The use of plasma and urine neutrophil gelatinase associated lipocalin (NGAL) and Cystatin C in early diagnosis of septic acute kidney injury in critically ill patients

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Abstract

AIM: To assess and compare the roles of plasma and urine concentrations of neutrophil gelatinase associated lipocalin (NGAL) and Cystatin C for early diagnosis of septic acute kidney injury (AKI) in adult critically ill patients.

METHODS: Patients were divided into three groups as sepsis-non AKI, sepsis-AKI and non sepsis-non AKI. Plasma samples for NGAL and Cystatin C were determined on admission and on alternate days and urinary samples were collected for every day until ICU discharge.

RESULTS: One hundred fifty one patients were studied; 66 in sepsis-non AKI, 63 in sepsis-AKI, 22 in non-sepsis-non-AKI groups. Although plasma NGAL performed less well (AUC 0.44), urinary NGAL showed significant discrimination for AKI diagnosis (AUC 0.80) with a threshold value of 29.5 ng/ml (88% sensitivity, 73% specificity). Both plasma and urine Cystatin C worked well for the diagnosis of AKI (AUC 0.82 and 0.86, thresholds 1.5 and 0.106 mg/L respectively).

CONCLUSION: Plasma and urinary Cystatin C and urinary NGAL are useful markers in predicting AKI in septic critically ill patients. Plasma NGAL raises in patients with sepsis in the absence of AKI and should be used with caution as a marker of AKI in septic ICU patients.

Keywords: NGAL protein, human, Cystatin C, Acute kidney injury, biomarkers, sepsis, Intensive Care Units, predictive value of tests

1. Introduction

Acute kidney injury (AKI) is a common clinical problem in critically ill patients [1–3]. It has quite high prevalence among the intensive care unit (ICU) patients varying between 30–50% with an attributed mortality rate varying between 28–90% [1–4]. Approximately 50% of all AKI is considered to be associated with sepsis and 15–20% of all patients admitted to ICUs are septic AKI patients [2].

According to RIFLE or AKIN criteria, the current diagnosis of AKI is mainly based on diagnostic increases in serum creatinine indicating the loss of excretory renal function [5]. In septic AKI, serum creatinine doesn’t accurately reflect the glomerular filtration rate (GFR), because the patient is not in steady state condition. Furthermore serum creatinine is also

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influenced by tubular creatinine secretion and non-renal factors such as muscle mass, liver function and non-renal gastrointestinal elimination [6–8]. Besides, Doi et al., found in their animal study that sepsis dramatically reduces the production of creatinine without changes in body weight, hematocrit and extracellular fluid volume [9].

Many clinical studies have demonstrated the ability of NGAL and Cystatin C to allow early identification of AKI i.e., 48 hours earlier than plasma creatinine level. They are investigated in different clinical settings including cardiac surgery [10], contrast agent use [11], critically ill patients [12–21] and in emergency department [22].

Among the studies performed in ICUs with critically ill patients there are still conflicting results about the use of NGAL and Cystatin C for the early diagnosis of AKI. Because SIRS, severe sepsis and septic shock are known to increase plasma NGAL and Cystatin C levels in the absence of AKI [17]. But, whether sepsis affects the specificity of urine NGAL and Cystatin C as an early AKI marker is still unclear. So in this study we aimed to assess and compare the utility of plasma and urine concentrations of NGAL and Cystatin C for early diagnosis of septic AKI in adult critically ill patients.

2. Methods

A prospective cohort study, performed in a seven beds pulmonary ICU of a university hospital between January 2008 and March 2010. The ethic committee of our institution approved the study and the procedures performed in this study were in accordance with the Helsinki Declaration. An informed consent was obtained either from patients (if competent) or from their family.

2.1. Patient selection

2.1.1. Inclusion criteria

The patients who were > 18 years old without known previous renal disease, who were admitted to our ICU and who did not have any one of the exclusion criteria were included in the study consecutively.

The patients who were included in the study were grouped into three as; sepsis and non-AKI group; sepsis and AKI group and non-sepsis, non-AKI group.

2.1.2. Exclusion criteria

It was shown previously in many clinical studies and has been accepted that NGAL and Cystatin C levels increase in different clinical settings leading to AKI development [10–22]. Since the aim of this study is to define the roles of Cystatin C and NGAL in predicting septic AKI development, the patients who had other risk factors, rather than sepsis, that would lead to AKI and increase the Cystatin C and NGAL levels (i.e., non-septic-AKI patients) were excluded from the study. Patients who were excluded were the ones with the diagnosis of:

1) Chronic kidney disease,
2) Urinary tract infection at admission,
3) AKI at the time of admission
4) Prerenal and postrenal causes of AKI
5) Being exposed to radiocontrast dye or nephrotoxic drugs (aminoglycoside, colistin, amphotericin) within at least one week before the admission to ICU.

Although initially included in the study, the ones who had urine and blood samples that could not be studied in laboratory for Cystatin C and NGAL due to technical problems were also excluded at the end of the study.

2.2. Definitions

Acute kidney injury (AKI), risk and failure were defined according to RIFLE criteria [5,23]. The daily glomerular filtration rate was evaluated and also hourly urine samples were collected and the RIFLE class was defined based on the worst of either glomerular filtration criteria or urine output criteria. Glomerular filtration criteria were calculated as an increase of serum creatinine above the baseline serum creatinine level. Among the patients who had previous medical records, the lowest serum creatinine level within the previous one or two years was accepted as baseline. Among the ones who did not have any previous medical records and who did not have chronic kidney disease requiring dialysis, the admission serum creatinine level (if it is within normal range) was accepted as baseline and then followed for the development of AKI. Acute kidney injury should be both abrupt (within 1–7 days) and sustained (more than 24 hours). In order to evaluate whether Cystatin C and NGAL can be used as an early predictor of AKI, all patients with ‘injury’ and ‘failure’ were included in the statistical analysis and evaluated in the group ‘sepsis and AKI’.

Chronic kidney disease was defined according to the definition of National Kidney Foundation as kidney damage or glomerular filtration rate (GFR) < 60 ml/min/1.73 m² for three or more months, irrespec-
tive of the cause [24]. For the evaluation of chronic kidney disease the previous medical records of the patients were used. The ones who were included to a routine dialysis program (2–3 times/week) before admission were also accepted as having chronic kidney disease and excluded from the study.

Sepsis and septic shock were defined according to consensus guidelines [25].

The possible underlying causes of sepsis such as community acquired pneumonia (CAP), ventilator associated pneumonia (VAP), hospital associated pneumonia (HAP), catheter related infections, blood stream related infections, bacteremia, urinary infections, abdominal and wound infections were defined according to consensus guidelines [26].

Patients were considered to have steroid treatment if they have received 40–80 mg/day of systemic methylprednisolone or equivalent therapy for at least five days.

2.3. Data collected

All data were prospectively collected on standardized study forms. Data variables collected included the demographic characteristics (age, gender, and body weight), admission diagnosis, and comorbidities. Admission baseline creatinine and BUN levels, leukocyte count, erythrocyte sedimentation rate, CRP, vital signs (heart rate, temperature, mean arterial blood pressure) were also noted for the evaluation of sepsis and renal failure. Numerous clinical and physiological details were also collected, including those that compose the APACHE II, severity of organ failure assessment (SOFA), pneumonia severity index (PSI) and Charlson comorbidity scores [27–30]. During ICU stay, daily creatinine, BUN, total daily fluid intake, total daily urinary output, medications (vasoactive drugs, steroids, antibiotics, nephrotoxic agents) were also recorded. The day of sepsis, etiology of sepsis, microorganisms causing sepsis, the day of risk, injury and failure development were recorded. Intensive care unit outcome parameters such as duration of mechanical ventilation, length of ICU stay, length of hospitalization and mortality were also evaluated for 3 patient groups.

2.4. Sample collection and analysis

The blood samples were obtained within first 24 hours of ICU admission and then in every other day from all patients and urine samples were collected daily either from spontaneous voids or from indwelling Foley catheters. Blood samples were not obtained daily in order not to cause anemia in patients who had longer ICU stay. In patients who did not develop AKI, samples were collected until ICU discharge. In patients who develop AKI sample collection was stopped at the day of AKI. After being centrifuged at 5000 rpm for 15 minutes, the urine and blood supernatant samples were aliquoted into 1.8 ml eppendorf tubes and frozen within 2 hours of collection were at −80°C. Plasma and urine NGAL levels were studied from these collected samples with enzyme linked immunosorbent assay method (Human Lipocalin-2/NGAL ELISA Biovendor™). Plasma and urine Cystatin C levels were analyzed with particle enhanced immunonephelometric assay with BNII nephelometer (Dade Behring GmbH, Marburg, Germany).

For the patients who developed AKI the results were numerated as giving ‘0’ to the day of AKI and then the samples were assessed as −2, −4, −6, −8, −10, −12 etc. days before the development of AKI. Then the value that is at least 20% higher than the baseline was taken into consideration for the statistical analysis and its’ day was noted. For the patients without AKI just the course of NGAL and Cystatin C were followed during their whole stay in the ICU department and a mean value was calculated for the statistical analysis.

2.5. Statistical analysis

SPSS for Windows 15.0 software was used for the statistical analysis of the results (SPSS for Windows; Chicago, IL, USA). Results are presented as mean ± SD and percentiles or median (range) values. The independent samples t-test, the Chi square and Mann-Whitney U tests were used for comparison of the categorical and continuous variables. NGAL and Cystatin C values and other parameters of non-sepsis non-AKI, sepsis and non-AKI, sepsis and AKI groups were evaluated and compared with Kruskal Wallis one-way analysis of variance (ANOVA) analysis. \( P < 0.05 \) was considered statistically significant. In order to identify the cutoff values of urine and plasma NGAL and Cystatin C tests for the prediction of septic AKI, receiver-operating characteristic (ROC) curves were used and specificities and sensitivities of the tests were assessed using the area under ROC curves (AUC).

3. Results

3.1. Study population characteristics

Between January, 2008 and March, 2010 a total of 384 patients were evaluated but 151 of them were in-
Fig. 1. Flow-chart showing numbers of screened/excluded/analyzed patients and study groups.

Excluded in the study according to inclusion and exclusion criteria (Fig. 1). Among them, 66 (44%) patients included in ‘sepsis’ group; 63 (42%) patients included in ‘sepsis and AKI’ group and 22 (15%) patients included in ‘non sepsis non AKI’ group. Totally, 978 samples (326 blood and 652 urine) were used for NGAL and 780 samples (260 blood and 520 urine) were used for Cystatin C analysis.

During their stay in intensive care unit, ‘risk’ for renal failure was developed in 63 (42%) patients at 5 ± 7 days of their ICU admissions; ‘injury’ (AKI) developed in 54 (36%) patients at 9 ± 8 days of their ICU admissions and ‘failure’ developed in 15 (10%) patients at 12 ± 11 days of their ICU admissions. Dialysis was performed in only 4 (3%) patients. A total of 63 (42%) patients developing both ‘injury’ and ‘failure’ were grouped as ‘sepsis and AKI’ group.

The demographic properties of the patient groups, their admission diagnosis, comorbidities and ICU outcomes were compared and summarized in Table 1. A remarkable result that was presented in Table 1 is that; the length of MV, the length of hospital and ICU stay and also the rate of mortality were significantly higher in ‘sepsis and AKI’ patient group when compared with the ‘sepsis non AKI’ patient group (p < 0.005). When the factors affecting mortality in the study group was evaluated the significant factors affecting the mortality was found as malignancy, sepsis, septic shock, any degree of renal function impairment (risk, AKI and failure), length of MV, ICU stay and hospitalization, higher CRP levels, higher urine NGAL and Cystatin C levels and serum Cystatin C in the univariate analysis (p < 0.005). In the multivariate logistic regression analysis the presence of septic shock (P = 0.010, OR:13, %95 CI: 1.8–86.9) and higher serum Cystatin C levels (P = 0.005, OR = 16, %95 CI: 2.3–108.9) were identified as independent predictors of mortality. Sepsis was existed in 129 (85%) patients while 55 (36) of them developed septic shock. Etiology of sepsis, sepsis related scores (SOFA, PSI), microbiological analysis were given in Table 2.
3.2. NGAL and Cystatin C analysis results

The urine and plasma NGAL and Cystatin C levels were compared between the 3 groups and no statistically significant difference was found for plasma NGAL levels. On the other hand urine NGAL levels, serum and urine Cystatin C levels were found significantly elevated in septic AKI patients when compared with non sepsis-non AKI and sepsis-non AKI patient groups ($p = 0.001$) (Table 3). The changes in plasma and urine NGAL and Cystatin C levels within 8 days before the development of AKI in septic AKI patients were shown in Figs 2 and 3. Similarly the changes in plasma and urine NGAL and Cystatin C values within 8 days after ICU admission in non AKI patients were shown in Figs 4 and 5.

ROC curves of plasma and urine NGAL and Cystatin C for the prediction of AKI were shown in Fig. 6. AUC for the diagnosis of AKI was 0.44 for plasma NGAL, 0.80 for urine NGAL, 0.82 for plasma Cystatin C and 0.86 for urine Cystatin C. Threshold level, sensitivity and specificity of test determined for urine NGAL, plasma and urine Cystatin C, but could not for plasma NGAL levels (Table 4).

Plasma Cystatin C increased $> 100\%$ in 11 patients, 50–100% in 3 patients, $< 50\%$ in 5 patients during their follow up. Urine NGAL levels increased $> 100\%$ in 2 patients, $< 50\%$ in 10 patients. Urine Cystatin C levels increased $> 100\%$ in 5 patients, $< 50\%$ in 2 patients.

4. Discussion

This study revealed the importance of using urine NGAL and plasma and urine Cystatin C as follow up markers of septic AKI in ICUs; on the other hand plasma NGAL was found unsuccessful. Besides, threshold levels were determined for both markers for the diagnosis of septic AKI.

Acute kidney injury is an important cause of mortality and morbidity in ICUs. Our study remarkably once
Table 2
Characteristics and etiologies of sepsis, infection and sepsis related scores of septic patients (sepsis and non AKI group, sepsis and AKI group)

| Parameter                                      | N = 129* mean ± SD |
|------------------------------------------------|---------------------|
| Pneumonia Severity Index (PSI) score           | 141 ± 39            |
| Clinical Pulmonary Infection Score (CPIS)      | 6 ± 2               |
| Sequential Organ Failure Assessment (SOFA) score| 6 ± 3               |
| C-Reactive Protein                             | 60 ± 92             |
| Leukocyte count                                | 13788 ± 7134        |
| Mean arterial pressure (mmHg)                  | 72 ± 14             |
| Source of sepsis** *, n (%)                    |                     |
| Any pulmonary infection**                      | 89 (69)             |
| Catheter infection                             | 27 (21)             |
| Urinary infection                              | 27 (14)             |
| Wound infection                                | 5 (4)               |
| Microbiologic etiology, n (%)                  |                     |
| Acinetobacter Baumanii                         | 38 (29)             |
| MRSA                                           | 10 (8)              |
| Pseudomonas aeruginosa                         | 8 (6)               |
| Echerishia Coli                                | 8 (6)               |
| Candida species                                | 6 (5)               |
| Klebsiella                                     | 3 (2)               |
| Enterobacter                                   | 2 (2)               |
| Stenotrophomonas maltophilia                   | 1 (1)               |

* N: 129; since non-sepsis non-AKI patient group was excluded. Statistics were performed among 129 septic patients; ** More than one source of sepsis was identified in some patients; *** Ventilator associated pneumonia, hospital acquired pneumonia, immunosuppressive pneumonia, health care associated pneumonia, community acquired pneumonia and infective bronchiectasis.

again pointed out this fact as the rate of mortality and the length of mechanical ventilation, ICU stay and hospitalization were significantly higher in ‘sepsis-AKI’ patient group when compared with ‘sepsis non AKI’ patient group. This result underlines the importance of earlier detection of AKI especially in emergency departments and ICUs. With the earlier detection of AKI, earlier interventions can be made such as fluid resuscitation, early antibiotic initiation and restricting intravenous contrast dye and nephrotoxic antibiotic use. After cardiac surgery and radiocontrast exposure the development of AKI is expected; so follow up of NGAL levels may be useful in early diagnosis. But in critically ill patients it is hard to estimate the etiology and timing of AKI development. In order to diagnose AKI earlier than creatinine, follow up of plasma or urine NGAL levels might be used in ICUs. Our results pointed out that follow up of plasma NGAL was nonspecific for the diagnosis of AKI with an AUC of 0.44 and it can also increase during sepsis in the absence of AKI. NGAL is an acute phase reactant and may be released from neutrophils, macrophages and other immune cells. Furthermore, any decrease in GFR resulting from AKI would be expected to decrease the renal clearance of NGAL, with subsequent accumulation in the systemic circulation. Although plasma NGAL is freely filtered by the glomerulus, it is largely reabsorbed in the proximal tubules by efficient megalin-dependent endocytosis [31]. Thus, any urinary excretion of NGAL is likely only when there is concomitant proximal renal tubular injury. So in sepsis, although plasma NGAL levels increase due to inflammation, urine NGAL increase only in the presence of AKI [31]. As AKI often is associated with sepsis [32], this might obstruct the predictive properties of plasma NGAL as a biomarker of AKI, at least in the general ICU setting.

We identified that urine NGAL levels were increased in septic patients but the increase in AKI patients was...
Table 3

Comparison of the mean plasma and urine NGAL and Cystatin C levels in three patient groups (non-sepsis non-AKI, sepsis and non-AKI, sepsis and AKI)

| Parameter       | Non-sepsis non-AKI | Sepsis and non-AKI | Sepsis and AKI | P     |
|-----------------|--------------------|--------------------|----------------|-------|
|                 | N: 22 mean ± SD    | N:66 mean ± SD     | N:63 mean ± SD |       |
| Plasma NGAL, ng/ml | 60.3 ± 37.9         | 63.1 ± 39.2        | 72.5 ± 33.1     | 0.244 |
| Urine NGAL, ng/ml     | 17.5 ± 24.4a,c     | 25.9 ± 20.9b,c     | 56.4 ± 28.1b     | 0.001 |
| Plasma Cystatin C, mg/L | 1.1 ± 0.31b,c     | 1.4 ± 0.38b,c     | 2.37 ± 1.21a,b     | 0.001 |
| Urine Cystatin C, mg/L   | 0.04 ± 0.07a,c     | 0.11 ± 0.11b,c     | 0.92 ± 0.98b     | 0.001 |

*a,b,c: p < 0.05 between nonseptic nonAKI group and septicAKI group; b,c: p < 0.05 between sepsis group and septicAKI group; c: p < 0.05 between nonseptic nonAKI group and sepsis group.

Table 4

Threshold levels, sensitivity and specificity of plasma and urinary NGAL and Cystatin C levels for predicting AKI development

| Parameter       | AUC | p  | Threshold value | Sensitivity | Specificity |
|-----------------|-----|----|----------------|-------------|-------------|
| Plasma NGAL (ng/ml) | 0.44 | 0.44 | 29.5         | 0.88        | 0.73        |
| Urine NGAL (ng/ml)     | 0.80 | 0.001 | 1.5          | 0.73        | 0.68        |
| Plasma Cystatin C (mg/l) | 0.82 | 0.001 | 0.106        | 0.85        | 0.80        |
| Urine Cystatin C (mg/l)   | 0.86 | 0.001 |              |             |             |

Fig. 4. The change of plasma and urine NGAL levels (mean values, ng/ml) within 8 days after admission to ICU in septic non-AKI patients. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/DMA-130966)

Fig. 5. The change of plasma and urine Cystatin C levels (mean values, mg/L) within 8 days after admission to ICU in septic non-AKI patients. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/DMA-130966)

much higher. There was a significant difference in urine NGAL levels of septic and septic-AKI patients and a threshold level can be useful in discriminating septic non-AKI and septic-AKI patients in ICUs. We determined a threshold value of 29.5 ng/ml for urine NGAL in order to diagnose AKI in septic critically ill patients. Urine NGAL measurement is an easy and a noninvasive method that necessitates only spontaneously produced urine sample. With the evolving technology it is now possible to measure urine NGAL at bed side with assay kits [6]. Since no blood is drawn daily, it will not contribute the anemia in ICUs.

Our results about the diagnostic role of NGAL in septic AKI patients were compatible with the results of Martensson et al. [17]. They stated in their study that in septic ICU patients, although plasma NGAL levels were not successful in predicting AKI development, urine NGAL levels were much more successful with an AUC of 0.86. They concluded that urine NGAL is probably a more robust marker of AKI than plasma NGAL in patients with septic shock since urine NGAL levels remain within normal limits even when plasma levels are high and signs of AKI are absent. Previous studies on pediatric ICU patients have also shown that serum NGAL is a nonspecific predictor [19] and urine NGAL is a good predictor of AKI [13]. Bagshaw et al., also studied plasma and urine NGAL levels in early diagnosis of AKI in septic patients [15]. They compared septic and nonseptic AKI patients and found higher plasma and urine NGAL levels in septic patients when compared with the nonseptic ones. Since their both groups had AKI, either septic or nonsep-
Fig. 6. The ROC curve showing the performance of plasma and urine NGAL and Cystatin C levels in predicting septic AKI. AUC for plasma and urine NGAL and Cystatin C levels were also presented under the graphic. Threshold values were identified as 29.5 ng/ml for urine NGAL, 1.5 mg/l for plasma Cystatin C and 0.106 mg/l for urine Cystatin C. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/DMA-130966)

tic, they showed no superiority of urine NGAL over plasma NGAL [15].

We identified a threshold value of 1.5 mg/L for plasma Cystatin C for the diagnosis of septic AKI with a sensitivity of 73% and specificity of 68%. For urine Cystatin C, 0.11 mg/L was the threshold level with a sensitivity of 85% and specificity of 80%. Nejad et al. in their heterogeneous adult ICU study population evaluated the use of plasma Cystatin C in early diagnosis of AKI and they identified that plasma Cystatin C levels identified AKI 5.8 hours earlier than creatinine [33]. This was median 2 days before in our study. Same researchers also studied urine Cystatin C in their studies and AUC for the diagnosis of sepsis was identified as 0.80, for the diagnosis of AKI was 0.70 [33]. In this study a threshold level of 0.12 mg/L was identified for urine Cystatin C levels and this was very similar with our findings. In contrast to our study Nejad et al. identified no difference in urine Cystatin C levels of patients with sepsis and septic AKI. They explained their findings with the possibility of masking of urine Cystatin C increase in AKI with the increased levels in sepsis and as it was shown in an experimental study of Doi et al. sepsis can decrease creatinin levels; if AKI definition was made according to an increase in creatinine level the sensitivity of urine Cystatin C decreases [9]. The authors suggested that a new threshold for urine Cystatin C can be defined for the prediction of AKI in these septic patients. But their results can also be due to not exactly dividing the groups.

The use of Cystatin C as a marker of AKI must be done with caution in critically ill patients. Because in these patients there are many factors that can affect the plasma and urine levels of Cystatin C such as abnormal thyroid function, use of immunosuppressive therapy (corticosteroids), sepsis, cardiac failure, renal injury, chronic renal failure and nephrotoxic drug use [34]. We excluded the patients with chronic kidney disease and receiving nephrotoxic drugs. As a limitation we did not exclude patients with cardiac problems but by having serial measurements of plasma and urine levels of Cystatin C and NGAL we partially eliminate this disadvantage. We also did not exclude patients receiving steroids since they were too much in number. But we evaluated the effects of steroid use on Cystatin C levels. Steroid use was significantly higher both in septic and septic AKI groups when compared with the non sepsis-non AKI group. But since there were no difference between the rates of steroid use in septic-non AKI and septic-AKI groups, the significant increase in plasma and urinary Cystatin C levels in septic AKI patients could not be explained only by the steroid use. As another limitation we did not evaluate the thyroid function tests which are also reported to be affecting Cystatin C levels.

In addition to limitations mentioned above there are also a few more important limitations of this study.
First of all, it is a single center study with low patient number. Secondly, urine NGAL and Cystatin C was not expressed as ng/mg creatinine in this study. This expression may be useful to standardize and correct for changes in urine concentration. On the other hand it is not practical and cost effective for our study and also for daily routine of ICUs to collect urine samples for 24 hours and to study daily creatinine levels.

5. Conclusion

As a conclusion; plasma and urine Cystatin C and urine NGAL can be used for the early diagnosis of AKI; on the other hand plasma NGAL should be used with caution since it can increase in septic patients in the absence of AKI in critically ill patients. This finding is quite much important for ICU patients and if this study will be supported with further studies, simple urine tests can be used noninvasively for the monitoring of AKI. In the future with new evolutions it might be easier to perform urine tests at bedside both for NGAL and Cystatin C.

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