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ORIGINAL ARTICLE

Early allograft inflammation and scarring associate with graft dysfunction and poor outcomes in renal transplant recipients with delayed graft function: a prospective single center cohort study

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SUMMARY

Early histological progression that associates with delayed graft function (DGF) and its relationship to graft outcomes is less well-understood. We systematically evaluated early acute and chronic histological changes associated with DGF through serial biopsies (protocol: 3 and 12 months; for-cause) and related them to graft outcomes. 56/294 (19.04%) of our patients had DGF. DGF was associated with a progressive increase in both Banff 't' and 'i' scores from 2 weeks to 3 and 12 months with a resultant increase in T cell mediated rejection (TCMR) that was significantly greater than those with primary graft function (PGF). This increase in TCMR was predominantly sub-clinical TCMR diagnosed on protocol biopsy. Furthermore, TCMR in patients with DGF was recurrent/persistent at 12 months. Importantly, the combination of DGF and TCMR was associated with significantly worse interstitial fibrosis and tubular atrophy (IFTA) and interstitial fibrosis with inflammation (IF + 'i') as early as 3 months and worse renal function. Finally, DGF with TCMR was associated with significantly worse graft loss. In this regard, DGF without TCMR had comparable chronic histology and outcomes to PGF. Thus, DGF with TCMR (predominantly sub-clinical), represents a high-risk patient group who may benefit from early novel immunosuppression augmentation strategies to improve graft outcomes.

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Key words
acute rejection, delayed graft function, graft loss, kidney transplantation

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Introduction

The diagnosis of delayed graft function (DGF) is common after kidney transplantation and is a manifestation of acute kidney injury in the immediate post-transplant phase. DGF is commonly defined as need for dialysis within the first post-transplant week [1,2]. Donor and/or recipient variables through prerenal, intrinsic or postrenal interactions lead to DGF. The lack of a unifying definition for DGF leads to variability in the reported rates of DGF between 5–50% [3–6]. Higher incidence of DGF up to 30% has been reported with the introduction of newer Kidney Allocation System [7]. DGF is generally accepted as a clinical entity associated with worse overall kidney transplant outcomes although this may not universally be the case [8].

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However, early acute rejection and allograft fibrosis can modulate the impact of DGF on graft outcomes [9–11]. Importantly DGF with acute rejection has been shown to be associated with a much worse graft outcome [3,12]. Ischemic injury that sets in even prior to organ implantation initiates a series of signaling events on the vascular endothelial cell surface, which stimulate synthesis of MHC-1 molecules. The adaptive immune system further participates in early reperfusion kidney injury through direct allorecognition. The chronological events that lead to graft failure in this setting and the timeframe for their occurrences are unclear. The current single center study was performed to compare recipients with DGF and those with primary graft function (PGF) for early allograft histological changes including acute inflammatory lesions and chronic damage as well as allograft dysfunction. We hypothesize that DGF is associated with a higher prevalence and degree of allograft tubulo-interstitial inflammation, evolving to allograft fibrosis and dysfunction leading to kidney transplant graft failure and these effects are pronounced in patients with DGF who developed rejection.

Material and methods

Study population

As shown in Fig. S1, a total of 378 adult patients who underwent kidney transplantation at the Thomas E. Stars Transplantation Institute, University of Pittsburgh from January 2013 to November 2014 were considered for this analysis. All kidney transplant recipients, from both genders and all racial and ethnic groups who had transplants were included for this study. Recipients who did not undergo allograft biopsy during the 1st year post-transplant (n = 84) were excluded. Thus, a total of 294 transplants were included and of these, 56 (19.04%) had DGF and the remaining 238 were classified into the PGF group.

Delayed graft function

DGF was defined as the requirement of at least 1 dialysis treatment within 1st week post-transplant (N = 56). Patients who did not require post-transplant dialysis were classified into the PGF group (N = 238). PGF patients were divided into those with PGF after LD transplantation (PGF-LD, N = 114) and those with PGF after DD (PGF-DD, N = 124) transplantation. Patients were also stratified by the presence or absence of Biopsy Proven Acute Rejection.

Immunosuppression

Nearly all patients received Thymoglobulin induction up to a total dose of 6 mg/kg, administered over 4–6 days. All patients received a rapid 7-day corticosteroid taper. Mycophenolate Mofetil (MMF) was started on day of transplant at a dose of 1000 mg BID, and Tacrolimus (TAC) was started within 72 h post-transplant. Maintenance steroids at 5 mg daily was administered only for highly sensitized patients with cPRA > 90%. Target trough levels for Tacrolimus for the first 3 months and beyond 3 months were 8–12 ng/ml and 6–10 ng/ml respectively.

Allograft biopsies

Based on our center protocol, patients who had persistent DGF at 2 weeks underwent a biopsy. During the follow-up period, protocol biopsies were performed at 3 and 12 months post-transplant in patients with stable allograft function. In addition, patients with graft dysfunction (delta creatinine of >0.3 mg/dl or 25% increase serum creatinine from baseline) had for-cause allograft biopsies.

Allograft inflammation and chronic damage

All biopsies were analyzed and graded for both acute and chronic damage based on 2013 Banff classification [13]. Acute (t, i, v, and g) and chronic (ct, ci, cv, and cg) scores were assessed and recorded for each biopsy. The chronic damage indices that were assessed were IFTA ('ct' + 'ci') and IF + 'i' ('ci' > 1 and i >1).

T/B cell flow cross match and post-transplant donor specific antibody (DSA) testing

T and B Flow cross-matches were performed before transplant and only patients with negative CDC and flow cross matches underwent transplantation. No patients underwent desensitization therapy. HLA antibodies were detected post-transplant by Lambda LAB Screen single antigen bead (SAB) assay using the HLA Fusion version 3.5.6. Serum samples were analyzed for DSA at 0, 1, 3, 6, 9, and 12 months post-transplant. Background normalized median fluorescence intensity (MFI) was determined for each DSA. A normalized MFI of >1000 within 1st post-transplant year were categorized positive for DSA. None of patients underwent de-sensitization treatment prior to transplantation.

Acute rejection

Acute T cell mediated rejection (TCMR) or antibody mediated rejection (AMR) were diagnosed based on 2013 Banff classification. Allograft inflammation was graded as Borderline Suspicious for TCMR and TCMR [Grade I (A/B), II (A/B) and III]. Patients with Grade I (A/B) and IIA were treated IV solumedrol (250 mg X3 doses) and then maintained on prednisolone 5 mg daily. Patients with Banff IIA refractory to IV steroids and those with Banff Grade IIB and III were treated with Thymoglobulin 4–6 mg/kg over 3–6 days. AMR was treated with plasmapheresis and IV Ig (4–6 doses).

Data collection and data variables

Data were collected on all patients including donor and recipient demographics as well as other transplant variables such as cause of ESRD, donor source (living versus deceased), HLA A, B, DR, total mismatch; pretransplant levels of Class I/II PRA, Kidney Donor Profile index (KDPI) scores, warm ischemia time (WIT) in minutes, cold ischemia time (CIT) in minutes, use of Thymoglobulin for induction antibody treatment, post-transplant DGF (yes or no) and post-transplant DSA, using Electronic Medical Records.

Follow-up

Patients were followed up for a mean period of 40 ± 10 months post-transplant.

Outcome measures

Acute allograft inflammation and chronic damage scores in patients with DGF were compared over time from 2 weeks, to 3 and 12 months post-transplant. Both acute and chronic scores during early (3 months) and late (6–12 months) post-transplant period were compared in patients with DGF versus PGF-LD versus PGF-DD groups. Allograft function was assessed by serum creatinine and actuarial kidney graft survival rates were compared amongst patients with DGF, PGF-LD, and PGF-DD groups and for patients with DGF/TCMR+ versus DGF/TCMR−. In addition to graft failure, a composite end-point of allograft loss and impending allograft loss (defined as eGFR < 30 ml/min with >30% decline from the baseline) was also analyzed [14,15].

Analysis and statistical methods

Data were presented either as means or medians with appropriate measures of dispersion. Unpaired *t* test or Mann Whitney U test were used for comparison of continuous variables and Chi-square test was used for proportions. Comparison of means across multiple groups was by one-way ANOVA with Dunnet's correction for multiple comparisons or the Kruskal–Wallis test for data with skewed distribution. Kaplan–Meier method with Log-Rank test was used to identify differences in actuarial graft survival between groups. Multivariate Cox Proportional Hazards models were used to assess the independent effect of DGF on graft outcomes. Univariate and multivariate logistic regression analysis were performed to identify risk factors for the development of DGF/TCMR+. Variables that achieved a P value <0.1 in the univariate analysis were included in the multivariate model. All P values were two tailed and a P value of <0.05 was considered significant. SPSS Version 21 (IBM Corp., Armonk, NY, USA) was used for statistical analysis.

Ethical guidelines

Patient information used for this analysis was obtained from the transplant registry through institutionally designated individuals at the UPMC and the Thomas E. Stars Transplantation Institute as regulated by the institutional review board guidelines at the University of Pittsburgh. This institution maintains a prospectively collected electronic database of all kidney transplant patients. Data with patient identifiers was collected under the IRB number PRO-13060220 provided to investigator as approved by the University of Pittsburgh. The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the 'Declaration of Istanbul on Organ Trafficking and Transplant Tourism.

Results

Study population and groups

Figure S1 illustrates the study cohort and subjects. A total of 378 kidney transplants were performed from January 2013 through November 2014. 84 who did not undergo kidney allograft biopsy within 1st year were excluded. Thus, a total of 294 kidney transplants were included in this study. Of these 56 patients had DGF post-transplantation and the remaining 238 had PGF.

Table 1 illustrates the donor and recipient demographics as well as transplant variables for patients who had DGF versus those with PGF-LD and PGF-DD kidney transplantation. Patients in the PGF-LD group were younger, with more female recipients and lower proportion of Non-Caucasians as opposed to the PGF-DD and DGF groups. CIT was much lower in patients with PGF-LD as opposed to those with DGF and PGF-DD group. Serum creatinine at 3 months was significantly lower in patients with PGF after LD/DD transplantation.

DGF, allograft tubulointerstitial inflammation and chronic changes

The evolution of tubulointerstitial inflammation (Banff 't' and 'i' scores) and chronic histological changes including interstitial fibrosis with tubular atrophy (IFTA, 'ct' + 'ci') and interstitial fibrosis with inflammation (IF + 'i', ci ≥ 1 & 'i' ≥ 1) in patients with DGF from 2 weeks through 3 and 12 months post-transplant are shown in Fig. 1. Both 't' and 'i' scores were significantly greater at 3 months and 12 months when compared to 2 weeks (Fig. 1a and b). Prevalence of acute rejection at 3 months and 12 months post-transplant remained higher in patients with DGF (Fig. 1c). At 2 weeks, patients had minimal IFTA that significantly increased at 3 and 12 months post-transplant (Fig. 1d). Amongst patients with DGF, <20% had IFTA ≥ 2 at 2 weeks post-transplant and this increased to >50% and 80% at 3 and 12 months post-transplant respectively. Similarly, 14% of patients had IF + 'i' at DGF and this significantly increased to 55% and 56% at 3 and 12 months respectively, (Fig. 1e). Thus, there was minimal inflammation and chronicity detected at DGF, but the inflammation and chronic changes were noted as early as 3 months and persisted at 12 months post-transplant.

DGF is associated with increased incidence of subclinical TCMR

Patients with DGF had significantly greater 't' and 'i' scores compared to PGF-LD and PGF-DD groups at 3 months (Fig. 2a and b). This translated to a significantly greater incidence of (≥Banff 1A) acute TCMR in the DGF group when compared to both the PGF groups (Fig. 2c). Greater incidence of TCMR noted in the DGF group was due to an increase in the incidence of subclinical TCMR diagnosed on a protocol biopsy at 3 months (Fig. 2d). A similar trend with the 't' and 'i' scores and incidence of biopsy proven acute TCMR

Table 1. Clinical characteristics- patients with DGF and those with PGF-LD and PGF-DD kidney transplantation

Characteristic	DGF	PGF (LD)#	PGF (DD)$	*P* value
Number (*n*)	56	114	124	
Recipient age in years (mean ± SD)	56 ± 13	45 ± 15	56 ± 13	<0.0001*
Recipient gender (male %)	71	48	64	0.006*
Non-Caucasian Ethnicity%	32	12	23	0.008
Primary renal diagnosis				
% Diabetes mellitus	18	24	29	NS
%GN	26	28	11	
%HTN	16	11	17	
%others	40	37	43	
Kidney Donor Profile index score > 20 (±SD)	63		67-	NS
HLA*AB mis match (mean ± SD)	3.2 ± 1.4	3.0 ± 1.4	3.1 ± 1.6	NS
HLA*DR mis match (mean ± SD)	1.26 ± 0.7	1.28 ± 0.7	1.08 ± 0.8	NS
PRA Class I ≥ 70 (%)	5	1	8	NS
PRA Class II ≥ 70	11	8	12	NS
Cold ischemia time (min) (mean ± SD)	602 ± 335	129 ± 167	641 ± 292	<0.0001*
Donor age (mean ± SD)	41 ± 14	39 ± 12	38 ± 12	NS
% re-transplant	18	18	19	NS
% thymoglobulin induction	95	98	92	0.09
Serum Creatinine (mg/dl) at 3 month	1.8 ± 0.6	1.4 ± 0.5	1.5 ± 0.6	0.002
Post-transplant donor specific antibody (%)	30.4	20	22	NS

*Statistically significant differences are seen between the delayed graft function (DGF) and primary graft function (PGF)-LD groups. Patients in the DGF and PGF-DD groups had comparable baseline characteristics. #Living donor (LD); $Deceased donor (DD).

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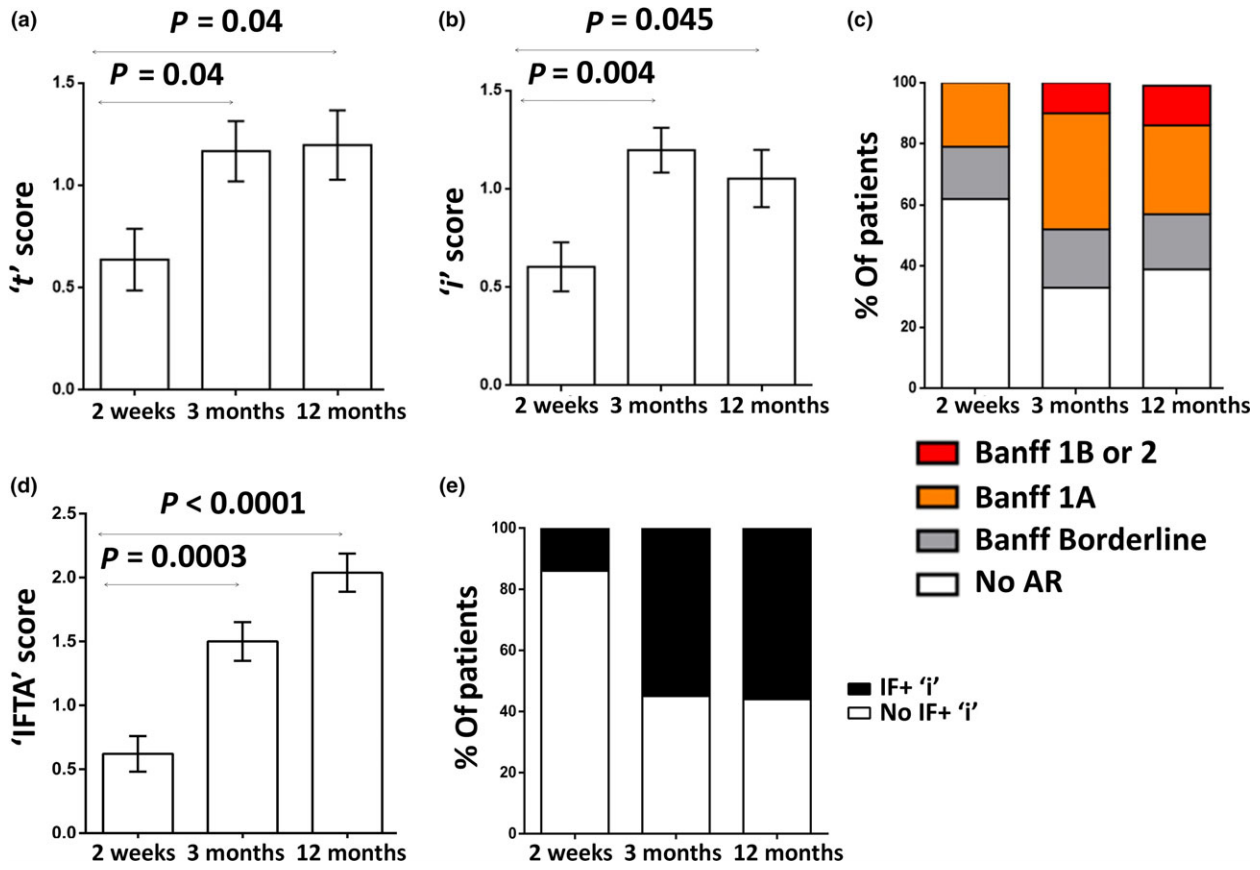

Figure 1 Histological progression in delayed graft function (DGF). Banff tubulointerstitial inflammation scores ('t' and 'i') along with IFTA and IF + 'i' were compared in patients with DGF at 2 weeks (DGF biopsy) versus 3 months and 12 months. Patients had a significantly greater 't' score (a) and 'i' score (b) at 3 months and 12 months compared to 2 weeks. Greater proportion of patients had ≥Banff 1A acute rejection at 3 months and 12 months in comparison to at 2 weeks ($P = 0.03$, (c). The mean IFTA score and the proportion of patients with IF + 'i' were compared between the three time-points and again, patients had significantly greater IFTA score (d) and a greater proportion had IF + 'i' (e, $P < 0.0001$) at 3 and 12 months when compared to 2 weeks. The error bars in the bar charts represent SEM.

(both clinical and sub-clinical) were also noted at 12 months (data not shown). We have next noted that a significantly greater proportion of patients with DGF and TCMR (DGF/TCMR+) at 3 months had either persistent or recurrent late TCMR (12 months) despite standard of care therapy for acute TCMR with steroids when compared to those with rejection in PGF-DD and PGF-LD groups (Fig. 2e). Thus, DGF was associated with an increased incidence of early sub-clinical TCMR and higher proportion had persistent TCMR at 12 months.

DGF, post-transplant DSA and AMR

Of the 294 patients analyzed, 67 (22.8%) had detectable DSA in the first year. A total of 108 patients (36.7%) had either clinical or sub-clinical TCMR diagnosed in the 1st year (protocol biopsies at 3 months and 12 months and all for-cause biopsies). Of these, only 11 patients (10.2% of all TCMRs) developed concomitant AMR, suggesting mixed T cell and AMR in a minority. None of them had pure AMR. There was a trend towards greater DSA detection in patients with DGF when compared to those with PGF (Fig. S2A) that did not reach statistical significance. Incidence of mixed rejection among patients with DGF and PGF were similar (Fig. S2B) Interestingly, a significantly greater proportion of patients with DGF had DSA and TCMR when compared to those with PGF (Fig. S2C).

DGF with TCMR is associated with allograft chronicity

Patients with DGF demonstrated significantly greater degree of IFTA as early as 3 months post-transplant (Fig. 3a), a trend that continued even at 12 months (Fig. 3c). Similarly, a significantly greater proportion of patients with DGF had IF + 'i' both at 3 and 12 months

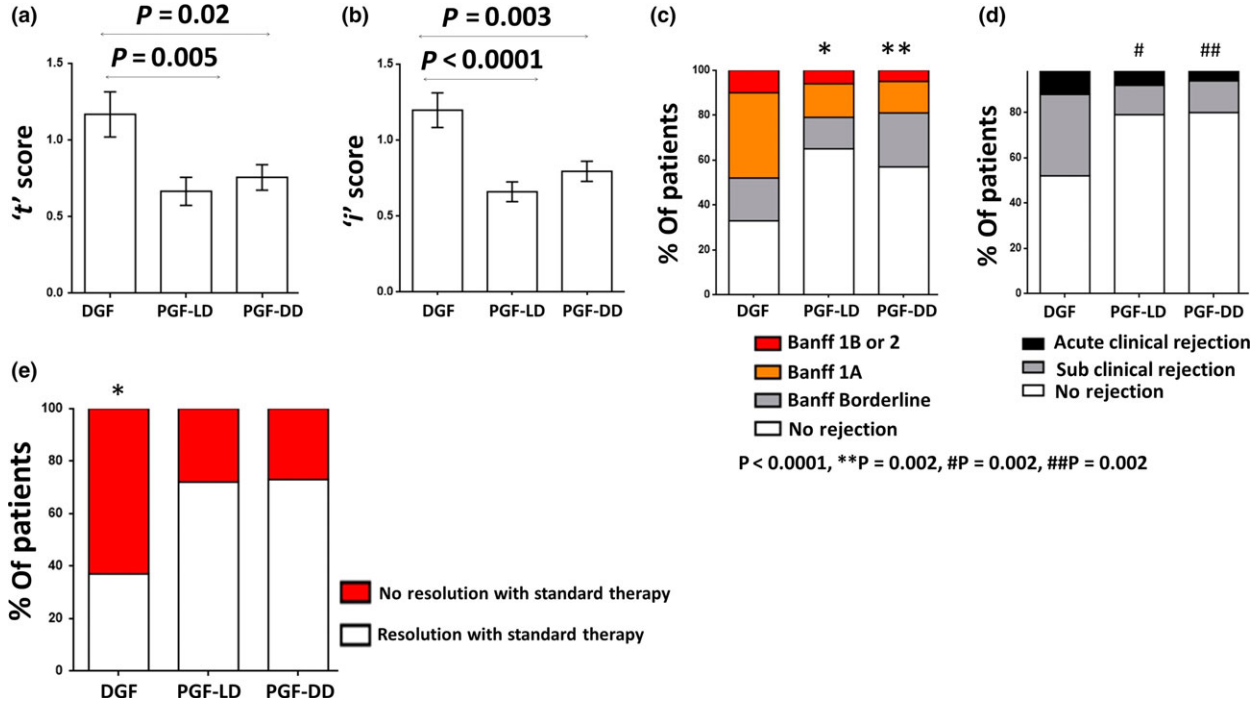

Figure 2 Tubulointerstitial inflammation- delayed graft function (DGF) versus primary graft function (PGF). Banff tubulointerstitial inflammation scores ('t' and 'i') were compared between the DGF and the two PGF groups (live donor recipients (LD) and deceased donor allograft recipients (DD)) at 3 months post-transplant. DGF was associated with a significantly elevated 't' and 'i' Banff scores at 3 months (a, b) when compared to either PGF-LD or PGF-DD groups. (c). DGF was associated with a significantly greater incidence of ≥Banff 1A acute rejection at 3 months (DGF versus DGF-LD, $P < 0.0001$ DGF versus PGF-DD, $P = 0.002$). DGF was associated with significantly greater incidence of subclinical rejection when compared to the two PGF groups, while the incidence of acute clinical rejection was comparable between the three patient groups at 3 months (d, SCR 3 months: DGF versus PGF-LD, $P = 0.002$; DGF versus PGF-DD, $P = 0.002$). The error bars in the bar charts represent SEM. (e) compares the incidence of late ≥Banff 1A rejection at 12 months in patients who were treated for ≥Banff 1A rejection episodes at 3 months in the DGF or the two PGF groups (DGF versus PGF-LD, $P = 0.04$; DGF versus PGF-DD, $P = 0.04$). The error bars in the bar charts represent SEM.

(Fig. 3b and d). Importantly, the group with DGF and TCMR (DGF/TCMR+) had the highest IFTA scores and IF + 'i' at 12 months (Fig. 3e and f). In this regard, DGF/TCMR− group had IFTA and IF + 'i' scores significantly different than the DGF/TCMR+ group and importantly, comparable to the PGF group Thus, DGF/TCMR+ group is associated with significantly more chronic allograft histological changes.

DGF, TCMR, and graft outcomes

DGF group had significantly higher serum creatinine values at 12 and 24 months (Fig. S3A and B) when compared to both the PGF groups. DGF/TCMR+ group had the worst serum creatinine at 12 and 24 months (Fig. S3C and D) when compared to other groups. DGF was also associated with significantly greater overall graft loss during follow-up (Fig. S4A) and both PGF-DD and PGF-LD groups had similar and higher graft survival rates than the DGF group (Fig. S4C). When composite end-point of allograft loss and impending allograft loss was analyzed patients with DGF had significantly greater incidence of impending graft loss when compared to the two PGF groups (Fig. S4B and D).

Since, DGF/TCMR+ group was associated with significantly worse allograft chronic changes and serum creatinine, we next analyzed the influence of the concomitant diagnosis of TCMR in patients with DGF on graft outcomes. As shown in Fig. 4, patients with DGF and TCMR (DGF/TCMR+ group) had significantly worse overall graft loss (Fig. 4a) and impending graft loss (Fig. 4b). Again, graft outcomes in patients with DGF in the absence of rejection (DGF/TCMR− group) were comparable to those without DGF.

Multivariate analyses

We assessed for the independent association of DGF and TCMR with graft outcomes in three multivariate

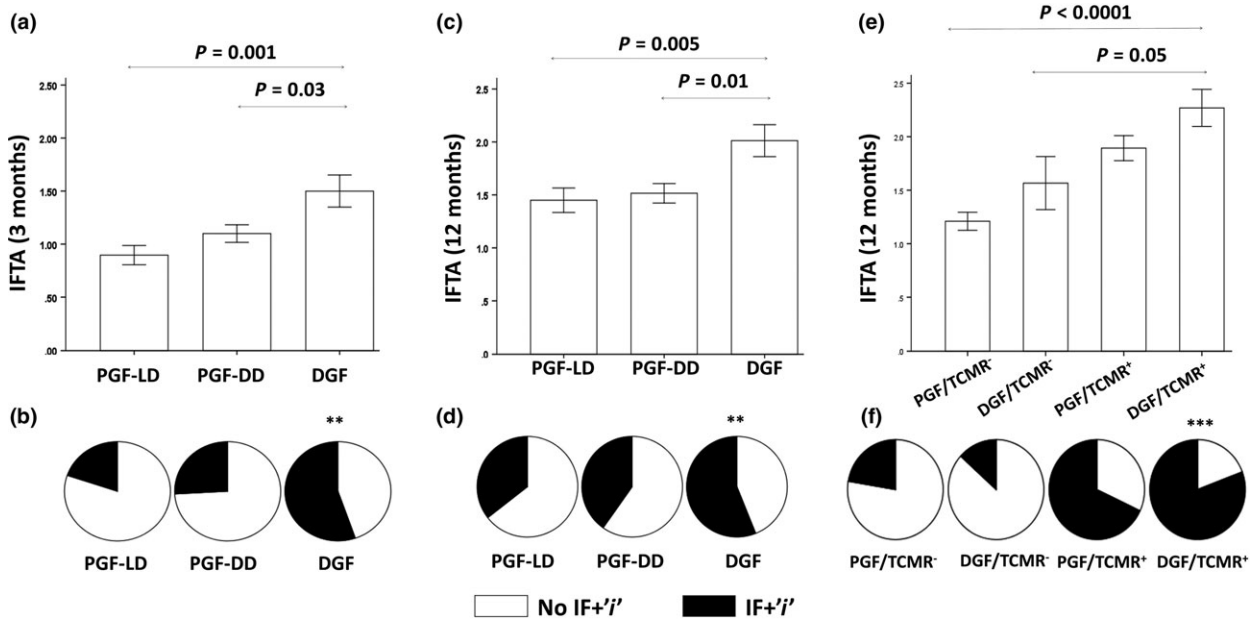

Figure 3 The association of delayed graft function (DGF) with allograft chronic histological changes. IFTA defined as a cumulative score of Banff 'ct' and 'ci' scores along with If + 'i' (Banff 'ci' score ≥ 1 & Banff 'i' score ≥ 1) were compared between the DGF and primary graft function (PGF) groups. DGF was associated with significantly increased 'IFTA" when compared to either PGF-LD or PGF-DD groups at both 3 months (a, DGF versus PGF-LD, $P = 0.001$; DGF versus PGF-DD, $P = 0.03$) and 12 months (c, DGF versus PGF-LD, $P = 0.005$; DGF versus PGF-DD, $P = 0.01$). Furthermore, a greater proportion of patients with DGF had IF + 'i' when compared to PGF-LD or PGF-DD groups at both 3 months (b, DGF versus PGF-LD, $P = 0.0001$; DGF versus PGF-DD, $P = 0.0003$) and 12 months (d, DGF versus PGF-LD, $P = 0.03$; DGF versus PGF-DD, $P = 0.09$). Finally, DGF/T cell mediated Rejection (TCMR)+ was associated with significantly increased 'IFTA" when compared to either PGF/Rej− or DGF/TCMR− groups (e, DGF/TCMR+ versus PGF/Rej−, $P < 0.0001$; DGF/TCMR+ versus PGF/TCMR+, P = NS; DGF/TCMR+ versus DGF/TCMR−, $P = 0.05$) and they had more IF + 'i' at 12 months (f, DGF/TCMR+ versus PGF/TCMR−, $P < 0.0001$; DGF/TCMR+ versus PGF/TCMR+, $P = 0.18$; DGF/TCMR+ versus DGF/TCMR−, $P = 0.0002$). Although there was a trend toward a greater IFTA score and IF+'i' in DGF/TCMR+ when compared to PGF/TCMR+, these results did not reach statistical significance.

Cox Proportional Hazards models (Table 2). Model 1 showed that DGF/TCMR+ was associated with a significantly increased hazard of impending graft loss (composite end-point) independent of prior sensitization, HLA mismatches, ethnicity and DSA detection. Model 2 also showed DGF/TCMR+ as an independent risk factor when recipient age, donor age, KDPI score and donor type (live versus DBD versus DCD) were included in the model and Model-3 even when post-transplant renal function measured at 3 months was included. Thus, per all the three multivariate models DGF/TCMR+ was associated with the heightened risk of the composite end-point of graft loss and impending graft loss. Thus, DGF/TCMR+ (TCMR) was associated with poor graft outcomes.

Risk factors associated with DGF/TCMR+/−

Since DGF/TCMR+ was associated with poor transplant outcomes, risk variables for its development within the first post-transplant year were therefore examined. It is possible that the differences noted in the rejection rates in patients with DGF could be attributed to changes in immunosuppression levels. We therefore compared tacrolimus trough levels along with mycophenolate and corticosteroid doses n the first 90 days of the transplant when early biopsies to assess inflammation were performed. Mycophenolate dose and prednisolone usage were comparable across patient groups stratified by DGF and TCMR (Fig. S5A–D). Patients with DGF had a significantly lower tacrolimus trough level on day 14 (at DGF biopsy, Fig. S5E) although, the trough levels were similar at day #7, 30, and 90 post-transplant. In addition, Tac levels were similar within the DGF groups with and without TCMR (Fig. S5F). Of the remaining variables examined by univariate analysis, Post-transplant DSA, CIT, KDPI score > 20% and DCD donor source were identified as significant risk variables among patients with DGF/TCMR+ (Table 3). In the multivariate model, post-transplant DSA was the only factor independently associated with the development of DGF/TCMR+ in this study cohort (Table 3).

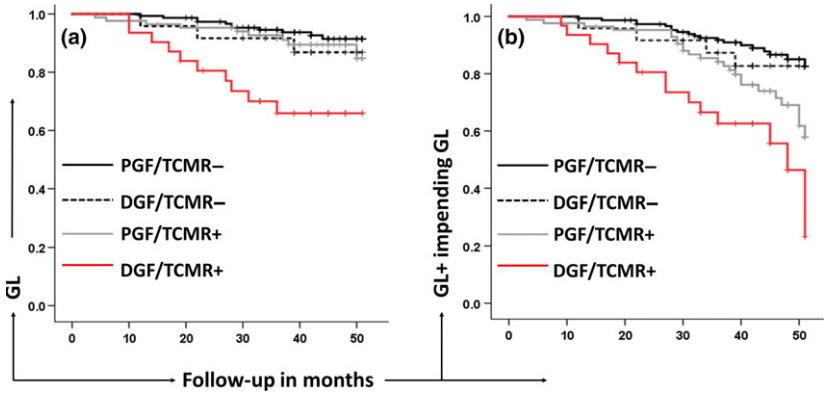

Figure 4 Delayed graft function (DGF)/T cell mediated Rejection (TCMR)+ and graft outcomes. Overall graft survival including death and a composite of graft loss and impending graft loss (eGFR < 30 ml/min & >30% decline from baseline) were compared between DGF and primary graft function (PGF) groups stratified by the presence or absence of acute rejection within the 1st post-transplant year. DGF/TCMR+ (n = 32) was associated with significantly worse overall graft survival (a, DGF/TCMR+ versus PGF/TCMR−, $P < 0.0001$; DGF/TCMR+ versus PGF/TCMR+, $P = 0.002$; DGF/TCMR+ versus DGF/TCMR−, $P = 0.07$), and increased incidence of the composite of graft loss and impending graft loss (b, DGF/TCMR+ versus PGF/TCMR−, $P < 0.0001$; DGF/TCMR+ versus PGF/TCMR+, $P = 0.02$; DGF/TCMR+ versus DGF/TCMR−, $P = 0.03$) when compared to either the PGF/TCMR− (n = 152), PGF/TCMR+ (n = 86) or DGF/TCMR− (n = 24) groups. Actuarial survival analysis was by the Kaplan Meier method and curves compared by Log-Rank test.

Thus, renal transplant recipients with DGF are a high-risk group of patients who have early and persistent tubulointerstitial inflammation, which progresses to early allograft chronicity, dysfunction and inferior graft survival. TCMR risk stratifies patients with DGF by identifying a subset of patients at risk for early chronic damage and poor graft outcomes.

Discussion

Early post-transplant events like DGF and acute rejection are detrimental to the long-term kidney transplant outcome [16]. DGF, which represents AKI in the early post-transplant period continues to pose a challenge and is an impediment to the long-term graft survival [17]. The new organ allocation rules have further increased the incidence of DGF in recent years (7). Although it is very well-established that DGF is associated with poor long-term clinical outcomes, the early chronology of events that are associated with DGF and lead to these poor outcomes are less well studied [1,2,11,18,19]. Furthermore, the influence of DGF on graft outcomes in the modern era of immunosuppression is less well-studied. In this prospective single center study, we present a systematic sequential analysis of progressive acute and chronic histological changes in patients with DGF in comparison to those with PGF.

DGF is a manifestation of ischemia reperfusion injury of the allograft with a resultant increase in the immunogenicity of the organ and thus a predilection for further alloimmune mediated graft injury [20]. Thus, several studies have shown a relationship between DGF and concomitant diagnosis of acute rejection [9,21,22]. Availability of serial biopsies (protocol at 3 and 12 months and any for-cause) allowed us to explore the temporal relationship between DGF and evolution of allograft tubulo-interstitial inflammation and chronic damage. We observed that increased tubulo-interstitial inflammation and acute rejection were predominantly seen not during DGF but at 3 months post-transplant. Furthermore, the increased incidence of rejection noted in these patients was mainly attributed to subclinical rejection diagnosed on a protocol biopsy. In this regard Jain *et al.*, in a small single center study showed a similar increase in the incidence of subclinical rejection within the first 90 days of transplantation [23]. It is important to note that most of these rejections would not have been diagnosed without the use of protocol biopsies. Further it was shown that timely intervention in patients with subclinical rejection could improve graft function. [24].

In contradiction to some previous reports that DGF was not associated with progressive fibrosis, we noted that DGF was associated with adverse chronic allograft changes including IFTA and IF + 'i' that progress through the first year [25]Of these chronicity indices, IF + 'i' was particularly shown to be a surrogate marker for poor outcomes [26]. Importantly, these chronic histological features were much worse in patients with DGF/TCMR+ versus other groups with a resultant poor allograft function and poor graft outcome at 4 years. Thus, we observed that minimal tubulo-interstitial

Table 2. DGF/TCMR+ and Composite end-point (graft loss +impending graft loss)

Variable	Hazard ratio	95% CI	*P* value
	Univariate analysis		
DGF	2.3	1.33–4.03	0.003
DGF and Rejection (TCMR)			
PGF/TCMR−	Ref	Ref	Ref
DGF/TCMR−	1.6	0.5–4.7	0.4
PGF/TCMR+	2.4	1.3–4.4	0.005
DGF/TCMR+	5.2	2.6–10.4	0.000003
	Multivariate Analysis		
Model-1: adjusted for ethnicity, re-transplantation, HLA m/m, DSA			
PGF/TCMR−	Ref	Ref	Ref
DGF/TCMR−	1.5	0.5–4.5	0.5
PGF/TCMR+	2.2	1.2–4.1	0.01
DGF/TCMR+	4.9	2.4–10.0	0.00001
Model-2: adjusted for recipient age, donor age, graft type, and Kidney Donor Profile index			
PGF/TCMR−	Ref	Ref	Ref
DGF/TCMR−	1.3	0.3–6.3	0.7
PGF/TCMR+	2.6	1.1–6.3	0.03
DGF/TCMR+	6.6	2.3–18.7	0.0004
Model-3: adjusted for renal function (serum creatinine at 3 months)			
PGF/TCMR−	Ref	Ref	Ref
DGF/TCMR−	1.5	0.5–4.4	0.5
PGF/TCMR+	2.3	1.3–4.2	0.007
DGF/TCMR+	4.4	2.1–9.1	0.00009

PGF, primary graft function; DGF, delayed graft function; HLA MM: HLA mismatch; DSA, donor specific antibody; TCMR, T cell mediated rejection.

Table 3. Risk factors for DGF/TCMR+ (DGF with TCMR) after kidney transplantation

Variable	OR (95% CI) Univariate analysis	*P* value	OR (95% CI) Multivariate analysis	*P* value
Recipient age (per year)	1.03 (0.99–1.05)	0.07	1.0 (0.98–1.04)	NS
Recipient gender (M versus F)	1.3 (0.6–2.8)	NS		
Recipient ethnicity (Caucasian versus Non-Caucasian)	2.01 (0.9–4.6)	0.09	1.6 (0.6–3.9)	NS
Primary renal disease				
Hypertension	Ref			
Diabetes mellitus	1.8 (0.3–9.8)	NS		
Glomerulonephritis	2.2 (0.4–11.7)	NS		
Inherited	1.9 (0.3–12.7)	NS		
Others	1.3 (0.2–7.2)	NS		
Re-transplant	1.3 (0.6–3.3)	NS		
Donor age (per year)	0.99 (0.96–1.03)	NS		
Donor type				
LD versus DBD	4.9 (1.6–14.8)	0.005	5.8 (0.3–110)	NS
LD versus DCD	8.4 (2.4–29.1)	0.001	6.7 (0.2–154)	NS
Kidney Donor Profile index score > 20	3.6 (1.6–7.8)	0.002	2.5 (0.8–8.0)	0.1
CIT				
Lowest versus middle tertile	2.4 (0.7–7.9)	NS	0.2 (0.01–4.0)	NS
Lowest versus highest tertile	4.9 (1.6–16.1)	0.006	0.4 (0.02–7.2)	NS
HLA m/m	1.1 (0.9–1.3)	NS		
Post-transplant DSA	2.8 (1.3–6.1)	0.009	3.8 (1.6–9.4)	0.004

HLA MM: HLA mismatch; DSA, donor specific antibody; TCMR, T cell mediated rejection; CIT, cold ischemia time; LD, live donor; DBD, donation after brain death; DCD, donation after circulatory death; DGF, delayed graft function.

inflammation that is associated with the diagnosis of DGF at 2 weeks progresses to early subclinical rejection within 3 months that persists at 12 months. This inflammation is a harbinger for early allograft chronicity and late allograft loss. Thus, early acute rejection complicating DGF represents a high-risk group of patients for poor long-term outcomes. It is tempting to speculate that reducing allograft immunogenicity prior to implantation and early inflammation immediately postimplantation could serve as potential therapeutic targets. In fact, approaches to prevent chronic changes in allografts with ischemic injury and DGF are being explored in animal models [2,27,28]. Furthermore, a NIH funded multi-center randomized controlled trial is underway analyzing the impact of minimizing early inflammation with anti-TNF on outcomes including acute rejection and DGF [29].

We have identified that post-transplant DSA is an independent risk factor for the development of DGF/TCMR. Given the ongoing debate about the clinical utility of protocol biopsies and the potential financial constraints, increase in clinical work load and lack of infra-structure required to perform these biopsies; patients with DGF and those with *denovo* DSA could represent a group that might benefit from aggressive monitoring with protocol biopsies [30,31]. Such recipients could also be a select population for future clinical trials with novel immunomodulatory agents with an aim to improve long-term outcome.

A systematic longitudinal analysis and comparable immunosuppression regimens between the patient groups provide strength to our analysis. Furthermore, a regimented approach through serial histological examination with for-cause biopsies and protocol biopsies within the 1st post-transplant year enabled us to evaluate early sequential histological changes to graft function and survival. Additional detailed analysis of the cellular infiltrate in patients with DGF/TCMR+ could provide new mechanistic insights. Our study has some limitations. Subclinical rejection was noted in a fair proportion of our patients with DGF and it is difficult to determine whether the subsequent chronic changes are secondary to the initial insult that led to DGF or to the concomitant inflammation. Furthermore, given the high rate of sub-clinical rejection noted in our study, it could be argued that the conclusions drawn might not be applicable to lower risk patient cohorts. Therefore, these findings have to be validated in a larger cohort of patients with longer follow-up through a multi-center study. Additionally, information on time zero biopsies would be useful to validate progression from time zero as opposed to 2 weeks post-transplant in patients with DGF. However, KDPI values were similar among patients with DGF and those with PGF-DD. Our findings provide novel insights into the early histological changes that are associated with DGF and their progression within the 1st post-transplant year. The fact that chronic allograft histological changes are seen as early as 3 months post-transplant has important clinical implications since it provides opportunities for novel interventional strategies which can be implemented at an early time point post transplantation.

In conclusion, DGF is associated with early tubulo-interstitial inflammation that leads to chronic allograft damage and can adversely affect kidney transplant outcome. Thus, DGF with tubulo-interstitial inflammation represents a high-risk group of patients in whom prompt early identification could provide a valuable therapeutic opportunity to improve kidney transplant outcomes.

Authorship

AC: conceptualized, designed, and conducted the study. Completed all analysis and drafted the manuscript. RM and PS: collected data, helped in study design and drafting the manuscript. SH: conceptualized, designed, conducted, and completed writing the manuscript. SH is the corresponding author.

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Conflicts of interest

The authors have declared no conflicts of interest.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Study schema illustrates the total number of kidney transplant recipients, those excluded, study subjects with DGF and PGF and biopsies at 3 and 6–12 months post-transplant.

Figure S2. Relationship between DGF, DSA, TCMR & Antibody Mediated Rejection (AMR).

Figure S3. The relationship between DGF and renal function.

Figure S4. DGF and graft outcomes.

Figure S5. Relationship between DGF, TCMR and immunosuppression.

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