Urinary Biomarkers IGFBP7 and TIMP-2 for the Diagnostic Assessment of Transient and Persistent Acute Kidney Injury in Critically Ill Patients

Delphine Daubin[1], Jean Paul Cristol[2,3], Anne Marie Dupuy[2], Nils Kuster[2], Noémie Besnard[1], Laura Platon[1], Aurèle Buzançais[1], Vincent Brunot[1], Fanny Garnier[1], Olivier Jonquet[1], Kada Klouche[1,3]*

1 Department of Intensive Care Medicine, Lapeyronie University Hospital, Montpellier, France, 2 Department of Biochemistry, Lapeyronie University Hospital, Montpellier, France, 3 PhyMedExp, Centre National de la Recherche Scientifique (CNRS 9214) - Institut National de la Santé et de la Recherche Médicale (INSERM U-1046), Montpellier University, Montpellier, France

* k-klouche@chu-montpellier.fr

Abstract

Objective

The capability of urinary TIMP-2 (tissue inhibitor of metalloproteinase) and IGFBP7 (insulin-like growth factor binding protein)—NephroCheck Test (NC) = ([TIMP-2] x [IGFBP7]) / 1000)—to predict renal recovery from acute kidney injury (AKI) has been poorly studied. The aim of this study was to assess the performance of measurements of ([TIMP-2] x [IGFBP7]) / 1000) over 24 hours to differentiate transient from persistent AKI.

Methods

Of 460 consecutive adult patients admitted to the ICU, 101 were prospectively studied: 56 men, 62 (52–71) years old. A fresh urine sample was collected at H_0, H_4, H_{12} and H_{24} to determine ([TIMP-2] x [IGFBP7]) / 1000) levels. Areas under the curves of Delta NC H_4-H_0 and H_{12}-H_4 and serum creatinine (sCr) for detection of AKI recovery were compared.

Results

Forty-one (40.6%) patient were diagnosed with AKI: 27 transient and 14 persistent AKI. At admission (H_0), AKI patients had a significantly higher NC score than patients without AKI (0.43 [0.07–2.06] vs 0.15 [0.07–0.35], p = 0.027). In AKI groups, transient AKI have a higher NC, at H_0 and H_4, than persistent AKI (0.87 [0.09–2.82] vs 0.13 [0.05–0.66] p = 0.035 and 0.13 [0.07–0.61] vs 0.05 [0.02–0.13] p = 0.013). Thereafter, NC level decreased in both AKI groups with a Delta NC score H_4-H_0 and H_{12}-H_4 significantly more important in transient AKI. Roc curves showed however that delta NC scores did not discriminate between transient and persistent AKI.

Conclusion

In our population, absolute urinary levels of NC score were higher at early hours after ICU admission (H_0 and H_4) in transient AKI as compared to persistent AKI patients. NC variations (Delta NC scores) over the first 12 hours may indicate the AKI's evolving nature with a more significant decrease in case of transient AKI but were not able to differentiate transient from persistent AKI.

Background

In critically ill patients, the occurrence of an acute kidney injury (AKI) is frequent and bears a bad prognosis especially when it requires renal replacement therapy (RRT) [1–4]. At early stage, diagnosing and foreseeing the nature of a renal dysfunction—transient or persistent-are a key challenge for routine clinical practice but are deemed difficult. RIFLE, AKIN and KDIGO staging systems have certainly prompted intensivists to follow hourly diuresis and to take into account small creatinine rise for the diagnosis of kidney dysfunction, but enable neither early diagnosis nor transient or persistent AKI diagnosis [3, 5].

Kidney damage is associated with tubular cells lesions and retention of protein waste in the tubular lumen [6]. An abnormal presence of these proteins would be a better marker of kidney lesions and would precede glomerular filtration rate (GFR) decrease and serum creatinine (sCr) increase. Indeed, creatinine rise is rather slow and may occur in other conditions than tubular damage including cardio- and hepato-renal syndromes or obstructive AKI. Several studies validated the clinical value of new biomarkers in AKI detection [7, 8]. Some of them, like plasma cystatin C, are GFR markers, while others, like the urinary cystatin, Neutrophil gelatinase-associated lipocalin [9, 10], urinary interleukin 18 [11] or KIM-1 [12] do mark tubular impairments. These new markers have been successfully tested in numerous clinical conditions including postsurgical cardiology, and admission to the emergency room [10, 13]. The studies conducted in ICUs led however to discordant results due to heterogeneous studied populations and several confounders [7, 14]. Indeed, none of the mentioned biomarkers are routinely used and no recommendation has been made about their use in daily practice to optimize the patient's care [15].

The use of the combination of two new markers, TIMP-2 (tissue inhibitor of metalloproteinase) and IGFBP7 (insulin-like growth factor binding protein), seems to improve the identification of patients at risk of AKI at 12 hours compared with previous biomarkers [16]. These preliminary results may have however to be confirmed by further investigations. Moreover, these studies did investigate the prediction of AKI occurrence in ICU but without foreseeing its scalability [16]. To the best of our knowledge, very few studies have evaluated ([TIMP-2] x [IGFBP7])/1000) urinary measurements, the AKIRisk/NephroCheck (NC) Kit's (Astute Medical, San Diego, CA—USA) performance to differentiate transient from persistent AKI. We therefore conducted this study to assess the capability of the urinary measurements of TIMP-2 and IGFBP7, within the first 24 hours in ICU, to early detect AKI and to differentiate transient from persistent AKI.

Patients and Methods

This observational, prospective, single-center study has been conducted in a medical ICU at the Lapeyronie University Hospital of Montpellier, France and was approved by the Ethic

Committee of this institution. A free, informed and signed approval was collected from patients or their relatives by the investigator.

Population

From March 2014 to May 2015, consecutive adult patients admitted to our ICU were prospectively included in the study. Exclusion criteria included age <18 years, previous chronic renal failure stage >3, stage-c cirrhosis, progressive neoplasia, organ transplant, AKI of obstructive, glomerular or vascular etiology, tumor lysis syndrome, severe rhabdomyolysis, stage-3 AKI according to KDIGO scoring [5], nephrotoxic treatment within the 72 hours before admission, corticotherapy > 1mg/kg, and presumed life expectancy of <48 hours. A bladder catheter was inserted for diuresis monitoring and urine sampling, as well as an arterial catheter for blood pressure monitoring and blood sampling.

Data collection and study design

Baseline patient characteristics were recorded including age, gender, and comorbidities. Severity of illness was determined using the simplified acute physiology score II (SAPSII), and the Sequential Organ Failure Assessment (SOFA) score. Main therapeutics including type and quantity of fluids, mechanical ventilation, and administration of vasopressor drugs, and RRT were listed.

Blood and urines samples were collected at admission (H_0), and 4 (H_4), 12 (H_{12}), 24 hours (H_{24}) later for blood determination of urea, creatinine (sCr), electrolytes and urine determination of electrolytes.

Fresh urines samples were also taken at each time for AKIRisk/NC score measurements. AKIRisk/NC score results in the measurement of two markers: TIMP-2 and IGFBP7 and is valued according to the formula: NC = ([TIMP-2] X [IGFBP7]/1000). The 2 markers have been measured thanks to an Astute 140 meter® (Astute Medical, San Diego, CA—USA) converting fluorescent signals from each of the two immunoassays. The NC test (Astute Medical, San Diego, CA—USA) requires a fresh urine sample of at least 100 μl. Samples were either immediately processed, the result coming after 20 minutes, or later on with sample storage and freezing at -20°C. In both cases, investigators were blinded to the results of NC scoring. All values for ([TIMP-2] x [IGFBP7])/1000)-NC- are reported in units of $[(ng/ml)^2/1000]$. In the validation study, a cut-off of AKIRisk/NC score: ([TIMP-2] x [IGFBP7])/1000)>0.3 $[(ng/ml)^2/1000]$ was found to be predictive of AKI within 12 hours [16].

Definitions

AKI was defined as urine volume < 0.5 ml/kg/hour for 6 hours or/and a sCr increase ≥ 26.4 μmol/L or a sCR increase of 1.5 times the baseline value (KDIGO≥1) [5]. Baseline sCr was defined as the usual value of sCr preceding the admission within at least 7days or as the nadir sCr after recovery of renal function. Transient and persistent AKI were defined by the recovery -or not- of renal function at the latest day 5 as previously reported [17] and according to our practice. Recovery of renal function was defined as a return to a sCR value <1.5 times the baseline value or < 26.4 μmol/L above the baseline value with reversal of oliguria and/or return to the baseline sCr. Persistent AKI was defined as a steady or higher AKI KDIGO stage and/or persistence of oliguria after day 5. Glomerular filtration rate was calculated by using the CKD-EPI equation [18].

Statistical analysis

Statistical analyses were performed using the software R 3.1.0 (R Foundation for Statistical Computing, Vienna, Austria). We first performed a descriptive analysis by computing the frequencies and the percents for categorial data; medians, quartiles (1st quartile-3rd quartile) and extreme values for continuous data. We also checked for the normality of the continuous data distribution using the Shapiro-Wilk's tests. The population was subdivided in two groups according to the occurrence of AKI: no AKI vs AKI. AKI group was then subdivided in two subgroups: transient and persistent AKI. The univariate analysis was performed using two-tailed Student t-test for continuous variables, Fisher and Chi 2 tests for categorial variables or two-tailed Mann-Whitney-Wilcoxon's test when appropriate. Receiver-operating characteristic curves (ROCs) were plotted to determine the prognostic values of the AKI markers AKIRisk/NC score and creatininemia, and area under the curves (AUC) values were compared using the DeLong's nonparametric approach [19]. The primary endpoint was AKI recovery within 5 days. A p-value less than 0.05 was considered statistically significant.

Results

Study population

During the study period, 460 patients were admitted to the ICU, and 133 of them were included. Among these patients, 32 patients were excluded (26 for missing NC dosages, and 6 for nephrotoxic treatments administered prior to ICU admission) leading to a complete analysis of 101 patients (Fig 1). Table 1 summarizes the main patient characteristics. Median sCr level at admission was 84 μmol/l. More than half of the patients were treated with vasoconstrictive drugs and 2/3 of them were intubated and ventilated. Median duration of ICU stay was 5 days and ICU mortality rate was 17%.

AKI, transient and persistent AKI occurrence

During ICU stay, AKI occurred in 41 patients (40.6%). Profiles of patients with (n = 41) or without (n = 60) AKI are displayed in Table 1. AKI patients were older (70 vs 59 years), had more comorbidities and a more altered baseline kidney function than others. They were also more severe as reflected by higher SAPSII and SOFA scores and more frequently treated with vasoconstrictive drugs. However, ICU mortality rate was not higher (24% vs 11%, p = 0.11) and ICU length of stay was not extended. Among AKI patients, 27/41 (66%) were detected with a transient AKI and 14/41 (34%) with a persistent AKI. Although older and with more comorbidities, persistent AKI patients were comparable to those with transient AKI in terms of severity. RRT was initiated for 6 patients that developed a persistent AKI. The occurrence of persistent AKI definitively increased ICU mortality (57% vs 7%) and extended ICU length of stay (10 vs 5 days) (Table 1).

AKIRisk/NC score analysis for the differentiation between transient and persistent AKI

At ICU admission, AKI patients have a higher NC score-([TIMP-2] x [IGFBP7])/1000)—with a median level > 0.3 [(ng/ml)^2/1000] as compared to no AKI patients. At the 4th hour, NC score was slightly comparable in both groups; it noticeably decreased in AKI group after fluid challenge (Table 2).

Regarding AKI groups (Table 3), NC score was higher in transient AKI than in persistent AKI group at admission (0.87 vs 0.13 [(ng/ml)^2/1000], p = 0.035). Conversely, sCr level and kidney function's grading were comparable between groups. At the 4th hour, sCr level, kidney
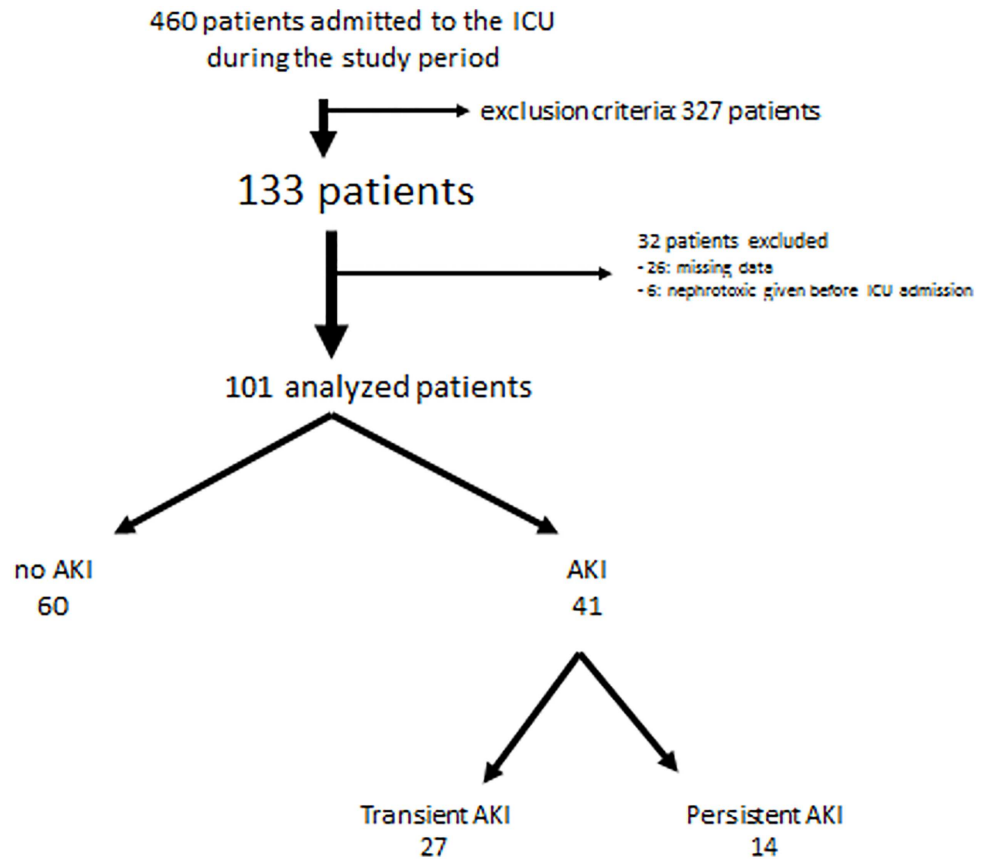
Fig 1. Flow chart of the study population.

doi:10.1371/journal.pone.0169674.g001

function grading, and urine volume remained comparable between both groups while NC score decreased in both groups but remained higher in transient AKI group (Fig 2). Over the next hours (H_{12} and H_{24}), sCr, KDIGO score, urine volume, and NC scores did not enable to differentiate the two groups.

Analysis of measurements at the successive time point's showed that NC score quickly decreased after H_0 to reach a level less than 0.1 in both groups (Table 3, Fig 2). However, Deltas of NC score H_4-H_0 (NC score H_4- NC score H_0) and H_{12}-H_4 (NC score H_{12}- NC score H_4) were more important in transient AKI group (-0.45 vs -0.07 [$(ng/ml)^2/1000$], p = 0.0002 and -0.02 vs 0.01 [$(ng/ml)^2/1000$], p<0.0001) as displayed in Fig 3. However, ROC curves displayed on Fig 4 showed that neither H_4-H_0 and H_{12}-H_4 Delta NC scores nor sCr discriminate transient from persistent AKI.

Discussion

In this study, patients with AKI have higher urinary levels of NC score than those without AKI at ICU admission. Also, NC score levels were higher in patients with transient AKI than in those with persistent AKI at ICU admission (H_0) and 4 hours later (H_4). The analysis of NC score at different time points showed that NC score decreased in the first 12 hours in case of transient AKI but remained at the same level, or even increased in case of persistent AKI. Deltas NC score H_4-H_0 and H_{12}-H_4 were indeed higher in transient AKI but did not enable to differentiate between transient and persistent AKI.

Table 1. Baseline characteristics of the studied population and differences between AKI and no AKI groups and between transient and persistent AKI groups of patients.

	Population, n = 101	no AKI, n = 60	AKI, n = 41	p*	Transient AKI, n = 27	Persistent AKI, n = 14	p**
Age, median [IQR], years	62 [52–71]	59 [47–68]	70 [61–77]	<0.05	67 [57–72]	73 [70–79]	<0.05
Men, n (%)	56 (55)	29 (48)	27 (66)	0.10	18 (67)	9 (64)	0.21
Comorbidities, n (%)							
HTA	44 (43.5)	19 (32)	25 (61)	<0.05	12 (44)	13 (93)	<0.05
Cardiac failure	30 (29.7)	13(22)	17 (41)	<0.05	7 (26)	10 (71)	<0.05
≥ 2 comorbidities	35 (34.6)	15 (25)	20 (49)	<0.05	9 (33)	11 (78)	<0.05
GFR, median [IQR], ml/min/1.73m²	85 [68–109]	98 [76–117]	78 [63–90]	<0.05	82 [70–94]	64 [50–80]	<0.05
SAPS II, median [IQR]	46 [35–57]	43 [30–55]	52 [45–69]	< 0.05	51 [44–64]	52 [46–71]	0.65
SOFA, median [IQR]	6 [4–9]	5 [4–9]	8 [6–10]	<0.05	6 [6–9]	8 [7–10]	0.31
MV, n (%)	69 (68.3)	44 (73)	25 (61)	0.21	14 (52)	11 (79)	0.22
Vasopressive drugs, n (%)	56 (55.4)	24 (40)	32 (78)	<0.05	21 (78)	11 (79)	0.35
RRT, n (%)	6 (5.9)	0	6 (15)	<0.05	0	6 (43)	<0.05
LOS, median [IQR], days	5 [3–10]	4.5 [3–9]	6 [3–11]	0.12	5 [3–9]	10 [6–24]	<0.05
Mortality, n (%)	17	7(11)	10 (24)	0.11	2 (7)	8 (57)	<0.05

AKI: acute kidney injury; IQR: interquartile 25–75; GFR: glomerular filtration rate; SAPS-II: simplified acute physiology score II, SOFA: sequential organ failure assessment; MV: mechanical ventilation; RRT: renal replacement therapy, LOS: length of stay.

* differences between no AKI and AKI patients;

**differences between transient and persistent AKI patients

doi:10.1371/journal.pone.0169674.t001

Numerous biomarkers of tubular impairment have been tested and developed to early detect AKI including NGAL, KIM-1, and interleukin 18. These biomarkers have already shown some limitations, especially their lack of specificity. Indeed, they may rise in settings like a non-complicated sepsis without occurred renal function alteration. It may due to their high sensibility to tubular injury and their fast kinetics unlike sCr [7, 14, 20]. Recently, several studies showed the superiority of AKIRisk/NC score- newly developed marker associating two biomarkers: TIMP-2 and IGFBP7- over other markers for an early detection of AKI [16]. A first single-centric study, counting 522 patients in ICU, led to select, among the 340 proteins

Table 2. Comparison between no AKI and AKI groups at ICU admission and 4 hours later.

	No AKI, n = 60	AKI, n = 41	p
H0			
Creat median [IQR], μmol/l	65 [53–80]	137 [109–196]	<0.05
Mechanical ventilation, n (%)	41 (68)	17 (41)	<0.05
Vasopressive drugs, n (%)	18 (30)	19 (46)	0.17
AKIRisk/NC score, median [IQR], (ng/ml)²/1000	0.15 [0.07–0,35]	0.43 [0.07–2,06]	0.027
H4			
Creat. median [IQR], μmol/l	61 [51–78]	129 [98–189]	<0.05
Diuresis, median [IQR], ml/h	30 [20–53]	21 [15–55]	0.31
Mechanical ventilation, n (%)	43 (72)	21 (51)	0.15
Vasopressive drugs, n (%)	25 (42)	22 (54)	0.21
AKIRisk/NC score, median [IQR], (ng/ml)²/1000	0.08 [0.04–0.29]	0.09 [0.04–0.78]	0.67

AKI: acute kidney injury; Creat: creatininemia; KDIGO: Kidney Disease Improving Global Outcomes; NC score: NephroCheck score.

IQR: interquartile 25–75; p: differences between AKI and no AKI patients.

doi:10.1371/journal.pone.0169674.t002

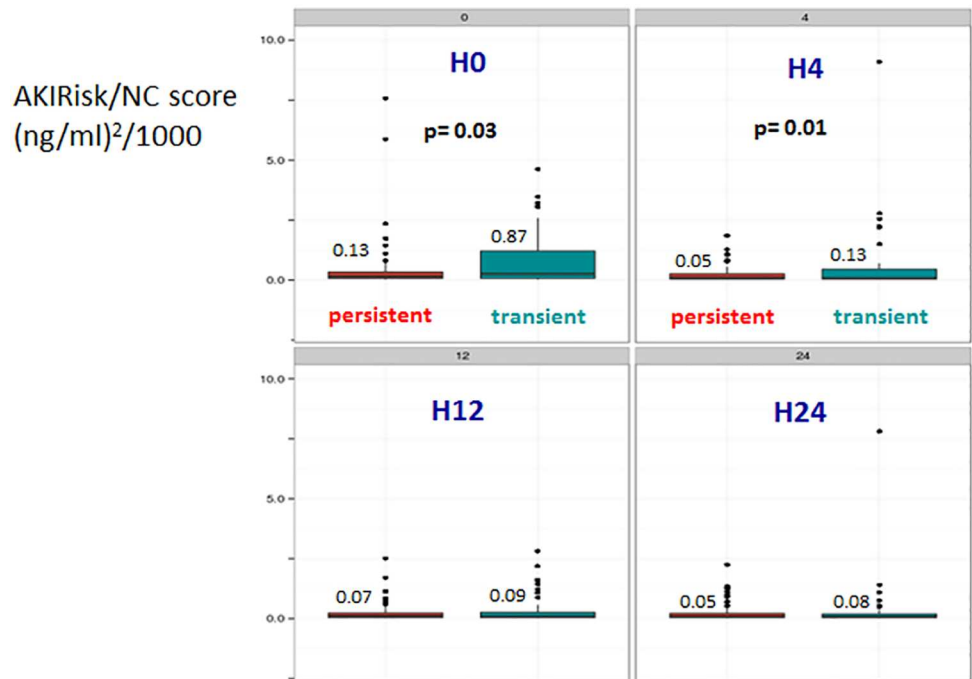**Table 3. Comparison of transient and persistent AKI groups at ICU admission and the following 24 hours.**

	Transient AKI, n = 27	Persistent AKI, n = 14	p
H0			
Creat. median [IQR], µmol/l	149 [106–205]	120 [109–145]	0.72
KDIGO score, median [IQR]	1 [1–2]	1 [0–1]	0.84
Mechanical ventilation, n (%)	9 (33)	8 (57)	0.35
Vasopressive drugs, n (%)	13 (48)	6 (43)	0.42
AKIRisk/NC score, median [IQR], $(ng/ml)^2/1000$	0.87 [0.09–2.82]	0.13 [0.05–0.66]	0.035
H4			
Creat. median [IQR], µmol/l	134 [100–194]	122 [99–143]	0.65
Diuresis, median [IQR], ml/h	20 [14–28]	36 [20–64]	0.17
KDIGO score, median [IQR]	1 [1–2]	1 [0–1]	0.54
Mechanical ventilation, n (%)	13 (48)	8 (57)	0.48
Vasopressive drugs, n (%)	15 (56)	7 (50)	0.45
AKIRisk/NC score, median [IQR], $(ng/ml)^2/1000$	0.13 [0.07–0.61]	0.05 [0.02–0.13]	0.013
H12			
Creat. median [IQR], µmol/l	130 [96–179]	129 [93–161]	0.78
Diuresis, median [IQR], ml/h	30 [22–41]	31 [20–46]	0.76
KDIGO score, median [IQR]	1 [0–2]	1 [0–1]	0.84
Mechanical ventilation, n (%)	14 (52)	8 (57)	0.46
Vasopressive drugs, n (%)	17 (63)	10 (71)	0.39
AKIRisk/NC score, median [IQR], $(ng/ml)^2/1000$	0.09 [0.05–0.59]	0,07 [0.02–0.21]	0.32
H24			
Creat. median [IQR], µmol/l	114 [81–156]	128 [94–176]	0.26
Diuresis, median [IQR], ml/h	41 [27–47]	28 [16–53]	0.11
KDIGO score, median [IQR]	1 [0–1]	1 [1–2]	0.39
Mechanical ventilation, n (%)	13 (48)	8 (57)	0.86
Vasopressive drugs, n (%)	14 (52)	11 (79)	0.22
SOFA, median [IQR]	6 [1–8]	7 [6–9]	<0.05
AKIRisk/NC score, median [IQR], $(ng/ml)^2/1000$	0.08 (0.06–0.34)	0.05 (0.02–0.09)	0.06

AKI: acute kidney injury; creat: creatininemia; KDIGO: Kidney Disease Improving Global Outcomes; SOFA: Sequential Organ Failure Assessment; NC score: NephroCheck score.

IQR: interquartile 25–75; sd: standard deviation, p: differences between transient and persistent AKI patients.
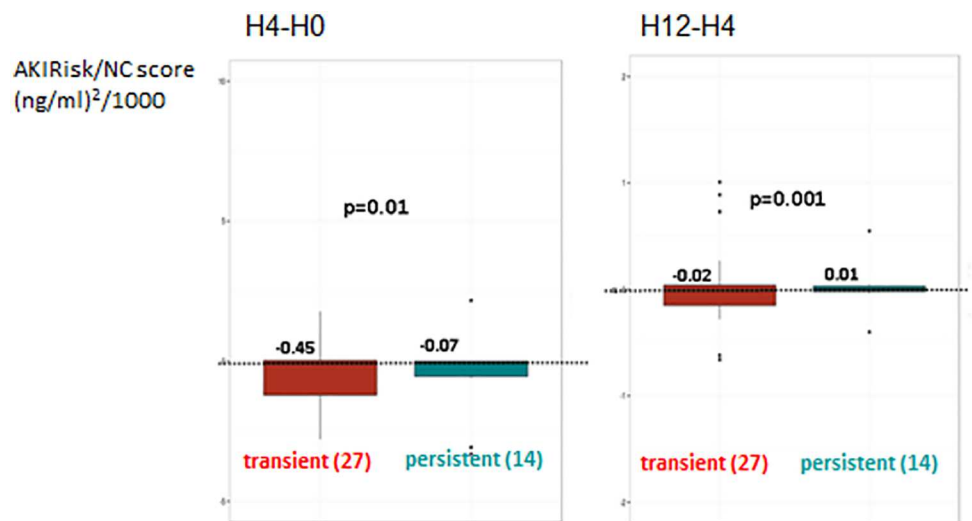
doi:10.1371/journal.pone.0169674.t003

identified as early markers, the most sensitive and most specific biomarkers of AKI. IGFBP7 and TIMP-2 were already known as having the best performance with an AUC of respectively 0.77 and 0.75. A second multicenter observational study (Sapphire Study) including 728 patients validated the performance of these markers with an AUC for IGFBP7 and for TIMP-2 statistically superior to those of KIM-1 and NGAL. More interestingly, their performance was enhanced when associated. The Sapphire study's results helped to set up two threshold values of the detection of AKI: AKIRisk/NC score of 0.3 for a better sensitivity (90%) and of 2 for specificity (95%) that were confirmed later [16, 21–23]. In our study, a AKIRisk/NC score at ICU admission was higher for patients with AKI with a median score> 0.3 $[(ng/ml)^2/1000]$. Our population was exclusively medical with many comorbidities and AKI is mostly multi-factorial and its origin prior to the admission [3, 4]. Indeed, the type and timing of the tubular aggression are rarely precisely identified conversely to post-operative AKI [24]. Our study shows that the AKIRisk/NC score is associated with a tubular aggression since it is higher in case of AKI but remains <0.2 $[(ng/ml)^2/1000]$ if no AKI occurs. We observed however a

Fig 2. AKIRisk/NephroCheck score at ICU admission (H$_0$) and the following 24 hours: comparison between transient and persistent AKI patients. AKI: acute kidney injury; NC: NephroCheck.

doi:10.1371/journal.pone.0169674.g002

decrease in this score from the fourth hour with medians < 0.1 [(ng/ml)^2/1000] despite the AKI. This decrease seems discordant especially with studies conducted in post-cardiac surgery [23, 24, 25]. Meersch et al [23] observed a significant increase in the AKIRisk/NC score from the 4th hour, earlier than the rise in creatinine and till the 12th hour after cardiac surgery in the group with AKI. One should however retain that the Bell's study does not exhibit such a

Fig 3. Deltas AKIRisk/NephroCheck score H$_4$-H$_0$ and H$_{12}$-H$_4$: comparison between transient and persistent AKI patients. AKI: acute kidney injury; NC: NephroCheck.
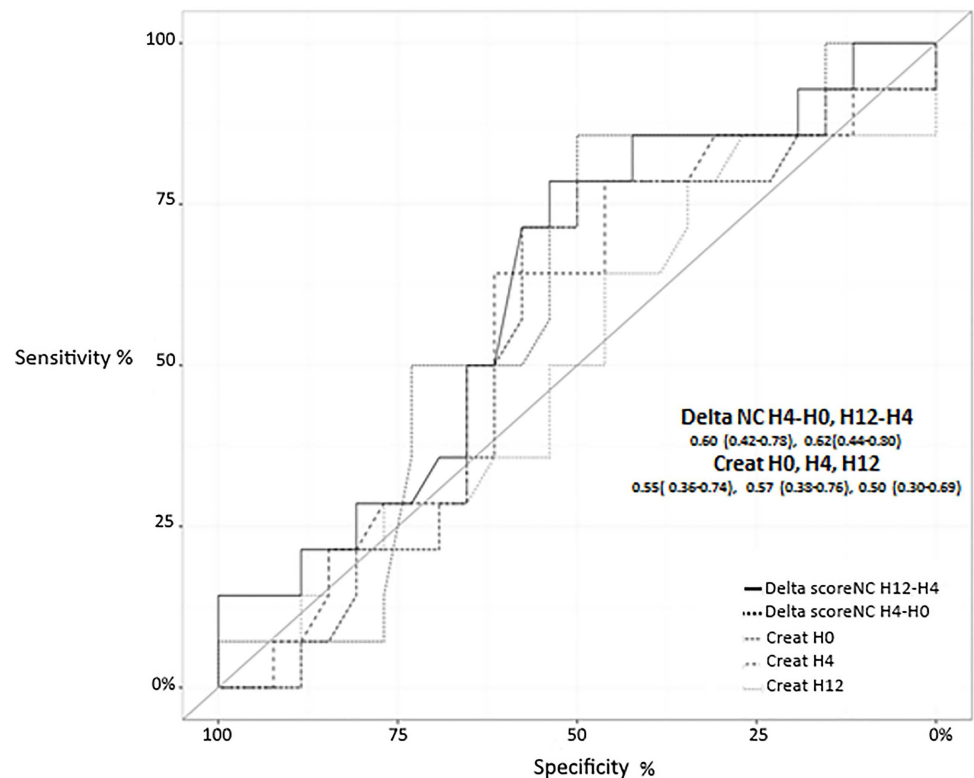
doi:10.1371/journal.pone.0169674.g003

Fig 4. Area under the curves for creatininemia (H$_0$, H$_4$,H$_{12}$) and for Deltas AKIRisk/NephroCheck score H$_4$-H$_0$ and H$_{12}$-H$_4$ to differentiate between transient or persistent acute kidney injury. AKI: acute kidney injury; NC: NephroCheck.

doi:10.1371/journal.pone.0169674.g004

good performance of AKIRisk/NC score in AKI prediction although few patients were included [25].

The main purpose of this study was to evaluate the performance of the NC score to differentiate transient from persistent AKI in the first 24 hours in ICU. Out of the 41 AKI patients, 27 transient AKIs and 14 persistent AKIs were identified. The differentiation between transient AKI and persistent AKI is crucial because it impacts both the care and prognosis [20, 26, 27]. Clinical and biological evidences (FE of sodium, FE of urea, U/P urea and osmolality...) are widely used for that matter but several studies have unveiled their lack of reliability especially in ICU [17, 28–30]. Echo-Doppler measurements of renal vascular resistance seemed to efficiently differentiate the 2 types of kidney impairment but results again disappointed [31, 32]. This emphasizes the value of an early identification and validation of new, performing urinary biomarkers. Previous studies did not enable to select one biomarker in this purpose [7, 9, 14, 33]. In our population, we found that the absolute values of NC score at different times (H$_0$ and H$_4$) were higher in transient AKI as compared to persistent AKI patients which may seem paradoxical regarding to previous studies. Indeed in a Sapphire's ancillary study, IGFBP7 was identified as an early prognostic marker of the AKI's severity, duration and associated mortality [34]. Also, Yamashita showed that TIMP-2 was performing in severe AKI detection and was predictive of poor prognosis [35]. More interestingly, Dewitte et al investigated NC score's kinetics and recently reported that NC score was significantly higher at H$_0$ and H$_{24}$ in persistent AKI patients [36]. The authors concluded to a good predictability of the NC score's kinetics over the first 24 hours in the recovery of the kidney function after the second day. Here

again, the populations are not comparable because the aforementioned study mostly included surgical patients. These discordant results led us to investigate further. We observed a decrease in NC score at H_4 and the following time measurements, mainly in the group with transient AKI. Moreover, Deltas NC score H_4-H_0 and H_{12}-H_4 were higher in transient AKI patients. It was mainly a fluid loading, as if relevant therapeutics, that triggered a NC score decrease in case of transient AKI but has a lesser impact in the case of persistent AKI.

The differentiation between pre-renal or functional AKI and renal or organic AKI was questioned, from 2007 by Bellomo [26, 37], for patients with severe sepsis. The functional and organic AKI may work the same way and only the duration of the degradation would define the impairment's severity namely its transient or persistent nature [38, 39]. Our results would then comply with this concept. Accordingly, the increase in the NC score at an early stage is a marker of tubular impairment whatever the type of transient or persistent AKI; even higher if AKI is transient. It may suggest that TIMP2 and IGFBP7 are released as an alarm signal of aggression with a greater response in case of hemodynamic changes like functional AKI and that a decrease or stabilization of these biomarkers, mostly dependent on the therapy, would characterize the AKI. Our observations may be put in line with the Zarbock's observations [40]. In a study of ischemic preconditioning, he demonstrated a rise in ([TIMP-2] x [IGFBP7]) / 1000) levels, but not of NGAL and HMGB-1, for all patients who went through a presurgical ischemic preconditioning versus a control group. Ischemic preconditioning would then induce a significant increase in AKIRisk/NC score in contrast with the other biomarkers, suggesting that the combination of [TIMP-2] and [IGFBP7] is more sensitive to transient hemodynamic variations [41]. The next hours, this rise was corrected for the patients preconditioned and unscathed from AKI but not for the patients who will later suffered from an AKI [40]. However, Deltas NC score H_4-H_0 and H_{12}-H_4, as reflected by ROC curve, were not discriminative of transient and persistent AKI in our population.

This study has numerous limitations. It is single-center, prospective but with a quite low number of patients, so it lacked power. This study was conducted exclusively on medical patients and cannot be therefore extrapolated to other surgical wards. Our recruitment was often "second hand", explaining the severity of patient cases. It made also the results hardly extrapolable to other ICUs. We defined persistent AKI as a non-recovery kidney function at D_5 while numerous thresholds were used: D_1 to D_3 [28, 29, 42, 43]. The D_5 milestone was also selected because it looks representative of our daily practice. Some authors like Vanmassenhove proceeded the same way [17] while some others like Brown postponed it to D_7, a timeframe definitely associated with a rise in mortality [30,44].

Conclusion

The NC score is a marker of tubular lesions with a greater increase in transient AKI as compared to persistent AKI suggesting that TIMP2 and IGFBP7 are more sensitive to hemodynamic changes. The investigation of the NC score's kinetics in the frame of an AKI may help for a better physiopathological understanding of the AKI and its transient or persistent mechanism. It remains however necessary to confirm these data with larger studies and targeted populations.

Author Contributions

Conceptualization: KK DD AMD.

Data curation: AB FG VB LP OJ NB.

Formal analysis: KK DD NK.

Investigation: AB FG VB LP OJ NB.

Methodology: KK JPC AMD DD.

Validation: KK NK.

Writing – original draft: KK DD.

Writing – review & editing: KK DD JPC.

References

1. Kellum JA, Angus DC. Patients are dying of acute renal failure. Crit Care Med. 2002; 30(9):2156–2157. PMID: 12352064

2. Metnitz PG, Krenn CG, Steltzer H, Lang T, Ploder J, Lenz K, et al. Effect of acute renal failure requiring renal replacement therapy on outcome in critically ill patients. Crit Care Med. 2002; 30(9):2051–2058. PMID: 12352040

3. Hoste EA, Kellum JA. Acute renal failure in the critically ill: impact on morbidity and mortality. Contrib Nephrol. 2004; 144:1–11.

4. Mehta RL, Pascual MT, Soroko S, Savage BR, Himmelfarb J, Ikizler TA, et al. Spectrum of acute renal failure in the intensive care unit: the PICARD experience. Kidney Int. 2004; 66(4):1613–1621. doi: 10.1111/j.1523-1755.2004.00927.x PMID: 15458458

5. Kellum JA, Lameire N. Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). Crit Care. 2013; 17(1):204. doi: 10.1186/cc11454 PMID: 23394211

6. Lameire N, Van Biesen W, Vanholder R. Lancet. 2008; 372:1863–1865. doi: 10.1016/S0140-6736(08)61794-8 PMID: 19041789

7. Ostermann M, Philips BJ, Forni LG. Clinical review: Biomarkers of acute kidney injury: where are we now? Crit Care. 2012; 16(5):233. doi: 10.1186/cc11380 PMID: 23014769

8. Hoste EA, Kellum JA. Acute kidney dysfunction and the critically ill. Minerva Anesthesiol. 2006; 72 (3):133–143.

9. Mishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, et al. Identification of NGAL as a novel urinary biomarker for ischemic injury. J Am Soc Nephrol. 2003; 14(10):2534–2543. PMID: 14514731

10. Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet. 2005; 365 (9466):1231–1238. doi: 10.1016/S0140-6736(05)74811-X PMID: 15811456

11. Parikh CR, Abraham E, Ancukiewicz M, Edelstein CL. Urine IL-18 is an early diagnostic marker for acute kidney injury and predicts mortality in the intensive care unit. J Am Soc Nephrol. 2005; 16(10):3046–3052. doi: 10.1681/ASN.2005030236 PMID: 16148039

12. Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre JV. Kidney injury molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. Kidney Int. 2002; 62(1):237–244. doi: 10.1046/j.1523-1755.2002.00433.x PMID: 12081583

13. Nickolas TL, O'rourke MJ, Yang J, Sise ME, Canetta PA, Barasch N, et al. Sensitivity and specificity of a single emergency department measurement of urinary neutrophil gelatinase-associated lipocalin for diagnosing acute kidney injury. Ann Intern Med. 2008. 148(11):810–819. PMID: 18519927

14. Legrand M, Darmon M, Joannidis M. NGAL and AKI: the end of a myth? Intensive Care Med. 2013; 39(10):1861–1863. doi: 10.1007/s00134-013-3061-2 PMID: 23949705

15. Murray PT, Mehta RL, Shaw A, Ronco C, Endre Z, Kellum JA, et al. Potentiel use of biomarkers in acute kidney injury: report and summary of recommendations from the 10th Acute Dialysis Quality Initiative consensus conference. Kidney Int. 2014; 85(3):513–521. doi: 10.1038/ki.2013.374 PMID: 24107851

16. Kashani K, Al- Khafaji A, Ardiles T, Artigas A, Bagshaw SM, Bell M, et al. Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury. Crit Care. 2013; 17(1):R25. doi: 10.1186/cc12503 PMID: 23388612

17. Vanmassenhove J, Glorieux G, Hoste E, Dhondt A, Vanholder R, Van Biesen W. Urinary output and fractional excretion of sodium and urea as indicators of transient versus intrinsic acute kidney injury during early sepsis. Crit Care. 2013; 17(5):R234. doi: 10.1186/cc13057 PMID: 24119730

18. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate filtration rate. Ann Intern Med. 2009; 150(9):604–612. PMID: 19414839

19. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics. 1988; 44(3):837–845. PMID: 3203132

20. Legrand M, Jacquemod A, Gayat E, Collet C, Giraudeaux V, Launay JM, et al. Failure of renal biomarkers to predict worsening renal function in high-risk patients presenting with oliguria. Intensive Care Med. 2015; 41(1):68–76. doi: 10.1007/s00134-014-3566-3 PMID: 25465906

21. Hoste EA, McCullough PA, Kashani K, Chawla LS, Joannidis M, Shaw AD, et al. Derivation and validation of cutoffs for clinical use of cycle arrest biommarkers. Nephrol Dial Transplant. 2014; 29(11):2054–2061. doi: 10.1093/ndt/gfu292 PMID: 25237065

22. Bihorac A, Chawla LS, Shaw AD, Al-Khafaji A, Davison DL, Demuth GE, et al. Validation of cell cycle arrest biomarkers for acute kidney injury using clinical adjudication. Am J Respi Crit Care Med. 2014; 189(8):932–939.

23. Meersch M, Schmidt C, Van Aken H, Martens S, Rossaint J, Singbartl K, et al. Urinary TIMP-2 and IGFBP7 as early biomarkers of acute kidney injury and renal recovery following cardiac surgery. Plos One. 2014; 9(3):e93460. doi: 10.1371/journal.pone.0093460 PMID: 24675717

24. Wetz AJ, Richardt EM, Wand S, Kunze N, Schotola H, Quintel M, et al. Quantification of urinary TIMP-2 and IGFBP7: an adequate diagnosis test to predict acute kidney injury after cardiac surgery? Crit Care. 2015; 19:3. doi: 10.1186/s13054-014-0717-4 PMID: 25560277

25. Bell M, Larsson A, Venge P, Bellomo R, Mårtensson J. Assessment of cell-cycle arrest biomarkers to predict early and delaye acute kidney injury. Dis Markers. 2015; 2015:158658. doi: 10.1155/2015/158658 PMID: 25866432

26. Perinel S, Vincent F, Lautrette A, Dellamonica J, Mariat C, Zeni F, et al. Transient and persistent acute kidney injury and the risk of hospital mortality in critically ill patients: results of multicenter cohort study. Crit Care Med. 2015; 43(8):e269–275. doi: 10.1097/CCM.0000000000001077 PMID: 25962084

27. Legrand M, Dupuis C, Simon C, Gayat E, Mateo J, Lukaszewicz AC, et al. Association between systemic hemodynamics and septic acute kidney injury in critically ill patients: a retrospective observational study. Crit Care. 2013; 17(6):R278. doi: 10.1186/cc13133 PMID: 24289206

28. Darmon M, Vincent F, Dellamonica J, Schortgen F, Gonzalez F, Das V, et al. Diagnostic performance of fractional excretion of urea in the evaluation of critically ill patients with acute kidney injury: a multicenter cohort study. Crit Care. 2011; 15(4):R178. doi: 10.1186/cc10327 PMID: 21794161

29. Pons B, Lautrette A, Oziel J, Dellamonica J, Vermesch R, Ezingeard E, et al. Diagnostic accuracy of early urinary index changes in differentiating transient from persistent acute kidney injury in critically ill pateints: multicenter cohort study. Crit Care. 2013; 17(2):R56. doi: 10.1186/cc12582 PMID: 23531299

30. Pepin MN, Bouchard J, Legault L, Ethier J. Diagnostic performance of fractional exretion of urea and fractional excretion of sodium in the evaluation of patients with acute kidney injury with or without diuretic treatment. Am J Kidney Dis. 2007; 50(4):566–573. doi: 10.1053/j.ajkd.2007.07.001 PMID: 17900456

31. Lerolle N, Guérot E, Faisy C, Bornstain C, Diehl JL, Fagon JY. Renal failure in septic shock: predictive value of doppler-based of renal arterial resistive index. Intensive Care Med. 2006. 32(10):1553–1559. doi: 10.1007/s00134-006-0360-x PMID: 16941165

32. Schnell D, Camous L, Guyomarch S, Duranteau J, Canet E, Gery P, et al. Renal perfusion assessment by renal doppler during fluid challenge in sepsis. Critical Care Med. 2013; 41(5):1214–1220.

33. Nejat M, Pickering JW, Devarajan P, Bonventre JV, Edelstein CL, Walker RJ, et al. Some biomarkers of acute kidney injury are increased in pre-renal acute injury. Kidney Int. 2012; 81(12):1254–1262. doi: 10.1038/ki.2012.23 PMID: 22418979

34. Aregger F, Uelinger DE, Witowski J, Brunisholz RA, Hunziker P, Frey FJ, et al. Identification of IGFBP-7 by urinary proteomics as a novel prognostic marker in early acute kidney injury. Kidney Int. 2014; 85(4):909–919. doi: 10.1038/ki.2013.363 PMID: 24067438

35. Yamashita T, Doi K, Hamasaki Y, Matsubara T, Ishii T, Yahagi N, et al. Evaluation of urinary tissue inhibitor of metalloproteinase-2 in acute kidney injury: a prospective observational study. Crit Care. 2014; 18(6):716. doi: 10.1186/s13054-014-0716-5 PMID: 25524453

36. Dewitte A, Joannès-Boyau O, Sidobre C, Fleureau C, Bats ML, Derache P, et al. Kinetic eGFR and novel AKI biomarkers to predict renal recovery. Clin J Am Soc Nephrol. 2015; 10 (11):1900–1910. doi: 10.2215/CJN.12651214 PMID: 26342047

37. Bellomo R, Bagshaw S, Langenberg C, Ronco C. Pre-renal azotemia: a flawed paradigm in critically ill septic patients? Contrib Nephrol. 2007; 156:1–9. PMID: 17464109

38. Schneider AG, Bellomo R. Urinalysis and pre-enal acute kidney injury: time to move one. Crit Care. 2013; 17(3):141. doi: 10.1186/cc12676 PMID: 23659200

39. Gomez H, Ince C, De Backer D, Pickkers P, Payen D, Hotchkiss J, et al. A unified theory of sepsis-induced acute kidney injury: inflammation, microcirculatory dysfunction, bioenergetics and the tubular cell adaptation to injury. Shock. 2014; 41(1):3–11. doi: 10.1097/SHK.0000000000000052 PMID: 24346647

40. Zarbock A, Schmidt C, Van Aken H, Wempe C, Martens S, Zahn PK, et al. Effect of remote ischemic preconditioning on kidney injury among high risk patients undergoing cardiac surgery: a randomized clinical trial. JAMA. 2015; 313(21):2133–2141. doi: 10.1001/jama.2015.4189 PMID: 26024502

41. Kellum JA, Chawla LS. Cell-cycle arrest and acute kidney injury: the light and the dark sides. Nephrol Dial Transplant. 2016; 31(1):16–22. doi: 10.1093/ndt/gfv130 PMID: 26044835

42. Uchino S, Bellomo R, Bagshaw SM, Goldsmith D. Transient azotaemia is associated with a high risk of death in hospitalized patients. Nephrol Dial Transplant. 2010; 25(6):1833–1839. doi: 10.1093/ndt/gfp624 PMID: 20054022

43. De Geus HR, Woo JG, Wang Y, Devarajan P, Betjes MG, le Noble JL et al. Urinary neutrophil gelatinase associated lipocalin measured on admission to the intensive care unit accurately discriminates between sustained and transient acute kidney injury in adult critically ill patients. Nephron Extra. 2011; 1 (1):9–23. doi: 10.1159/000330428 PMID: 22470375

44. Brown JR, Kramer RS, Coca SG, Parikh CR. Duration of acute kidney injury impacts long-term survival after cardiac surgery. Ann Thorac Surg. 2010; 90(4):1142–1148. doi: 10.1016/j.athoracsur.2010.04.039 PMID: 20868804