Table 6. KIM-1 results in AKI studies evaluated.

IL-18											
Title	**Authors**	**Date**	**Cohort (n)**	**Age**	**AKI Outcome**	**Cut-off**	**Sensitivity (%)**	**Specificity (%)**	**ROC-AUC**	**PPV**	**NPV**
Postoperative biomarkers predict acute kidney injury and poor outcomes after pediatric cardiac surgery	Parikh C, Devarajan P, Zappitelli M *et al.*	2011	Multicenter (311)	1 month - 18 years	Development of severe AKI TP: first post-op measure	125 pg/mL	69	68	0.72	30	92
Urine biomarkers predict acute kidney injury and mortality in very low birth weight infants.	Askenazi D, Montesanti A, Hundley H *et al.*	2011	Two-centers (30)	Preterm infants	AKI development	NR	NR	NR	0.60	NR	NR
			Two-centers (123)		Mortality	NR	NR	NR	0.47	NR	NR
Temporal relationship and predictive value of urinary acute kidney injury biomarkers after pediatric cardiopulmonary bypass	Krawczeski C, Goldstein S, Woo J *et al.*	2011	Single-center (220)	< 18 years	AKI development TP: 6h post-CPB	NR	NR	NR	0.78	NR	NR

Comparison of urinary biomarkers for early detection of acute kidney injury after cardiopulmonary bypass surgery in infants and young children	Zheng J, Xiao Y, Yao Y *et al.*	2013	Single-center (58)	< 3 years	AKI development TP: 4h post-CPB	49 pg/mL	96.6	62.1	0.835	71.8	94.7
Urinary Biomarkers and Renal Near-Infrared Spectroscopy Predict ICU Outcomes Following Cardiac Surgery in Infants Under 6 Months of Age	Hazle M, Gajarski R, Aiyagari R *et al.*	2014	Single-center (49)	< 6 months	Poor Outcome: death within 30d post-CPB, need for RRT, prolonged time for 1st extubation or ICU stay	0.8 ng/mL	59	86	0.76	68	80
Urinary biomarkers of cell cycle arrest are delayed predictors of acute kidney injury after pediatric cardiopulmonary bypass	Dong L, Ma Q, Bennett M *et al.*	2017	Single-center (150)	< 18 years	All forms of AKI TP: 24h post-CPB initiation	NR	NR	NR	~0.83	NR	NR
Predictive ability of urinary biomarkers for outcome in children with acute kidney injury	Mishra O, Rai A, Srivastava P *et al.*	2017	Single-center (46)	3 months - 14 years	Mortality	179.6 pg/mg	65.5	70.8	0.688	NR	NR
Urine biomarkers of acute kidney injury in noncritically ill, hospitalized children treated with chemotherapy	Sterling M, Al-Ismaili Z, McMahon K *et al.*	2017	Two centers (30)	3 months - 18 years	Post-Cis-Carb infusion (day 1) AKI	NR	NR	NR	0.41	NR	NR
			Two centers (27)		Post-Ifos infusion (day 1) AKI	NR	NR	NR	0.73	NR	NR
Urine KIM-1 as a Potential Biomarker of Acute Renal Injury after Circulatory Collapse in Children	Assadi F, Sharbaf F	2019	Single-center (86)	7 months - 14 years	AKI at day-6 post-PICU admission	NR	NR	NR	0.69	NR	NR
Early diagnosis and prognostic value of acute kidney injury in critically ill patients	Dobilienė D, Masalskienė J, Rudaitis Š *et al.*	2019	Single-center (32)	1 month - 18 years	AKI progression	69.24 pg/mL	62.59	83.3	0.734	NR	NR

Table 7. IL-18 results in AKI studies evaluated.

L-FABP											
Title	**Authors**	**Date**	**Cohort (n)**	**Age**	**AKI Outcome**	**Cut-off**	**Sensitivity (%)**	**Specificity (%)**	**ROC-AUC**	**PPV**	**NPV**
Liver fatty acid-binding protein as a biomarker of acute kidney injury after cardiac surgery	Portilla D, Dent C, Sugaya T *et al.*	2008	Single-center (40)	Children	AKI development TP: 4h post-CPB	486 ng/mg Cr	71.4	68.4	0.810	NR	NR
Temporal relationship and predictive value of urinary acute kidney injury biomarkers after pediatric cardiopulmonary bypass	Krawczeski C, Goldstein S, Woo J *et al.*	2011	Single-center (220)	< 18 years	AKI development TP: 12h post-CPB	NR	NR	NR	0.78	NR	NR
Biomarkers of acute kidney injury in pediatric cardiac surgery	Peco-Antić A, Ivanišević I, Vulićević I *et al.*	2013	Single-center (112)	< 18 years	AKI development TP: 2h post-CPB	NR	NR	NR	0.89	NR	NR
Urinary biomarkers of cell cycle arrest are delayed predictors of acute kidney injury after pediatric cardiopulmonary bypass	Dong L, Ma Q, Bennett M *et al.*	2017	Single-center (150)	< 18 years	All forms of AKI TP: 12h post-CPB initiation	NR	NR	NR	0.85	NR	NR
Novel Urinary Biomarkers for Acute Kidney Injury and Prediction of Clinical Outcomes After Pediatric Cardiac Surgery	Yoneyama F, Okamura T, Tlragiku K *et al.*	2019	Single-center (103)	< 18 years	AKI development TP: ICU admission	90 ng/mL	64	93	0.82	NR	NR

Table 8. L-FABP results in AKI studies evaluated.

TIMP-2*IGFBP-7											
Title	**Authors**	**Date**	**Cohort (n)**	**Age**	**AKI Outcome**	**Cut-off**	**Sensitivity (%)**	**Specificity (%)**	**ROC-AUC**	**PPV**	**NPV**
Urinary biomarkers of cell cycle arrest are delayed predictors of acute kidney injury after pediatric cardiopulmonary bypass	Dong L, Ma Q, Bennett M *et al.*	2017	Single-center (150)	< 18 years	All forms of AKI TP: 12h post-CPB initiation	NR	NR	NR	~0.83	NR	NR
Kinetics of the cell cycle arrest biomarkers (TIMP-2*IGFBP-7) for prediction of acute kidney injury in infants after cardiac surgery	Gist K, Goldstein S, Wrona J *et al.*	2017	Multicenter (94)	< 1 year	KDIGO AKI TP: 12h post-CPB	NR	~70	~70	0.71	NR	83
Urinary acute kidney injury biomarkers in very low-birth-weight infants on indomethacin for patent ductus arteriosus	Waldherr S, Fichtner A, Beedgen B *et al.*	2019	Single-center (32)	Preterm infants	KDIGO AKI TP: 12h post-indomethacin initiation	NR	NR	NR	1.00	NR	NR

Table 9. TIMP-2*IGFBP-7 results in AKI studies evaluated.