# Early Diagnostic Markers for Detection of Acute Kidney Injury in Allogeneic Hematopoietic Stem Cell Transplant Recipients

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## Abstract

***Objectives:*** **Acute kidney injury is a relatively frequent complication of allogenic hematopoietic stem cell transplant, resulting in increased risk of morbidity and mortality. Early diagnosis and management of acute kidney injury is of great importance for prevention of poor outcomes in these transplant recipients.**

***Materials and Methods:*** **Fifty consecutive patients, hospitalized for allogenic hematopoietic stem cell transplant at the Bone Marrow Transplantation Unit of Gazi University Faculty of Medicine, were included in this prospective study. Serial measurements of serum creatinine and creatinine clearance were obtained before administration of conditioning regimen and at 0, 7, 14, 21, and 28 days after start of conditioning. Blood and urine samples were also obtained for the measurement of serum cystatin C and urine neutrophil gelatinase-associated lipocalin levels before conditioning and 24 hours before each serum creatinine measurement.**

***Results:*** **During the median 25 days of follow-up, acute kidney injury developed in 19 patients: 10 patients had stage 1, 7 had stage 2, and 2 had stage 3 acute kidney injury according to the Acute Kidney Injury Network classification. There were significant positive correlations between serum cystatin C levels and serum creatinine levels and negative correlations with creatinine clearance levels at each time point ($P < .001$), whereas no statistically significant associations were observed with urinary neutrophil gelatinase-associated lipocalin levels. Both univariate and multivariate Cox regression models showed a statistically significant association between serum cystatin C levels and development of acute kidney injury, whereas urine neutrophil gelatinase-associated lipocalin levels did not show any significant associations.**

***Conclusions:*** **Serum cystatin C levels might be a useful marker for early detection of acute kidney injury in adult allogenic hematopoietic stem cell transplant recipients. Close monitoring of kidney function by sensitive biomarkers might provide early recognition and timely management of acute kidney injury in high-risk patient populations.**

**Key words:** *Hematopoietic stem cell transplantation, Serum cystatin C, Urine neutrophil gelatinase-associated lipocalin*

## Introduction

Hematopoietic stem cell transplantation (HSCT) has gained worldwide acceptance as a curative treatment modality for both malignant and benign hematologic and immunologic diseases.[1] However, complications limit the success of HSCT, particularly in allogeneic HSCT (AHSCT), which is related to greater incidences of transplant-related morbidity and mortality.[2] Acute kidney injury (AKI) is one of the most common complications associated with AHSCT, with an incidence of 21% to 73%,[3-6] particularly within the first 100 days after transplant.[3] Recent reports have demonstrated that AKI after AHSCT is an important risk factor for the development of chronic kidney disease, with a strong impact on progression-free survival.[7-9] Thus, close monitoring and early recognition of kidney damage is crucial for the prevention of poor outcomes in this patient population.

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In current clinical practice, AKI is typically diagnosed by measuring serum creatinine levels. However, serum creatinine responses to kidney damage are usually only shown within 48 hour after injury.[10] Serum creatinine is an untrustworthy marker during AKI, especially during the early stage after transplant, which has been termed as the "creatinine blinded period." The lack of a consensus on how to quantitatively diagnose AKI has restrained the ability to compare research reports in this field. Hence, the Acute Dialysis Quality Initiative group has created RIFLE criteria (Risk, Injury, Failure, Loss, End-Stage Renal Disease), and subsequently the Acute Kidney Injury Network (AKIN) has proposed 3 systems modified from RIFLE for standardization of AKI diagnosis.[11,12] Although these staging systems provide homogenization of studies in the AKI field, new biomarkers are still needed for early prediction of AKI, as all of these staging systems are based on serum creatinine levels.

Several new biomarkers have been introduced for evaluating renal dysfunction. Cystatin C, a recent marker, is a low-molecular-weight protein, completely filtrated from the glomerular filtration barrier and almost completely reabsorbed from the proximal tubule.[13] This protein is not only a biomarker for glomerular filtration, but it also offers a good prediction for early renal injury in different settings. Although serum cystatin C levels have been previously determined as a useful tool for AHSCT patients who have increased risk of AKI,[14] limited data exist on its use in adult AHSCT recipients. Recent research studies have also shown that neutrophil gelatinase-associated lipocalin (NGAL), a 25-kDa molecular-weight protein, highly accumulates in blood, the renal cortical tubule, and urine, after ischemic and toxic injury of the kidney. Thus, it seems to be a sensitive, noninvasive biomarker for early detection of AKI.[15,16]

In the present study, we evaluated the incidence of AKI in our AHSCT patient group and investigated whether serum cystatin C and urine NGAL levels might be useful biomarkers for early detection of AKI after AHSCT in adult recipients.

## Materials and Methods

### Study population

Fifty consecutive patients undergoing AHSCT at the Bone Marrow Transplantation Unit of Gazi University Faculty of Medicine were prospectively enrolled in this study between December 2009 and June 2011. Patients younger than 18 years old, patients with preexisting chronic renal insufficiency, and patients with diabetes mellitus were excluded from the study. Information gathered from medical records included age, sex, underlying disorder, date of diagnosis, conditioning regimen, and disease status at transplant. This study was approved by the Institutional Review Board of Gazi University Faculty of Medicine, and written informed consent was obtained from each patient before enrollment.

### Conditioning regimen

Myeloablative conditioning regimens consisted of cyclophosphamide + busulfan or total body irradiation + cyclophosphamide or total body irradiation + cyclophosphamide + thiotepa + melphalan. Nonmyeloablative regimens consisted of antithymocyte globulin + cyclophosphamide + fludarabine or fludarabine + melphalan or fludarabine + antithymocyte globulin + busulfan. All patients received cyclosporine and methotrexate for graft-versus-host disease (GVHD) prophylaxis. Infection prophylaxis consisted of fluconazole, levofloxacin, trimethoprim/sulfamethoxazole, and acyclovir.

### Laboratory assessments

Hemoglobin levels, white blood cell count, platelet count, serum urea nitrogen, serum creatinine levels, and serum electrolyte levels, along with glomerular filtration rate, were monitored on day -7, on the day of infusion (day 0), and on days +7, +14, +21, and +28. Serum creatinine levels and glomerular filtration rate measurements were also obtained on days +6, +13, +20, and +27 post-AHSCT. Glomerular filtration rate was calculated by the Modified Diet in Renal Disease equation.[17]

### Evaluation of acute kidney injury

Study patients were followed for 30 days for acute kidney injury. Renal dysfunction was evaluated according to the AKIN staging criteria[12] (Table 1).

**Table 1.** Criteria for Acute Kidney Injury

| Acute Kidney Injury Network Classification | Glomerular Filtration Rate Criteria |
|---|---|
| Stage 1 | Increased serum creatinine ≥ 0.3 mg/dL or serum creatinine baseline ≥ 1.5-2 |
| Stage 2 | Increased serum creatinine ≥ 2-2.9 from baseline |
| Stage 3 | Increased serum creatinine ≥ 3 from baseline or 0.5 mg/dL increase in patient with serum creatinine > 4 mg/dL |

Acute kidney injury was evaluated using only glomerular filtration rate criteria, as urine output could not be determined for all patients.

**Determination of blood cystatin C and urine neutrophil gelatinase-associated lipocalin levels**

Urine and blood samples were collected at day -7, infusion day (day 0), and on days +6, +13, +20, and +27. Samples were centrifuged at 3000g for 5 minutes, and the supernatants were stored at -80°C. Serum cystatin C (Biovendor Laboratorni Medicina, Brno, Czech Republic) and urine NGAL (Biovendor Laboratorni Medicina) levels were analyzed by commercially available enzyme-linked immunosorbent assay kits with sandwich monoclonal enzyme-linked immunosorbent assay procedures. All tests were performed according to the manufacturer's instructions. Sensitivities specified by the manufacturer are 0.02 ng/mL for NGAL levels and 0.25 ng/mL for serum cystatin C levels.

**Statistical analyses**

All values are expressed as means ± standard deviation or median with interquartile range (IQR) depending on their distribution. Nonparametric Friedman test was performed to compare the levels of serum creatinine, creatinine clearance, serum cystatin C, and urine NGAL levels for each measurement. The correlation coefficients between creatinine, creatinine clearance, cystatin C, and urine NGAL were evaluated using Spearman correlation analysis. The predictors of AKI were assessed in a time-dependent manner by regression analysis. For multivariable analyses, general risk factors such as age, sex, and transplant type along with each renal function marker were adjusted to avoid overfitting in the regression model. Log-rank analyses were performed for comparisons between groups. Statistical significance was assessed at the 95% confidence interval. Analyses were performed using SPSS version 21 for Windows (SPSS, Chicago, IL, USA).

## Results

**Patient characteristics**

Baseline characteristics of the 50 patients are shown in Table 2. Median age was 29 years (IQR: 18, 62 y), with overall male predominance. Four patients underwent unrelated donor transplant. Thirty patients received myeloablative conditioning, and 20 patients received nonmyeloablative conditioning regimens. Five patients developed sinusoidal obstruction syndrome, 1 at day 7 and 4 at day 14 after transplant. Four patients developed hepatic GVHD, 6 patients developed gastrointestinal GVHD, and 7 patients developed skin GVHD during follow-up. Baseline laboratory characteristics of study patients are presented in Table 3.

**Table 2.** Demographic Characteristics of Study Population (N = 50 Patients)

| Characteristic | Result |
|---|---|
| Median age, years (interquartile range) | 29 (18, 62) |
| No. of male/No. of female patients | 35/15 |
| Primary disorder, No. of patients (%) | |
| Acute myeloid leukemia | 13 (26%) |
| Non-Hodgkin lymphoma | 5 (10%) |
| Myelodysplastic syndrome | 5 (10%) |
| Aplastic anemia | 5 (10%) |
| Acute lymphocytic leukemia | 13 (26%) |
| Paroxysmal nocturnal hemoglobinuria | 2 (4%) |
| Multiple myeloma | 4 (1%) |
| Hodgkin lymphoma | 1 (2%) |
| Biphenotypic leukemia | 1 (2%) |
| Chronic myelocytic leukemia | 1 (2%) |
| Type of AHSCT, No. of patients (%) | |
| Myeloablative | 30 (60%) |
| Nonmyeloablative | 20 (40%) |
| Disease status at AHSCT, No. of patients (%) | |
| Complete remission | 27 (61.4%) |
| Minimal response | 2 (4.5%) |
| Active disease | 15 (34.1%) |
| HCT-CI, % | |
| 0 | 15.8% |
| 1, 2 | 63.2% |
| 3, 4, 5 | 21% |
| Conditioning regimen, myeloablative | |
| Cyclophosphamide + busulfan | 18 (36%) |
| TBI + cyclophosphamide | 11 (22%) |
| TBI + cyclophosphamide + thiotepa | 1 (2%) |
| Thiotepa + melphalan + fludarabine | 1 (2%) |
| Conditioning regimen, nonmyeloablative | |
| ATG + cyclophosphamide + fludarabine | 6 (12%) |
| Fludarabine + melphalan | 11 (22%) |
| Fludarabine + ATG + busulfan | 2 (4%) |

***Abbreviations:*** AHSCT, allogenic hematopoietic stem cell transplant; ATG, antithymocyte globulin; HCT-CI, hospital cell transplant comorbidity index; TBI, total body irradiation

**Table 3.** Baseline Laboratory Parameters of Study Population (N = 50 Patients)

| Parameter | Result |
|---|---|
| Serum urea nitrogen, mg/dL | 11.75 (4, 91) |
| Creatinine, mg/dL | 0.60 (0.44, 1.1) |
| 24-hour proteinuria, mg/day | 171 (39, 779) |
| 24-hour proteinuria, No. of patients (%) | |
| < 150 mg/day | 21 (42%) |
| 150-300 mg/day | 14 (28%) |
| > 300 mg/day | 15 (30%) |
| Creatinine clearance, mL/min/1.73 $m^2$ | 147 ± 31.3 |
| Uric acid, mg/dL | 3.65 ± 1.57 |
| Albumin, g/dL | 3.57 ± 0.48 |
| Cholesterol, mg/dL | |
| LDL cholesterol | 83 ± 32.6 |
| HDL cholesterol | 34.2 ± 11.7 |
| TG | 137 (45, 546) |

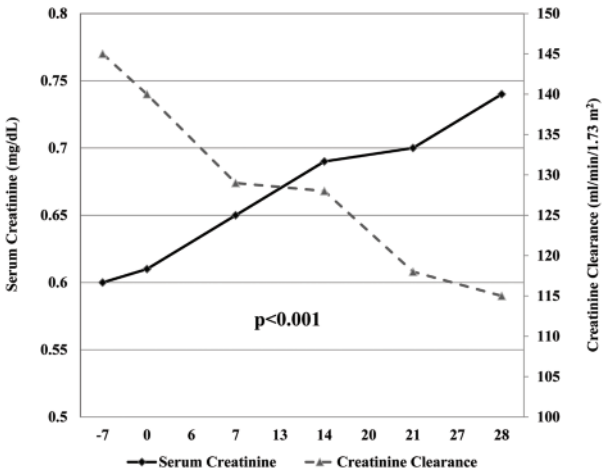
***Abbreviations:*** HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; TG, triglycerides

Results are displayed as median (IQR) or means ± standard deviation as indicated.

### Changes in kidney function

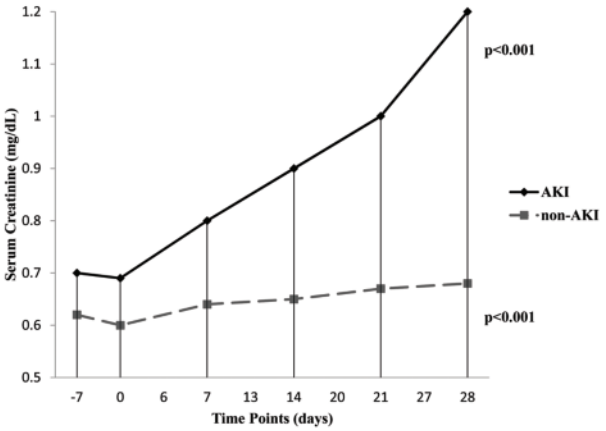
Median follow-up was 25 days (IQR: 7, 30). We observed a significant increase in creatinine levels between each time point after transplant compared with the previous level. In addition, there was a significant and steady decrease in creatinine clearance levels at each time point (Figure 1). Nineteen patients (38%) developed AKI. Among these, 10 patients were classified as stage 1, 7 patients as stage 2, and 2 patients as stage 3 AKI according to the AKIN classification. The incidence of AKI was 55% in those who received myeloablative and 26.7% in those who received nonmyeloablative conditioning regimens. Baseline creatinine, creatinine clearance, proteinuria, age, sex, and cholesterol levels were similar in patients with and without AKI. Both AKI and non-AKI subgroups showed significantly increased creatinine levels during follow-up ($P < .001$ for each subgroup) (Figure 2).

**Figure 1.** Time-Dependent Changes in Renal Function




Median serum creatinine concentration was 0.60 (IQR: 0.44, 1.11) mg/dL before transplant, 0.61 (IQR: 0.41, 0.96) on day of infusion (day 0), 0.66 (IQR: 0.39,1.65) on day 7, 0.67 (IQR: 0.44, 1.25) on day 14, 0.68 (IQR: 0.5, 1.8) on day 21, and 0.73 (IQR: 0.47, 1.44) on day 28. There was a steady and statistically significant increase ($P < .001$) during follow-up and statistically significant difference at each time point compared with the previous time measurements ($P = .018$, $P = .001$, $P = .005$, $P = .002$, and $P = .003$). Mean creatinine clearance level was 147 ± 31.3 mL/min/1.73 m$^2$ before transplant, 142 ± 37.7 mL/min/1.73 m$^2$ on day of infusion, 127 ± 43 mL/min/1.73 m$^2$ on day 7, 126 ± 41 mL/min/1.73 m$^2$ on day 14, 114 ± 35 mL/min/1.73 m$^2$ on day 21, and 110 ± 30.3 mL/min/1.73 m$^2$ on day 28. There was a steady and significant decrease in creatinine clearance at each time point ($P < .001$ for each comparison).

**Figure 2.** Creatinine Levels in Patients With and Without Acute Kidney Injury




Patients with and without acute kidney injury (AKI) showed a steady rise in their serum creatinine levels (Friedman analyses, $P < .001$ for both subgroups). Creatinine level was 0.7 (IQR: 0.44, 1.09) mg/dL before transplant, 0.67 (IQR: 0.45, 1.26) mg/dL on day of infusion, 0.81 (IQR: 0.39, 1.87) mg/dL on day 7, 0.93 (IQR: 0.52, 2.6) mg/dL on day 14, 1.01 (IQR: 0.5, 2.6) mg/dL on day 21, and 1.2 (IQR: 0.62, 1.44) mg/dL on day 28 in patients with AKI. Creatinine level was 0.61 (IQR: 0.5, 1.1) mg/dL before transplant, 0.6 (IQR: 0.41, 0.96) mg/dL on day of infusion, 0.64 (IQR: 0.44, 0.96) mg/dL on day 7, 0.65 (IQR: 0.44, 1.06) mg/dL on day 14, 0.67 (IQR: 0.5, 1) mg/dL on day 21, and 0.68 (IQR: 0.47, 0.92) mg/dL on day 24 in patients without AKI.

### Serum cystatin C and urine neutrophil gelatinase-associated lipocalin measurements

Serum cystatin C and urine NGAL levels were measured on day -7, on infusion day (day 0), and on days +6, +13, +20, and +27. Both the AKI and non-AKI subgroups showed significantly increased serum cystatin C levels, although with a more prominent rise in the AKI subgroup ($P < .001$ for the AKI and $P = .001$ for the non-AKI subgroup). Among the measured urine NGAL levels at each time point, the highest urine NGAL level was found on day +13 in both patients with and those without AKI (Table 4). Serum cystatin C levels were significantly increased earlier than the significant elevation in serum creatinine levels shown among all study

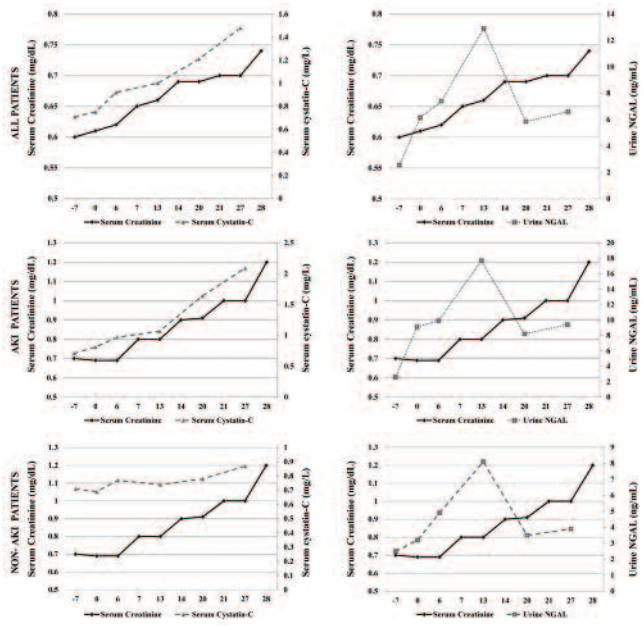
**Table 4.** Serum Cystatin C and Urine Neutrophil Gelatinase-Associated Lipocalin Levels at Each Time Point

| | Before Conditioning Regimen | Infusion Day (Day 0 | Day 6 | Day 13 | Day 20 | Day 27 | *P* Value |
|---|---|---|---|---|---|---|---|
| AKI group | | | | | | | |
| Cys-C, mg/L | 0.71 ± 0.2 | 0.81 ± 0.2 | 0.97 ± 0.5 | 1.07 ± 0.6 | 1.64 ± 1.3 | 2.09 ± 1.2 | < .001 |
| NGAL, ng/mL | 2.6 (2.1, 5.2) | 9.1 (2.1, 80) | 9.9 (2.2, 44) | 17.7 (2.2, 85) | 8.2 (2, 36) | 9.41 (2.3,29) | .003 |
| Non-AKI group | | | | | | | |
| Cys-C, mg/L | 0.71 ± 0.1 | 0.69 ± 0.1 | 0.77 ± 0.3 | 0.74 ± 0.1 | 0.78 ± 0.1 | 0.87 ± 0.3 | .001 |
| NGAL, ng/mL | 2.5 (2.1, 33) | 3.2 (2.1, 18) | 4.9 (2.1, 75) | 8.1 (2.2, 58) | 3.5 (2.3, 3.2) | 3.9 (2.2, 19) | .015 |

***Abbreviations:*** AKI, acute kidney injury; Cys-C, cystatin C, NGAL, neutrophil gelatinase-associated lipocalin
Results are displayed as median interquartile range or means ± standard deviation as indicated.

patients and also in subgroup analyses (Figure 3). Although the incremental change in urine NGAL levels was earlier than the significant rise in serum creatinine levels, this decrease was significant and sustained at the same levels in all study patients and in subgroup analyses (Figure 3).

**Figure 3.** Serum Creatinine, Serum Cystatin C, and Urine Neutrophil Gelatinase-Associated Lipocalin Changes During Follow-Up Among Study Groups




*Abbreviations:* AKI, acute kidney injury; NGAL, neutrophil gelatinase-associated lipocalin

Serum cystatin C levels showed statistically significant positive correlations with serum creatinine levels and significant negative correlations with creatinine clearance levels at each time point. Although the correlation directions were similar with urine NGAL levels, these associations did not reach statistical significance. The Spearman correlation results among the variables are summarized in Figure 4.

## Predictors of acute kidney injury after allogeneic hematopoietic stem cell transplant

Both univariate and multivariable Cox regression analyses indicated that sinusoidal obstruction syndrome was the only significant clinical predictor for the development of AKI among several clinical factors (univariate analysis: odds ratio of 4.325; 95% confidence interval, 1.395, 13.404; $P$ = .001; multivariable analysis adjusted by age, sex, and transplant type: odds ratio of 4.973; 95% confidence interval, 1.485, 16.648; $P$ = .009) (Table 5).

**Table 5.** Association Between Patient Characteristics and Acute Kidney Injury (Univariate Cox Model)

| | Odds Ratio (95% CI) | *P* Value |
|---|---|---|
| Age | 1.013 (0.976, 1.052) | .484 |
| Female sex | 0.610 (0.202, 1.840) | .380 |
| Transplantation type | | |
| Nonmyeloablative | 0.447 (0.180, 1.114) | .084 |
| Disease status at transplant | | |
| Minimal response | 1.733 (0.628, 4.785) | .289 |
| Active disease | 2.342 (0.292, 18.792) | .423 |
| Development of SOS | 4.325 (1.395, 13.404) | .001 |
| Antifungal therapy | 1.013 (0.407, 2.520) | .979 |
| Acute GVHD | 1.228 (0.446, 3.232) | .678 |
| Blood cyclosporine levels | 1.000 (0.992, 1.009) | .941 |

*Abbreviations:* CI, confidence interval; GVHD, graft-versus-host disease; SOS, sinusoidal obstruction syndrome

There were no statistically significant associations between the development of AKI and baseline serum creatinine levels and also as shown with baseline creatinine clearance regression analyses adjusted by age, sex, and transplant type (Table 6). Among all time point measurements, serum cystatin C levels over 0.9 mg/L at days 13, 20, and 27 post-AHSCT

**Figure 4.** Spearman Correlation Analyses Between Serum Creatinine and Serum Cystatin C and Urine Neutrophil Gelatinase Associated Lipocalin Levels

| Serum Creatinine (mg/dL) | Serum Cystatin- C (mg/L) | | | | | |
|---|---|---|---|---|---|---|
| | Pre-Trans | Day 0 | Day +6 | Day +13 | Day+20 | Day+27 |
| Pre-Trans | 0.496* | | | | | |
| Day 0 | | 0.616** | | | | |
| Day +7 | | | 0.665* | | | |
| Day +14 | | | | 0.777* | | |
| Day +21 | | | | | 0.786** | |
| Day +28 | | | | | | 0.773* |

| Creatinine Clearance (ml/min/1.73 m²) | Serum Cystatin- C (mg/L) | | | | | |
|---|---|---|---|---|---|---|
| | Pre-Trans | Day 0 | Day +6 | Day +13 | Day+20 | Day+27 |
| Pre-Trans | -0.747* | | | | | |
| Day 0 | | -0.770** | | | | |
| Day +7 | | | -0.715* | | | |
| Day +14 | | | | -0.860* | | |
| Day +21 | | | | | -0.915** | |
| Day +28 | | | | | | -0.898* |

| Serum Creatinine (mg/dL) | Urine NGAL (ng/mL) | | | | | |
|---|---|---|---|---|---|---|
| | Pre-Trans | Day 0 | Day +6 | Day +13 | Day+20 | Day+27 |
| Pre-Trans | 0.114 | | | | | |
| Day 0 | | 0.060 | | | | |
| Day +7 | | | 0.197 | | | |
| Day +14 | | | | 0.120 | | |
| Day +21 | | | | | 0.039 | |
| Day +28 | | | | | | 0.055 |

| Creatinine Clearance (ml/min/1.73 m²) | Urine NGAL (ng/mL) | | | | | |
|---|---|---|---|---|---|---|
| | Pre-Trans | Day 0 | Day +6 | Day +13 | Day+20 | Day+27 |
| Pre-Trans | -0.182 | | | | | |
| Day 0 | | -0.014 | | | | |
| Day +7 | | | -0.398 | | | |
| Day +14 | | | | -0.018 | | |
| Day +21 | | | | | -0.053 | |
| Day +28 | | | | | | -0.111 |

*Abbreviations:* NGAL, neutrophil gelatinase-associated lipocalin
*$P$ < .001; **$P$ < .01.

**Table 6.** Predictors of Acute Kidney Injury

| | Univariate | | Multivariate* | |
|---|---|---|---|---|
| | Odds Ratio (95% CI) | *P* Value | Odds Ratio (95% CI) | *P* Value |
| Baseline creatinine | 3.992 (0.259, 61.5) | .321 | 4.106 (0.086, 195.1) | .479 |
| Baseline creatinine clearance | 0.994 (0.980, 1.008) | .410 | 0.992 (0.975, 1.010) | .400 |
| Blood cystatin C | | | | |
| Day 13 > 0.9 | 2.666 (1.009, 7.004) | .048 | 2.620 (0.923, 7.433) | .07 |
| Day 20 > 0.9 | 4.752 (1.639, 13.778) | .004 | 6.413 (1.945, 21.131) | .002 |
| Day 27 > 0.9 | 6.489 (1.777, 23.702) | .005 | 6.508 (1.725, 24.551) | .006 |
| Urine NGAL level | | | | |
| Day 13 | 1.019 (0.975, 1.066) | .399 | 1.015 (0.995, 1.025) | .140 |
| Day 20 | 1.025 (0.950, 1.105) | .522 | 1.013 (0.961, 1.067) | .637 |
| Day 27 | 0.994 (0.882, 1.119) | .318 | 1.031 (0.969, 1.097) | .330 |

***Abbreviations:*** CI, confidence interval; NGAL, neutrophil gelatinase-associated lipocalin
*Multivariate analysis adjusted by age, sex, and transplant type.

were significant predictors of AKI in univariate regression analyses. These associations remained statistically significant even after adjustment by age, sex, and transplant type (Table 6). However, none of the NGAL measurements showed significant associations in regression models.

## Discussion

Our study shows that AKI defined by AKIN criteria is a frequent complication of AHSCT, and serum cystatin C measurements allow the prediction of AKI in adult AHSCT patients earlier than measurements of changes in serum creatinine levels. However, urine NGAL levels did not show any significant association with the development of AKI. These data indicate that close monitoring of kidney function by serum cystatin C measurements might be a useful tool for early recognition and timely management of AKI after AHSCT.

With the recognition of need for a uniform definition for AKI, RIFLE11 criteria and subsequently AKIN12 criteria have been proposed for the classification of AKI. The AKIN criteria were used for staging kidney injury in our present study, which showed that 38% of the patients developed AKI after AHSCT within the first 30 days. To the best of our knowledge, there are few studies in the literature that have evaluated the development of AKI by using AKIN criteria in adult AHSCT patients. A 5-year follow-up retrospective study reported that 53.6% of HSCT patients developed AKI by using AKIN criteria.[18] The same group also showed that AKIN criteria is the better diagnostic system for determining acute renal injury in this patient population.[19] The determined incidence of AKI in our study is slightly lower than shown in the previous studies, which might be explained by the different follow-up periods. Our study analyzed the first 30 days after AHSCT, whereas the previous studies used follow-ups of 100 days.[18,20] However, it should be noted that the presence of AKI is more common within the first 2 to 3 weeks after AHSCT due to toxicities with the high-dose regimens, which can trigger the development of AKI in the early posttransplant period.

There are conflicting results in the literature regarding the laboratory predictors for the development of renal dysfunction after HSCT. Two previous studies have claimed that low baseline creatinine levels were found to be associated with development of AKI.[3,4] Although this association was not completely explained, it has been concluded that loss of lean body mass due to the malignant process might be the cause of low pretransplant creatinine levels. In another study, baseline low creatinine clearance levels were found as a risk factor for AKI.[21] Here, we demonstrated that baseline higher serum creatinine levels were associated with 3.9-fold increased risk of AKI. An important point of our finding was the observed trend toward an increase in creatinine levels in the non-AKI subgroup with cystatin C levels showing a significant rise. One should conclude that there is a subclinical kidney injury in all AHSCT patients with a potential of progressing to advanced kidney failure with the addition of contributing factors in the long-term AHSCT settings. In addition, the use of serial serum creatinine readings alone would cause an underestimate or misrepresent the timing of AKI, thus stressing the need of other biomarkers such as cystatin C to identify even mild kidney damage. Also, regardless of the biomarker type used during follow-up, clinicians should be careful with fluid administration and drug management after transplant, even in patients who have no apparent clinical or laboratory signs of kidney injury.

Almost all complications after AHSCT could lead to development of AKI in the early posttransplant period. Previous risk management studies have shown that GVHD, sinusoidal obstruction syndrome, and cyclosporine toxicity are the major important risk factors for renal injury in these patients. Our study did not show any relation between serum cyclosporine levels and AKI. However, administration of cyclosporine to all patients and keeping blood levels at a similar range are potential explanations for this finding. Consistent with previous studies,[4,22] patients with acute GVHD in our study showed 1.2-fold increased risk for developing AKI, although this was not statistically significant. We also found that sinusoidal obstruction syndrome caused a 4.9-fold increased risk for development of AKI, similar with the previous literature.[23] It should also be noted that, besides the casual relation between sinusoidal obstruction syndrome and AKI, the degree of renal injury is the most important prognostic factor for survival in patients who developed sinusoidal obstruction syndrome. Thus, it is important to identify patients who are at risk of AKI, for both prevention of its development and also for early initiation of effective treatment to improve outcomes.

We performed serial measurements of blood cystatin C weekly for the first 30 days to evaluate the performance of this test as an early kidney injury marker in the AHSCT setting. Cystatin C has been suggested to be a precise marker, even as a marker in mild renal dysfunction.[13] It has been demonstrated that cystatin C is more sensitive than serum creatinine for detection of renal dysfunction in renal and liver transplant patients.[24] Although cystatin C has been studied in those with liver and kidney transplants and in oncologic patients, few studies exist on its use in AHSCT patients. Hazar and associates showed that serum cystatin C is as sensitive as diethylenetriaminepentaacetic acid-based glomerular filtration rate measurement in pediatric HSCT patients.[25] In our study, we found strong correlations between serum cystatin C levels and routine clinical practice markers (serum creatinine and creatinine clearance measurements), which were measured 24 hours after cystatin C measurements. Similarly, Muto and associates[14] demonstrated a strong negative correlation between serum cystatin C and creatinine clearance levels ($r = 0.682$; $P < .001$). Furthermore, an earlier increase in serum cystatin C levels versus serum creatinine concentrations during the creatinine blinded period suggests that cystatin C has a higher sensitivity in identifying early kidney dysfunction, which may be missed by relying on serum creatinine concentrations alone. Although the baseline cystatin C levels could not predict development of AKI in our analysis, the significant risk prediction of serum cystatin C levels over 0.9 mg/L with other time points led us to conclude that sequential serum cystatin C measurements after AHSCT may be necessary for determining AKI during the creatinine blinded period.

To the best of our knowledge, this is the first prospective study that evaluated kidney injury by cystatin C and urine NGAL measurements concurrently. We found that serial measurements of NGAL levels showed an increased trend within the first 2 weeks after AHSCT in patients with and without AKI. Although there were positive correlations between urine NGAL levels and serum creatinine and negative correlations with creatinine clearances, none of these reached statistically significance. We were also unable to find significant associations in our regression model, in contrast to that conducted by Taghizadeh-Ghehi et al.[26] The relatively small sample size may have masked the possible value of urine NGAL levels in predicting AKI in our study. In addition, more frequent measurements of urine NGAL levels could be more effective for documenting renal function decline, since urine NGAL levels might change more rapidly due to the possible effects of various factors, such as direct drug toxicity, portal hypertension due to sinusoidal obstruction syndrome, renal hypoperfusion, and renal hemodynamic changes due to infections and sepsis after AHSCT.

Our study has several limitations, primarily the relatively small sample size and being a single center study. These limitations preclude us from drawing solid conclusions. A secondary limitation is the absent use of histopathology, which was due to the potential hemorrhagic risk with renal biopsy. Third, we limited our prospective design to the first 30 days, which may have led to an underestimate of the incidence of AKI. However, it should be mentioned that AKI usually occurs within the first 2 to 4 weeks after transplant.[27] Finally, we measured the biomarkers in a weekly manner, as more frequent follow-up of these markers would be worth the extra cost. On the other hand, the prospective nature and

use of the AKIN classification system, a proven sensitive method for AKI diagnoses, are the strong parts of our present study. In addition, to our knowledge, this is the first study that used urine NGAL together with serum cystatin C measurements with traditional clinical markers in an adult AHSCT population.

In conclusion, our results indicate that serum cystatin C measurements could be a useful tool for the early detection of AKI. Further studies with larger sample sizes are required to determine the value of urine NGAL levels in predicting AKI in the AHSCT setting.

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