Impact of estimated glomerular filtration rate based on plasma cystatin C and serum creatinine levels before allogeneic hematopoietic cell transplantation

Hidenori Wada*, Junya Kanda*, Yu Akahoshi, Hirofumi Nakano, Tomotaka Uegai, Ryoko Yamasaki, Yuko Ishihara, Koji Kawamura, Kana Sakamoto, Masahiro Ashizawa, Miki Sato, Kiriko Terasako-Saito, Shur-ichi Kimura, Misato Kikuchi, Hideki Nakasone, Rie Yamazaki, Shinichi Kako, Aki Tanihara, Junji Nishida and Yoshinobu Kanda

Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama City, Japan

ABSTRACT

Background: No standard method for measuring renal function has been established in allogeneic hematopoietic cell transplantation (allo-HCT).

Methods: We retrospectively analyzed 80 patients with hematological diseases who underwent allo-HCT at our center. We assessed renal function using creatinine clearance (Ccr), estimated glomerular filtration rate (eGFR) based on creatinine (eGFRcre), eGFR based on cystatin C (eGFRcys), and the average of eGFRcre and eGFRcys (eGFRave). We then evaluated the impact of pre-transplant renal function on the exacerbation of renal function and non-relapse mortality after transplantation.

Results: There was a significant correlation between Ccr and eGFRcre, eGFRcys, and eGFRave. eGFRave best predicted the exacerbation of renal function according to the area under the receiver-operating characteristic curve. The cumulative incidence of renal function exacerbation at 1 year was higher in the lower eGFRave group (<90 ml/min/1.73 m²) than in the higher eGFRave group (≥90 ml/min/1.73 m²; 0.85 vs. 0.39, \( p < 0.001 \)), which was confirmed by a multivariate analysis (HR 2.75, \( p = 0.001 \)). A lower eGFRave value was a marginally significant factor for non-relapse mortality (HR 3.29, \( p = 0.076 \)).

Conclusion: Among the four parameters, eGFRave best predicted the exacerbation of renal function in allo-HCT. Further, the marginal association between low eGFRave and high non-relapse mortality warrants further study in a prospective study in allo-HCT.

KEYWORDS

Allogeneic transplantation; creatinine clearance; cystatin C; estimated glomerular filtration rate

Introduction

Renal function is a critical factor that affects clinical decision-making and outcomes before and after allogeneic hematopoietic cell transplantation (allo-HCT) [1–6]. It needs to be accurately evaluated before transplantation, since some chemotherapeutic agents, antibiotics, and calcineurin inhibitors for graft-versus-host disease (GVHD) prophylaxis need to be dose-adjusted depending on the glomerular filtration rate (GFR) [7–9]. The gold standard for the evaluation of renal function has been a measurement of the GFR using inulin or a nuclear isotope, such as \(^{51}\)Cr-EDTA or \(^{99m}\)Tc-DPTA [10,11]. However, the availability of these methods is limited because of their cost and inconvenience. An alternative to these approaches is the measurement of creatinine clearance (Ccr) based on serum and 24-hour urine creatinine levels, but this requires urine collection [12]. Instead, the estimated GFR based on serum creatinine alone (eGFRcre) is now widely used because it is a simple and easy-to-use approach; however, eGFRcre is significantly influenced by muscle volume [10,13,14]. GFR in pre-transplant patients tends to be overestimated because multiple intensive chemotherapies often require prolonged bed rest, resulting in a loss of muscle volume. An estimated GFR based on cystatin C (eGFRcys) has been introduced to overcome the disadvantages of measuring eGFRcre. Cystatin C is a 13-kDa protein produced in nucleated cells, and the level of serum cystatin C is not affected by muscle volume. After cystatin C is released from cells, it does not bind to other proteins and is filtered through glomeruli. After it is reabsorbed through the proximal renal tubule, it is metabolized into amino acids. Therefore, the concentration of cystatin C in blood is dependent on GFR. The estimated GFR based on serum cystatin C is comparable to or even superior to that based on serum creatinine, not only in the general population, but also in cancer patients receiving chemotherapy [15–18]. The estimation of GFR based on both cystatin C and creatinine, such as by the average of eGFRcre and eGFRcys (eGFRave), further improves the estimation of GFR [19,20]. However, the impact of eGFRcys has not been well-studied in the setting of allo-HCT [21–23]. Therefore, we retrospectively analyzed 80 patients...
who received allo-HCT to evaluate renal function using Ccr, eGFRcys, eGFRcre, and eGFRave and their associations with transplant outcomes.

Patients and methods

Patients

We included 80 consecutive patients with hematological diseases who underwent allo-HCT between June 2008 and November 2012 at our center, and for whom blood samples before transplantation were available. This retrospective study was approved by the Institutional Review Board of Saitama Medical Center, Jichi Medical University, and was performed in accordance with the ethical standards in the 1964 Declaration of Helsinki and its later amendments.

Measurement of renal function

Renal function before transplantation was evaluated using either Ccr or estimated GFR based on the creatinine or cystatin C level. Ccr was calculated from the serum creatinine and 24-hour urine creatinine level, and corrected for the actual body surface area using the following formula:

\[
\text{Ccr} = \frac{\text{Ucr} \times \text{UV}}{(\text{Scr} \times 1440)} - \frac{\text{BSA}}{73}\text{(m}^2\text{)}
\]

Ucr: 24-hour urine creatinine (mg/dl), UV: urine volume (ml/day), BSA: actual body surface area (m²).

Ccr was measured up to three times consecutively before transplantation. An average value of Ccr was used if it was measured more than once. Among the 80 patients, Ccr was measured twice in 33 patients and three times in 34. The serum creatinine level was measured using an enzymatic creatinine assay.

We calculated eGFRcre using the following formula adjusted for a Japanese population [24]:

\[
\text{eGFRcre} = 194 \times \text{Scr}^{-1.094} \times \text{age}^{-0.287} \times 0.739 \text{if female}
\]

The exacerbation of renal function after transplantation was assessed using eGFRcre, and was defined as the first day of eGFRcre <60 ml/min/1.73 m² that continued for ≥7 days, which corresponds to chronic kidney disease stage 3.

eGFRcys was calculated using the following formula [18]:

\[
\text{eGFRcys} = \frac{104 \times \text{CysC}^{-1.019}}{0.996^{\text{age}}} \times 0.929 \text{if female}
\]

CysC: serum/plasma cystatin C (mg/l)

Further, eGFRave was calculated, since eGFRave was reported to be more accurate than either eGFRcre or eGFRcys alone [19,20].

Statistical analysis

Correlations between two continuous variables were evaluated using the Pearson correlation coefficient. We assessed the potential of Ccr and eGFR levels for predicting renal function exacerbation after transplantation by the area under the receiver-operating characteristic curve. The cutoff value was also determined by a receiver-operating characteristic curve analysis. The abilities of Ccr and eGFR levels to predict the exacerbation of renal function were compared using the respective area under the curve (AUC) values. The cumulative incidence was estimated by considering death before an event as a competing risk. The groups were compared using Gray’s test [25]. A competing risk regression analysis was used to evaluate the impact of Ccr and eGFR levels on outcomes in a multivariate analysis by adjusting for factors with at least borderline significance (p < 0.10) in univariate analyses [26]. Potential confounding variables were age (<45 vs. ≥45 years), sex, Eastern Cooperative Oncology Group performance status (ECOG PS; 0 vs. 1 vs. 2), disease risk (standard vs. high), conditioning regimen (myeloablative vs. reduced intensity), total body irradiation (0 vs. 2–4 vs. 8–12 Gy), donor (related vs. unrelated), positivity of protein and occult blood in urinalysis, and HLA matching (match vs. mismatch). Untreated myelodysplastic syndrome (MDS), aplastic anemia, and disease in complete remission at transplant were defined as standard risk, and others were defined as high risk. All tests were 2-sided, and p ≤ 0.05 was considered statistically significant. All statistical analyses were performed with Stata version 13 software (Stata Corp., College Station, TX, U.S.A.) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [27].

Results

Patient and treatment characteristics

The median age (range) of the 80 patients was 45 years (18–64 years) (Table 1). Diagnoses were acute myelogenous leukemia (AML) in 38 patients, acute lymphoblastic leukemia (ALL) in 13, MDS in 11, aplastic anemia in 6, and others in 12. Forty-one patients received myeloablative conditioning. The median values of pre-transplant eGFRcys, eGFRcre, and Ccr
were 93.1, 111.9, and 105.1 ml/min/1.73 m², respectively. The coefficient of variation for Ccr was 13% (0.6–38%) for the 34 patients in whom Ccr was measured three times.

**Table 1.** Patient and transplant characteristics.

| Age (years), median (range) | 45 (18–64) |
|-----------------------------|------------|
| Sex, n (%)                  |            |
| Male                        | 51 (64)    |
| Female                      | 29 (36)    |
| Height (cm), median (range) | 166 (146–187) |
| Weigh (kg), median (range)  | 58 (36–87) |
| BMI (kg/m²), median (range) | 21 (14–33) |
| BSA (m²), median (range)    | 1.67 (1.28–2.02) |
| PS, n (%)                   |            |
| 0                           | 52 (65)    |
| 1                           | 24 (30)    |
| 2–4                         | 8 (10)     |
| Disease, n (%)              |            |
| AML                         | 38 (48)    |
| ALL                         | 13 (16)    |
| MDS                         | 11 (14)    |
| AA                          | 6 (8)      |
| ATL                         | 5 (6)      |
| NHL                         | 4 (5)      |
| Other                       | 3 (4)      |
| Disease risk, n (%)         |            |
| Standard risk               | 58 (73)    |
| High risk                   | 22 (28)    |
| Conditioning regimen, n (%) |            |
| Myeloabative                | 43 (54)    |
| TBI/CY                      | 34         |
| Other TBI regimen           | 8          |
| Bu/CY                       | 1          |
| Reduced-intensity           | 37 (46)    |
| Flu/Flu based               | 15         |
| Flu/Mel based               | 16         |
| Flu/CY based                | 5          |
| Flu/TBI                     | 1          |
| TBI (Gy), n (%)             |            |
| 0                           | 19 (24)    |
| 2–4                         | 18 (23)    |
| 8–12                        | 43 (54)    |
| Stem cell source, n (%)     |            |
| Matched related PBSC        | 25 (31)    |
| Mismatched related PBSC     | 20 (25)    |
| Matched unrelated BM        | 16 (20)    |
| Mismatched unrelated BM     | 10 (13)    |
| Mismatched CB               | 9 (11)     |
| Urinalysis                  |            |
| Protein, n (%)              |            |
| 0                           | 65 (82)    |
| +                           | 11 (14)    |
| 2+                          | 3 (4)      |
| Occult blood, n (%)         |            |
| 0                           | 6 (86)     |
| +                           | 4 (5)      |
| 2+                          | 3 (4)      |
| 3+                          | 3 (4)      |
| 4+                          | 1 (1)      |
| Pre-transplant renal parameters |          |
| Cre (mg/dl), median (range) | 0.60 (0.28–1.30) |
| CysC (mg/l), median (range) | 0.86 (0.46–1.87) |
| eGFRcre (ml/min/1.73 m²), median (range) | 111.9 (38.4–201.1) |
| eGFRcys (ml/min/1.73 m²), median (range) | 93.1 (35.4–186.4) |
| eGFRave (ml/min/1.73 m²), median (range) | 101.4 (40.3–169.5) |
| Ccr (ml/min/1.73 m²), median (range) | 105.1 (37.7–182.2) |

**Abbreviations:** BMI, body mass index; BSA, body surface area; ECOG PS, Eastern Cooperative Oncology Group performance status; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; AA, aplastic anemia; ATL, adult T cell leukemia; NHL, non-Hodgkin lymphoma; TBI, total body irradiation; Bu, busulfan; CY, cyclophosphamide; Flu, fludarabine; Mel, melphalan; PBSC, peripheral blood stem cells; BM, bone marrow; CB, cord blood; Cre, creatinine; CysC, cystatin C; eGFRcre, estimated glomerular rate based on creatinine; eGFRcys, estimated glomerular rate based on cystatin C; eGFRave, average of eGFRcre and eGFRcys; Ccr, creatinine clearance.

**Correlation between Ccr and estimated GFR**

First, we assessed the correlations between Ccr and eGFRcre, eGFRcys, and eGFRave. As shown in [Figure 1](#image), there were significant correlations between Ccr and eGFRcre, eGFRcys, and eGFRave (Ccr vs. eGFRcre, correlation coefficient = 0.600, \( p < 0.001 \); Ccr vs. eGFRcys, correlation coefficient = 0.519, \( p < 0.001 \); Ccr vs. eGFRave, correlation coefficient = 0.627, \( p < 0.001 \)).

**Prediction of renal function exacerbation after transplantation**

To evaluate the potential of baseline renal function for predicting the exacerbation of renal function, we performed receiver-operating characteristic curve analyses and evaluated the AUC. The AUC was highest when
eGFRave was used for calculations; the AUC values using Ccr, eGFRcre, eGFRcys, and eGFRave were 0.71 (95% CI: 0.60–0.82), 0.79 (95% CI: 0.69–0.89), 0.78 (95% CI: 0.67–0.88), and 0.82 (95% CI: 0.73–0.92), respectively (Figure 2). The sensitivity and specificity for eGFRave were 64 and 87%, respectively, with a cutoff value of 114 ml/min/1.73 m². We also performed separate analysis according to conditioning intensity, TBI dose, donor, patient age, and HLA matching, and found that the AUC was highest in most of the subcategories when eGFRave was used. This was also consistent regardless of the acute and chronic GVHD.

Since 90 ml/min/1.73 m² is used as the cutoff for chronic kidney disease stages 1 and 2, we used this cutoff value of eGFRave in the later analysis.

The cumulative incidence of renal function exacerbation at 1 year after allo-HCT was 0.39 (95%CI 0.25–0.52) in the high eGFRave group (≥90 ml/min/1.73 m²) and 0.85 (95%CI 0.61–0.95) in the low eGFRave group (<90 ml/min/1.73 m²). This difference was statistically significant (p < 0.001; Figure 3). In the multivariate analysis, the pre-transplant eGFRave level was a significant factor (HR 2.75, 95%CI 1.48–5.13, p = 0.001), after adjusting for patient age (≥45 vs. <45 years, HR 1.60, 95%CI 0.84–3.03, p = 0.152) and positivity of occult blood in urinalysis (yes vs. no, HR 1.38, 95%CI 0.78–2.44, p = 0.271).

### Prediction of non-relapse mortality after transplantation

The cumulative incidence of non-relapse mortality at 1 year after allo-HCT was 0.08 (95%CI 0.02–0.17) in the...
It should be noted that serum cystatin C (eGFRcys) improves the estimation of GFR in general populations 19,20. eGFRave further showed a stronger correlation with GFR based on an equation for a Japanese population, possibly due to insufficient urine collection, and found that these estimated GFRs are well-correlated with Ccr, although there are some discrepancies. Therefore, renal function, directly or indirectly, affects the risk of transplant-related mortality. Pretransplant renal function may determine the transplant indication or could be used to detect high-risk patients who need preemptive treatment and care. Severe renal impairment has been recognized as one of the comorbidities associated with non-relapse mortality in allogeneic transplantation. In a hematopoietic cell transplantation comorbidity index analysis, mild renal impairment (defined as creatinine 1.2–2.0 mg/dl) was not associated with an increased risk of non-relapse mortality [2,3]. On the other hand, moderate to severe renal impairment (defined as creatinine more than 2.0 mg/dl, renal dialysis, or renal transplant) was associated with an increased risk of non-relapse mortality. However, in the current study, only two patients (2.5%) met the criteria of mild renal comorbidity, and none met the criteria of moderate to severe renal comorbidity. In our study, low eGFRave was marginally associated with high non-relapse mortality. Although this association was not statistically significant, most likely due to our small sample size, eGFRave was the best biomarker for predicting renal failure and treatment-related mortality.

Discussion

In the present study, we evaluated pre-transplant renal function using eGFRcre, eGFRcys, eGFRave, and Ccr, and found that these estimated GFRs are well-correlated with Ccr, although there are some discrepancies. Among these four parameters, eGFRave best predicted the exacerbation of renal function in the setting of allo-HCT. Further, there was a marginal association between eGFRave and non-relapse mortality.

Few studies have compared methods for measuring pre-transplant renal function in allo-HCT [21–23]. Patients who received allogeneic transplantation included those who required prolonged bed rest due to chemotherapy-related complications, and therefore lost muscle volume. The renal function in these patients may have been overestimated if assessed by eGFRcre. In such a population, eGFRcys may be more appropriate because eGFRcys is not affected by muscle volume. Laskin et al. [21] reported that, in pediatric populations, pre-transplant eGFRcys showed a stronger correlation with GFR based on a nuclear isotope clearance than eGFRcre. eGFRave further improves the estimation of GFR in general populations 19,20. It should be noted that serum cystatin level can be affected by thyroid function and medications. All patients had normal thyroid function (data not shown). However, we could not deny the effect of medications on the cystatin level, since most of the patients took various medications through chemotherapy. In our analysis, eGFRcys and eGFRcre both showed good correlations with Ccr, but eGFRave showed the highest correlation coefficient. These findings suggest eGFRave may also be useful in allo-HCT.

The assessment of pre-transplant renal function is clinically important. In addition to calcineurin inhibitors for GVHD prophylaxis, various potential nephrotoxic agents, such as vancomycin, teicoplanin, and amphotericin B, may be used simultaneously to control transplant-related complications. If renal function is exacerbated in such a situation, the use of these agents will be limited, which then exposes patients to a high risk of GVHD or a breakthrough of bacterial or fungal infection. In an extreme situation, emergent dialysis may be needed to support renal function, which further increases the risk of transplant complications. Therefore, renal function, directly or indirectly, affects the risk of transplant-related mortality. Pretransplant renal function may determine the transplant indication or could be used to detect high-risk patients who need preemptive treatment and care. Severe renal impairment has been recognized as one of the comorbidities associated with non-relapse mortality in allogeneic transplantation. In a hematopoietic cell transplantation comorbidity index analysis, mild renal impairment (defined as creatinine 1.2–2.0 mg/dl) was not associated with an increased risk of non-relapse mortality [2,3]. On the other hand, moderate to severe renal impairment (defined as creatinine more than 2.0 mg/dl, renal dialysis, or renal transplant) was associated with an increased risk of non-relapse mortality. However, in the current study, only two patients (2.5%) met the criteria of mild renal comorbidity, and none met the criteria of moderate to severe renal comorbidity. In our study, low eGFRave was marginally associated with high non-relapse mortality. Although this association was not statistically significant, most likely due to our small sample size, eGFRave was the best biomarker for predicting renal failure and treatment-related mortality.

This study has several limitations. First, our interpretation of renal function may have been limited, because we did not assess renal function using inulin or nuclear isotope clearance. However, we used Ccr as a reference. Although there was a deviation in Ccr for some patients, possibly due to insufficient urine collection, the coefficient of variation of Ccr was acceptable (13%). Second, we used an equation of eGFRcre based on an equation for a Japanese population, since this is more strongly correlated with the results with other equations [24]. Therefore, our findings should be validated in other racial populations. Lastly, the retrospective study design, small sample size, and heterogeneous disease and transplantation background may have biased the results.
In conclusion, eGFRave best predicted the exacerbation of renal function after allogeneic stem cell transplantation. This indicates that eGFRave is clinically effective in the setting of allo-HCT. Further, the marginal association between low eGFRave and high non-relapse mortality warrants further study in a larger prospective study in patients undergoing allo-HCT.

Disclosure statement
No potential conflict of interest was reported by the authors.

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