Associations of pre-transplant anemia management with post-transplant delayed graft function in kidney transplant recipients


Abstract: Background: Delayed graft function (DGF) complicates kidney allograft outcomes in the immediate post-transplantation period. We hypothesized that in hemodialysis patients more severe anemia, iron deficiency, the requirement for higher doses of erythropoietin-stimulating agents (ESA), or blood transfusions prior to transplantation are associated with higher risk of DGF.

Methods: Linking five-yr hemodialysis patient data of a large dialysis organization to the Scientific Registry of Transplant Recipients, we identified 11 836 hemodialysis patients. Using logistic regression analyses we examined the association between pre-transplant parameters and post-transplant DGF.

Results: Patients were 49 ± 14 (mean ± SD) yr old and included 38% women, 27% blacks, and 26% diabetics. After adjusting for relevant covariates, pre-transplant blood transfusion was associated with 33% higher DGF risk (odds ratio [OR] = 1.33; 95% confidence interval [CI]: 1.19–1.48); and each 5000 U/wk increase of pre-transplant ESA dose with 5% higher DGF (OR = 1.05; 95% CI: 1.02–1.09). Compared to pre-transplant blood hemoglobin of 12–12.99 g/dL, there was 25% higher risk of DGF with blood hemoglobin 10–10.99 g/dL (OR = 1.25; 95% CI: 1.01–1.55), whereas blood hemoglobin ≥ 13 g/dL exhibited 15% higher risk of DGF (OR = 1.15; 95% CI: 0.98–1.34).

Conclusions: Pre-transplant blood transfusion, higher ESA dose, and either high or low blood hemoglobin but not iron markers are associated with higher risk of DGF.

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Delayed graft function (DGF) is an important complication affecting kidney allograft outcomes in the immediate post-transplantation period and is defined as the need for at least one session of dialysis treatment in the first week after receiving a kidney transplant (1). DGF is attributed to
ischemia-reperfusion and immunological injury of the graft (2). The prevalence of DGF varies from 4% to 10% in living donors (2) and 5% to 50% in deceased donor kidney transplants (3–7). The occurrence of DGF may significantly complicate the immediate post-transplant management by increasing morbidity and mortality (8, 9), prolonging patient hospitalization (10), and inflating health care costs (10–12).

DGF is attributed to ischemia-reperfusion and immunological injury of the graft (2), which could be induced through a variety of mechanisms. The transfusion of blood products before transplantation may affect the development of DGF through its effect on the recipient’s immune response. Higher blood hemoglobin level might be protective against ischemia-reperfusion injury after transplantation (13, 14). Few studies examined the association between pre-transplant administration of erythropoietin-stimulating agents (ESA) and DGF (13, 15, 16).

In hemodialyzed patients, iron deficiency is one of the most common causes of anemia. Additionally, iron deficiency is associated with higher platelet counts in hemodialyzed patients (17). Platelet reactivity plays a central role in thrombo-embolic events such as graft thrombosis, which is more frequent in patients with DGF (16). To our knowledge, no study has yet examined the association between iron deficiency and DGF.

To our knowledge, no large study examined the association between pre-transplant anemia, iron deficiency, its therapeutic options (blood transfusion or ESA), and DGF in kidney transplant recipients. Given the foregoing inconsistent data, we sought to examine whether recipients’ low pre-transplant hemoglobin, iron deficiency, blood transfusion, or ESA dose has a bearing on early post-transplant graft function in a large and contemporary incident cohort of kidney transplant recipients throughout the United States.

Patients and methods

Patients

We linked data on all kidney transplant recipients listed in the Scientific Registry of Transplant Recipients (SRTR) up until June 2007 to a list of individuals with chronic kidney disease stage 5D, who underwent maintenance hemodialysis (MHD) treatment from July 2001 to June 2006 in one of the outpatient dialysis facilities of a US-based large dialysis organization (DaVita Inc, prior to its acquisition of former Gambro dialysis facilities). The study was approved by the Institutional Review Committees of both Los Angeles Biomedical Research Institute at Harbor-UCLA and DaVita Clinical Research. The study conformed to the principles of the Declaration of Helsinki. Because of the large sample size, the anonymity of the patients studied, and the non-intrusive nature of the research, the requirement for informed consent was waived.

Clinical and demographic measures

The creation of the merged SRTR and DaVita MHD patient cohort has been described previously (18–23). Demographic data and details of medical history were collected, including information on age, gender, race, type of insurance, marital status, presence of diabetes, height, post-hemodialysis dry weight (to calculate averaged body mass index [BMI]), and dialysis vintage. Dialysis vintage was defined as the duration of time between the first day of dialysis treatment and the day of kidney transplantation.

To minimize measurement variability, all repeated measures for each patient during any given calendar quarter, i.e., over a 13-wk or three-month interval, up to the time of kidney transplantation, were averaged and the quarterly means in each of the 20 calendar quarters were used in our analyses. All values were averaged into one single quarterly value per patient per each calendar quarter.

Laboratory measures

Blood samples were drawn using uniform techniques in all of the DaVita dialysis clinics and were transported to the DaVita Laboratory in Deland, Florida, typically within 24 h. All laboratory values were measured by automated and standardized methods in the DaVita Laboratory. Most laboratory values were measured monthly, including serum urea, creatinine, albumin, calcium, phosphorus, bicarbonate, and total iron binding capacity (TIBC). Serum ferritin was measured at least quarterly. Hemoglobin was measured at least monthly in essentially all patients and weekly to biweekly in most patients. Most blood samples were collected pre-dialysis with the exception of the post-dialysis serum urea nitrogen that was obtained to calculate urea kinetics. Kt/V (single pool) was calculated using urea kinetic modeling equations as described elsewhere (24). Albumin-corrected calcium was calculated by subtracting 0.8 mg/dL for each g/dL serum albumin below 4.0 g/dL (25).

Anemia and delayed graft function
The last three-month-averaged pre-transplant serum ferritin, iron saturation ratio, and blood hemoglobin were used in our analyses. All values were averaged into one single value. We divided pre-transplant blood hemoglobin (<10, 10–11, 11–12, 12–13, and ≥ 13 g/dL), serum ferritin (<100, 100–299, 300–499, 500–799, and ≥ 800 ng/mL), and iron saturation ratio (<20, 20–29, 30–39, 40–49, and ≥ 50%) into five categories. We used the last three-month-averaged pre-transplant ESA dose and divided into five categories (<500, 500–999, 10 000–14 999, 15 000–19 999, and ≥ 20 000 U/wk) to analyze the association between ESA dose and DGF. We also analyzed the pre-transplant blood transfusion variable from SRTR. The physician was asked: “Did patients receive any pre-transplant blood transfusion? Yes/No,” and the answers were recorded in SRTR. We did not have data about number of blood transfusions and the time period of blood transfusions recorded.

Definition of DGF

DGF was defined as the need for any dialysis therapy in the first week after transplantation (1).

Statistical methods

Data were summarized using proportions, means (+standard deviation [SD]), or medians (interquartile range [IQR]) as appropriate. Categorical variables were analyzed with chi-square tests and continuous variables were compared using Student’s t-tests or the Mann–Whitney U-tests, Kruskal–Wallis H tests, or ANOVA as appropriate. In all statistics, two-sided tests were used and the results were considered statistically significant if p was <0.05. Logistic regression models were employed to estimate the odds ratio (OR) (and 95% confidence interval [95%CI]) of post-transplant DGF based on pre-transplant serum ferritin, iron saturation ratio, blood hemoglobin categories, and ESA dose during the calendar quarter preceding the kidney transplantation and pre-transplant transfusion. Additionally, we tested the non-linearity by adding the quadratic term of blood hemoglobin, serum ferritin, iron saturation ratio, and ESA dose to the models already containing the linear term. We also tested the non-linearity all of our models using “mvrs” STATA command to see instantaneously if any of the co-variables have a non-linear association with outcome.

For each analysis, four models were examined based on the level of multivariate adjustment: (I) an unadjusted model; (II) case-mix adjusted models included age, gender, race-ethnicity (African Americans and other self-categorized blacks, non-Hispanic whites, Asians, Hispanics, and others), diabetes mellitus, dialysis vintage, primary insurance (Medicare, Medicaid, private, and others), marital status (married, single, divorced, widowed, and other or unknown), the standardized mortality ratio of the dialysis clinic during entry quarter, dialysis dose as indicated by Kt/V (single pool), presence or absence of a dialysis catheter; and (III) malnutrition-inflammation-complex syndrome (MICS) adjusted models which included all of the covariates in the case-mix model as well as 12 surrogates of nutritional status and inflammation, including 10 laboratory variables with known association with clinical outcomes in HD patients, i.e., nPCR as an indicator of daily protein intake, also known as the normalized protein nitrogen appearance (nPNA) (26), body mass index, serum albumin, creatinine, TIBC, ferritin (except ferritin models), phosphorus, calcium, bicarbonate, peripheral white blood cell count (WBC), lymphocyte percentage, and hemoglobin (except hemoglobin models); and (IV) case-mix, MICS, and transplant data adjusted models included all of the above plus 7 transplant-related variables: (1) donor type (deceased or living), (2) donor age, (3) panel reactive antibody (PRA) titer (last value prior to transplant), (4) number of HLA mismatches, (5) cold ischemia time, (6) transfusion before transplantation, and (7) extended donor criteria (EDC) using standard definition (donor age >60 yr or donor age >50 yr and donor history of hypertension and/or serum creatinine of donor >1.5 mg/dL and/or cause of death in donor is cerebrovascular event).

Missing covariate data in multivariate logistic regression models were imputed by medians or means as appropriate. All analyses were carried out using STATA version 11.1 (STATA Corporation, College Station, TX, USA).

Results

The original five-yr (July 2001–June 2006) national database of all DaVita dialysis patients included 164 789 adult subjects. This database was linked via unique identifiers to the national SRTR registry that included all transplant waitlisted people and kidney transplant recipients until 06/2007 (Fig. S1). Of 37 766 DaVita dialysis patients who were identified in the SRTR database, 17 629 had undergone one or more kidney transplantations during their life time, including 14 508 patients who had undergone their first kidney transplantation between 7/2001 and 7/2007. After excluding...
those without electronically recorded data (n = 1), peritoneal dialysis patients (n = 2092) subjects who lacked data from the baseline quarter or those with outlier values for age (>99 or <16 yr; n = 579), there were 11 836 hemodialysis patients who met all inclusion and exclusion criteria and who subsequently underwent their first kidney transplantation during the observation period.

Table 1 compares the demographic, clinical, transplant-related, and pre-transplant laboratory characteristics of the patients with (n = 2628) and without (n = 9208) DGF. Patients with DGF were two yr older and more likely to be diabetic or African American or to have Medicare as their primary insurance. Patients with DGF had higher serum ferritin and lower blood hemoglobin levels and were more likely to receive kidneys from deceased donors with longer cold ischemic time.

Table 2 shows the results of multivariate logistic regression analyses for pre-transplant transfusion,
ESA dose, blood hemoglobin, and iron deficiency markers. Pre-transplant transfusion was a significant predictor of DGF in univariate analysis, being associated with a 30% higher risk of DGF ($OR = 1.30; 95\% CI: 1.18–1.44$). After adjusting for case-mix, MICS, and transplant-related variables, pre-transplant transfusion remained an independent and significant predictor of DGF ($OR = 1.33; 95\% CI: 1.19–1.48$) (Table 2). Fig. 1 shows the association between pre-transplant transfusion and DGF in different subgroups. The OR of DGF across almost all examined subgroups was greater than one, indicating a higher risk. Moreover, the association between pre-transplant transfusion and DGF was stronger in females than in males.

We detected linear association between pre-transplant ESA dose and DGF. Incrementally higher pre-transplant ESA dose over 5000 U/wk was associated with higher risk of DGF (Fig. 2). Each 5000 U/wk increase of pre-transplant ESA dose was associated with 7% higher risk of DGF ($OR = 1.07; 95\% CI: 1.04–1.10$) in our unadjusted model. After adjusting for case-mix and MICS and transplant-related variables, pre-transplant ESA dose remained an independent and significant predictor of DGF ($OR = 1.05; 95\% CI: 1.02–1.09$) (Table 2). The OR of DGF across almost all examined subgroups was greater than one, indicating a higher risk (Fig. 3). Moreover, the association between pre-transplant ESA dose and DGF was stronger in patients with hemoglobin $<11$ g/dL (Fig. 3 and Table S2).

In our final multivariate model, the association between pre-transplant blood hemoglobin and risk of DGF was U-shaped (Fig. 4).
supported by the fact that the squared hemoglobin term was a significant predictor of DGF ($p = 0.038$) (Table 2). Compared to pre-transplant blood hemoglobin of 12–12.99 g/dL, there was 25% higher risk of DGF with blood hemoglobin 10–10.99 g/dL ($OR = 1.25; 95\% CI: 1.01–1.55$), whereas blood hemoglobin $\geq 13$ g/dL exhibited 15% higher risk of DGF ($OR = 1.15; 95\% CI: 0.98–1.34$) (Fig. 4).

We also analyzed the association between serum ferritin and DGF. Incrementally higher pre-transplant serum ferritin over 100 ng/mL was associated with higher risk of DGF (Fig. 5) in our unadjusted model. However, after adjusting for case-mix, MICS, and transplant-related variables, this association abolished.

In our unadjusted model, the association between pre-transplant iron saturation ratio and risk of DGF was inverse U-shaped (Fig. 6). This notion is supported by the fact that the squared iron saturation ratio term was a significant

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**Fig. 1.** Multivariate analysis of fully adjusted (for case-mix, MICS, and transplant covariates) logistic regression models showing pre-transplant transfusion and OR (and 95% CI as error bars) of delayed graft function in different sub-group of patients.

**Fig. 2.** Multivariate analysis of logistic regression models showing pre-transplant weekly ESA dose and OR (and 95% CI as error bars) of delayed graft function in four different models (Reference: ESA dose: 5000–9999 [U/wk]).

**Fig. 3.** Multivariate analysis of fully adjusted (for case-mix, MICS, and transplant covariates) logistic regression models showing the ESA dose and OR (and 95% CI as error bars) of delayed graft function for every +5000 U/wk increase of ESA dose in different sub-group of patients.
predictor of DGF (p = 0.009) (Table 2). However, after adjusting for case-mix, MICS, and transplant-related variables, this association abolished.

Discussion

In 11 836 kidney transplant recipients with comprehensive pre- and post-transplant data, transfusion before transplantation and higher pre-transplant ESA dose and low and also the high pre-transplant blood hemoglobin level was associated with higher risk of DGF during the first post-transplant week. The associations between pre-transplant transfusion, ESA dose, and DGF were rather consistent across diverse demographic, clinical, and laboratory subgroups. These findings may have important clinical implications for pre-transplant management of waitlisted dialysis patients.

Whereas DGF is likely an important predictor of poorer short- and long-term graft survival (27–30), less is known about the risk factors which predict the development of DGF. Some of the well-known post-transplant complications, such as calcineurin inhibitor toxicity, vascular or urological complication, rejection and volume depletion, are characteristically present in patients with DGF (1). An important donor-related risk factor for DGF can be the modality of organ procurement. Avoidance of inotropic support (31), short cold ischemic time (14), and younger donor age (15) may also contribute to a lower risk of DGF. Additional recipient-related factors such as recipient hypovolemia (32), type of renal replacement therapy (33), and inherited thrombophilia (34, 35) have a bearing on the risk of DGF; however, very little is known about the association of DGF with pre-transplant-related factors during dialysis treatment era such as pre-transplant anemia, iron deficiency, and its treatment.

We found that transfusions before transplantation were associated with a DGF risk more than 30% even after we adjusted for PRA. This association could be explained by the immune-sensitization of the recipients, as DGF is attributed to immunological injury of the graft (2), but could also be the result of higher hemoglobin levels in patients who received a blood transfusion. Theoretically, higher blood hemoglobin level may be protective against ischemia-reperfusion injury after transplantation. However, Schmidt et al. (13) reported opposite
results. It is possible, though, that the observed association between high hematocrit level and DGF was present because pre-transplant transfusions were not accounted for in these analyses (13), even though 30% of the patients with impaired graft function and 18% of the patients with DGF in that study received blood transfusion intraoperatively (13). Another recent study did not show a significant difference between DGF in patients with high vs. low pre-transplant blood hemoglobin (14).

Our study indicated an association between pre-transplant blood hemoglobin concentration and DGF risk is U-shaped. It is conceivable that blood hemoglobin level has a U-shaped effect in that it could be protective against ischemia-reperfusion injury after transplantation by virtue of decreasing ischemia, but levels that increase beyond a certain cutoff could also contribute to graft thrombosis (which is more frequent in patients with DGF [16]) owing to increased viscosity. Additionally, dialysis with ultrafiltration before transplantation can elevate hemoglobin level and it can easily cause hypovolemia in recipient, which is a known risk factor of DGF (32). In our study, we used blood hemoglobin value from the last calendar quarter; therefore, this mechanism cannot explain our results.

We detected linear association between pre-transplant ESA dose over 5000 U/wk and DGF. In 1988, one case of allograft renal artery thrombosis was reported in patients receiving ESA before transplantation (15). However, other studies did not report increased incidence of DGF associated with ESA use (13, 16). Streja et al. (17) postulated that high doses of ESA can cause relative thrombocytosis by promoting iron depletion in MHD patients. Platelet reactivity plays a central role in thromboembolic events such as graft thrombosis, which are more frequent in patients with DGF (16). Based on the hypotheses that iron deficiency could induce thrombocytosis, we also postulated that there may be an association between low iron stores and DGF; but we did not find any in our observational study, as neither pre-transplant serum ferritin, nor iron saturation associated significantly with DGF after adjustment for confounders.

There are potential limitations to our study. Like all observational studies, ours too cannot prove causality. Patients who were excluded from analyses were likely different from the included ones (excluded peritoneal dialysis patients are different than hemodialysis patients), but their proportion was relatively small. In the SRTR dataset, more detailed data about immunosuppression therapy or laboratory data, which may have an effect on the risk of DGF, do not exist. The data about transfusion came from SRTR, which is based on the physicians’ recollection. To the best of our knowledge, our study is the first examining the association between pre-transplant anemia, iron deficiency and its treatment, and post-transplant DGF. Strengths of this study include the high number of patients and the multilevel adjustments which include several important covariates.

Conclusions
In our large and contemporary national cohort of 11 836 kidney transplant recipients, pre-transplant transfusion and the administration of high ESA doses were associated with higher risk of DGF post-transplantation. Moreover, the association between pre-transplant blood hemoglobin and risk of DGF was U-shaped.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Flow chart of the patient selection (see text).

Table S1. Multivariate analysis of fully adjusted (for case-mix, MICS, and transplant covariates) logistic regression models showing pre-transplant transfusion and OR (and 95% CI as error bars) of delayed graft function in different sub-group of patients.

Table S2. Multivariate analysis of fully adjusted (for case-mix, MICS, and transplant covariates) logistic regression models showing the ESA dose...
and OR (and 95% CI as error bars) of delayed graft function for every +5000 units/wk increase of ESA dose in different sub-group of patients.

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