Delayed graft function is correlated with graft loss in recipients of expanded-criteria rather than standard-criteria donor kidneys: a retrospective, multicenter, observation cohort study

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Abstract

Background: Although the use of expanded-criteria donors (ECDs) alleviates the problem of organ shortage, it significantly increases the incidence of delayed graft function (DGF). DGF is a common complication after kidney transplantation; however, the effect of DGF on graft loss is uncertain based on the published literature. Hence, the aim of this study was to determine the relationship between DGF and allograft survival.

Methods: We conducted a retrospective, multicenter, observation cohort study. A total of 284 deceased donors and 541 recipients between February 2012 and March 2017 were included. We used logistic regression analysis to verify the association between clinical parameters and DGF, and Cox proportional hazards models were applied to quantify the hazard ratios of DGF for kidney graft loss.

Results: Among the 284 deceased donors, 65 (22.8%) donors were ECD. Of the 541 recipients, 107 (19.8%) recipients developed DGF, and this rate was higher with ECD kidneys than with standard-criteria donor (SCD) kidneys (29.2% *vs.* 17.1%; *P* = 0.003). The 5-year graft survival rate was not significantly different between SCD kidney recipients with and without DGF (95.8% *vs.* 95.4%; *P* = 0.580). However, there was a significant difference between ECD kidney recipients with and without DGF (71.4% *vs.* 97.6%; *P* = 0.001), and the adjusted hazard ratio (HR) for graft loss for recipients with DGF was 1.885 (95% confidence interval [CI] = 1.305–7.630; *P* = 0.024). Results showed that induction therapy with anti-thymocyte globulin was protective against DGF (odds ratio = 0.359; 95% CI = 0.197–0.652; *P* = 0.001) with all donor kidneys and a protective factor for graft survival (HR = 0.308; 95% CI = 0.130–0.728; *P* = 0.007) with ECD kidneys.

Conclusion: DGF is an independent risk factor for graft survival in recipients with ECD kidneys, but not SCD kidneys.

Keywords: Chronic kidney disease; Delayed graft function; Expanded-criteria donors; Graft survival; Standard-criteria donors

Introduction

Kidney transplantation is the most cost-effective therapy for end-stage renal disease (ESRD).[1] However, the number of new registrations continues to grow more rapidly than the number of transplants performed, and there is no indication that these trends will change in the near future.[2] Due to limited supplies and increased demand, organs from expanded-criteria donors (ECD) are used to expand the pools of cadaver kidney donors.[3] Moreover, the aging general population has resulted in a constant and dramatic increase in ECDs. In the future,

ECD transplantation could be the main source for kidney and other solid-organ transplants.[4]

Although the use of ECD kidneys has alleviated the problem of organ shortage, it is associated with dramatic increase in delayed graft function (DGF) risk, which occurs in more than 50% of ECD transplants,[5-8] compared with 2% to 50% of standard- criteria donor kidney transplants. Numerous studies have reported the deleterious effects of DGF on graft survival.[9-12] A paired kidney registry

Access this article online

Quick Response Code:

Website:
www.cmj.org

DOI:
10.1097/CM9.0000000000000666

analysis showed that recipients with DGF experienced increased overall graft loss compared with individuals without DGF (14% *vs.* 4%).[11] Further, a recent systematic review involving 151,194 kidney transplant recipients demonstrated that the pooled relative risk for graft loss in recipients with DGF was 1.41 compared to that in individuals without DGF.[12] In contrast, Boom *et al*[13] revealed that DGF affects renal function but not graft survival, and other studies found that DGF has no effect on outcome.[8,14-20] Thus, the exact contribution of DGF to kidney graft loss remains controversial. Despite the potential for ECD, many of these harvested organs are ultimately refused or discarded by transplant teams.[21] To optimize the use of kidneys from ECDs, the association between DGF and graft survival must be understood.

The objectives of this study were to examine the association between DGF and graft survival after kidney transplantation and to identify the protective factors for graft survival to ensure evidence-based kidney allocation and that kidneys within transplant centers are used effectively, which will ultimately prolong transplant survival.

Methods

Ethical approval

The study was approved by the Human Organ Transplantation and Ethics Committee of Sun Yat-sen University and in accordance to the *Declaration of Helsinki*. Written informed consent was obtained from the participants for the publication of their individual details and accompanying images in this manuscript.

Study design

This retrospective, multicenter, observation cohort study included 541 kidney transplants from February 2012 to March 2017 (with follow-up until March 2018). This study was performed in three kidney transplant institutions, namely the Third Affiliated Hospital of Sun Yat-sen University, the Third Affiliated Hospital of Guangzhou Medical University, and the First Affiliated Hospital of Jilin University. We obtained donor data from the China Organ Transplant Response System and reviewed organ procurement organization charts to obtain additional donor information that was not available through the China Organ Transplant Response System. All recipient data were obtained from patient medical records.

The main outcome after transplantation was DGF, which was defined as the requirement for dialysis during the first week after transplantation. Graft loss was defined as a requirement for dialysis after kidney transplantation, excluding patients who had a functioning graft but died of other causes. ECD included all deceased donors ≥60 years and donors >50 years with at least two of the following conditions: history of hypertension, serum creatinine >1.5 mg/dL, and cerebrovascular cause of death.[22,23] Urine protein classification standards are <0.2 g/L: 0; urine protein is 0.2 to 1.0 g/L: 1+; 1.0 to 2.0 g/L: 2+; ≥2 g/L: 3+.

All organs were procured from donors in accordance with the *Declaration of Helsinki* and the *Declaration of Istanbul on Organ Trafficking and Transplant Tourism* and approved by the Human Organ Transplantation and Ethic Committee of each institution. Donation after cardiac death (DCD) was legally defined as irreversible cessation of circulatory function.[24] Organ allocation was according to the China Organ Transplant Response System. All data were searchable. The perfusion solution was stored in a refrigerator at 4°C. After the organ was obtained, the first perfusion was performed on the operating table with a hypertonic citrate purine solution, and all the blood in the organ was rinsed. The organs were stored in an ice cube filled with ice; the kidneys were kept in ice cubes until the blood vessels were opened.

Statistical analysis

Continuous variables are reported as the mean ± standard deviation or as medians (interquartile ranges), and categorical variables are reported as frequencies (%). Chi-squared tests or Fisher exact tests were used to assess between-group differences in categorical variables, and a Student's *t* test was used to assess between-group differences in continuous variables. The Mann-Whitney *U* test was used to assess between-group differences for non-normally distributed variables.

Kidney allograft survival according to ECD and DGF status was plotted using Kaplan-Meier curves and compared using the log-rank test. Univariate analysis and multivariate logistic regression analysis were used to verify the association between clinical parameters and DGF. Cox proportional hazards models were applied to quantify the hazard ratios (HRs) and the 95% confidence intervals (CIs) for kidney graft loss. The multivariate Cox model was obtained by entering risk factors from the univariate model that met the threshold of $P \leq 0.10$ in a single multivariate proportional hazards model.

All statistical analyses were performed with SPSS version 20.0 (IBM Corp., Armonk, NY, USA), and $P < 0.05$ was considered statistically significant.

Results

Characteristics of donors and recipients

A total of 284 deceased donors and 541 recipients were included. The mean age of the donors was 40.7 years. In total, 65 (22.8%) donors were ECD, and the mean recipient age was 55.8 years [Table 1]; 107 (19.8%) recipients developed DGF. The average follow-up time was 36.7 months, with the shortest being ten months and the longest being 77 months. 31 people died, 54 were lost to follow-up.

Four distinct populations were identified based on donor characteristics (standard-criteria donor [SCD] or ECD) and recipient status on day 7 post-transplantation (immediate graft function [IGF] or DGF) as follows: patients receiving SCD transplants with IGF (SCD + IGF, *n* = 349); patients receiving SCD transplants with DGF

Table 1: Donor and recipients characteristics of kidney transplants, stratified by donor type.

Characteristics	All (n = 541)	SCD (n = 421)	ECD (n = 120)	Statistics	P
Donor characteristic					
Age (years)	43 ± 13	40 ± 9	55 ± 4	−17.615*	<0.001
Female	84 (16)	64 (15)	20 (17)	0.153†	0.671
BMI (kg/m²)	23 [21, 24]	23 [21, 24]	23 [22, 25]	−2.340‡	0.19
Cause of death				0.144†	0.930
Cerebrovascular accident	174 (32)	137 (33)	37 (31)		
Head trauma	320 (59)	248 (59)	72 (60)		
Others	47 (9)	36 (8)	11 (9)		
Terminal serum creatinine (μmol/L)	143 [106, 185]	143 [107, 185]	143 [104, 189]	−0.028‡	0.978
Donor proteinuria level				6.509†	0.089
0	294 (54)	240 (57)	54 (45)		
1+	146 (27)	110 (26)	36 (30)		
2+	57 (11)	41 (10)	16 (13)		
3+	44 (8)	30 (7)	14 (12)		
Warm ischemia time				20.622†	0.001
≤18 min	383 (71)	318 (76)	65 (54)		
>18 min	158 (29)	103 (24)	55 (46)		
Recipient characteristic					
Age (years)	42.9 ± 11.2	42.2 ± 11.1	45.1 ± 11.2	−2.439*	0.855
Male	346 (64)	267 (63)	79 (66)	0.236†	0.627
BMI (kg/m²)	22 [21, 25]	22 [21, 25]	22 [21, 25]	−0.628‡	0.569
Cause of ESRD				1.127†	0.890
Hypertension	112 (20)	86 (20)	26 (22)		
Diabetes	131 (24)	106 (25)	25 (21)		
GN	187 (35)	145 (35)	42 (35)		
PKD	59 (42)	45 (11)	14 (12)		
Others	52 (9)	39 (9)	13 (10)		
Mode of dialysis				0.623†	0.430
HD	466 (86)	360 (85)	106 (88)		
PD	75 (14)	61 (15)	14 (12)		
Dialysis duration (months)	12 [7, 24]	12 [7, 24]	12 [8, 24]	−0.547‡	0.584
Cold ischemia time (h)	4 [2, 4]	4 [2, 6]	5 [2, 6]	−0.575‡	0.565
Number of HLA mismatches				0.586†	0.746
Level 1	424 (78)	327 (78)	97 (81)		
Level 2	64 (12)	51 (12)	13 (11)		
Level 3	53 (10)	43 (10)	10 (8)		
Panel active antibody				0.125†	0.724
Positive	54 (10)	41 (9)	13 (11)		
Negative	487 (90)	380 (91)	107 (89)		
Induction regimen				0.069†	0.792
ATG	424 (78)	331 (79)	93 (77)		
Basiliximab	117 (22)	90 (21)	27 (23)		
CNI				0.377†	0.539
Cyclosporin	161 (30)	128 (31)	33 (27)		
Tacrolimus	380 (70)	293 (69)	87 (73)		
Rejection				0.311†	0.577
Antibody mediated rejection	32 (6)	24 (6)	8 (7)		
T cell mediated rejection	28 (5)	21 (5)	7 (6)		
Infection				0.449†	0.503
Urinary tract infection	40 (7)	30 (7)	10 (8)		
Pneumonia	57 (11)	43 (10)	14 (12)		
Proteinuria rate 5-year post-transplantation	63 (12)	40 (9)	23 (19)	8.479†	0.004
Renal functions 5-year post-transplantation (Serum creatinine, μmol/L)	110 [91, 140]	107 [90, 134]	120 [98, 159]	−3.550‡	<0.001

Continuous variables according to the Shapiro test, if P > 0.05 the data are expressed as mean ± SD, otherwise data are expressed as median [P25, P75]; Categorical variables are described by numbers and percentages (%). Continuous variables were compared using Student's t test or Mann-Whitney U test, categorical variables were compared using the χ² test and Fisher exact test. *t values; †χ² values; ‡Z values. DGF: Delayed graft function; BMI: Body mass index; ESRD: End-stage renal disease; GN: Glomerulonephritis; PKD: Polycystic kidney disease; HD: Hemodialysis; PD: Peritoneal dialysis; HLA: Human leukocyte antigen; CNI: Calcineurin inhibitor; ATG: Anti-thymocyte globulin; SD: Standard deviation.

Table 2: Univariate logistic regression analyses for the parameters of delayed graft function.

	All recipients			SCD			ECD		
Items	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Male donor	1.057	0.533–2.095	0.873	1.412	0.600–3.321	0.429	0.545	0.156–1.907	0.342
Donor BMI	1.012	0.943–1.086	0.747	1.043	0.964–1.128	0.299	0.855	0.722–1.012	0.169
Donor cause of death	0.865	0.749–0.999	0.148	0.938	0.790–1.113	0.462	0.765	0.572–1.024	0.272
Donor proteinuria level (3+)	1.281	1.102–1.462	0.076	1.315	1.005–1.536	0.045	1.448	1.125–2.113	0.055
Terminal serum creatinine	1.005	1.003–1.008	0.001	1.005	1.003–1.008	0.001	1.006	1.002–1.010	0.006
Warm ischemia time (>18 min)	3.356	2.165–5.203	0.001	2.464	1.442–4.210	0.001	5.579	2.312–13.459	0.001
Cold ischemia time	1.041	0.997–1.087	0.066	0.944	0.850–1.049	0.125	1.082	0.965–1.213	0.176
Induction therapy (ATG)	0.580	0.357–0.944	0.028	0.466	0.267–0.815	0.007	0.953	0.334–0.922	0.042
PRA (positive or negative)	0.972	0.388–2.432	0.951	1.058	0.388–2.882	0.913	1.221	0.107–13.912	0.872
HLA mismatches level	0.853	0.605–1.204	0.366	0.980	0.667–1.442	0.920	0.563	0.256–1.235	0.149
Recipient age	0.990	0.972–1.009	0.323	0.982	0.960–1.005	0.127	0.999	0.965–1.035	0.961
Male recipient	1.083	0.695–1.688	0.725	1.025	0.604–1.737	0.928	1.190	0.513–2.759	0.685
Mode of dialysis (HD)	0.585	0.290–1.181	0.135	0.486	0.201–1.176	0.110	0.968	0.284–3.320	0.958
Duration of dialysis before transplantation	1.004	0.992–1.016	0.534	1.001	0.987–1.015	0.871	1.015	0.989–1.041	0.273
Donor type (ECD)	1.996	1.250–3.188	0.004						

OR: Odds ratio; CI: Confidence interval; BMI: Body mass index; SCr: Serum creatinine; SCD: Standard criteria donor; ECD: Expanded criteria donor; ATG: Anti-thymocyte globulin; HLA: Human leukocyte antigen; PRA: Panel active antibody; HD: Hemodialysis.

Table 3: Multivariate logistic regression analyses for the parameters of delayed graft function.

	All recipients			SCD			ECD		
Items	OR	95% CI	P	OR	95%CI	P	OR	95%CI	P
Donor proteinuria level (3+)	1.665	1.498–4.707	0.001	1.965	1.327–6.626	0.008			
Terminal serum creatinine	1.006	1.002–1.011	0.005	1.006	1.001–1.010	0.013			
Warm ischemia time (>18 min)	1.562	1.275–6.427	0.001	1.284	1.006–9.150	0.001	1.721	1.363–6.839	0.045
Induction therapy (ATG)	0.359	0.197–0.652	0.001	0.363	0.189–0.699	0.002	0.125	0.018–0.840	0.032

Multivariate logistic regression analysis was performed with a backward selection procedure. SCD: Standard criteria donor; ECD: Expanded criteria donor; OR: Odds ratio; CI: Confidence interval; ATG: Anti-thymocyte globulin.

(SCD + DGF, $n = 72$); patients receiving ECD transplants with IGF (ECD + IGF, $n = 85$); patients receiving ECD transplants with DGF (ECD + DGF, $n = 35$). The characteristics of the kidney recipients and their donors among the four groups are presented in Supplementary Table 1, http://links.lww.com/CM9/A169. We also stratified patients with induction therapy, and the characteristics of kidney recipients are shown in Supplementary Table 2, http://links.lww.com/CM9/A170.

### Association between clinical parameters and DGF

Univariate analysis of the parameters analyzed for their association with DGF is shown in Table 2, whereas multivariate logistic regression analysis is summarized in Table 3. For all recipients, induction therapy with anti-thymocyte globulin (ATG) (odds ratio [OR] = 0.359; 95% CI = 0.197–0.652; $P = 0.001$) was a protective factor against DGF. However, donor proteinuria level (3+) (OR = 1.665; 95% CI = 1.498–4.707; $P = 0.001$), donor terminal serum creatinine (OR = 1.006; 95% CI = 1.002–1.011; $P = 0.005$), and warm ischemia time (WIT) (OR = 1.562; 95% CI = 1.275–6.427; $P = 0.001$) were risk factors for DGF.

For patients receiving SCD transplants, induction therapy with ATG (OR = 0.363; 95% CI = 0.189–0.699; $P = 0.002$) was protective against DGF; donor proteinuria level (3+) (OR = 1.965; 95% CI = 1.327–6.626; $P = 0.008$), donor terminal serum creatinine (OR = 1.006; 95% CI = 1.001–1.010; $P = 0.013$), and WIT (OR = 1.284; 95% CI = 1.006–9.150; $P = 0.001$) were risk factors for DGF.

For patients receiving ECD transplants, induction therapy with ATG (OR = 0.125; 95% CI = 0.018–0.840; $P = 0.032$) was a protective factor against DGF, whereas WIT (OR = 1.721; 95% CI = 1.363–6.839; $P = 0.045$) was a risk factor for DGF.

DGF is associated with graft loss in ECD, but not SCD recipients

The rates of DGF for ECD and SCD groups were 29.2% and 17.1%, and graft loss rates were 10.0% and 4.5%, respectively. Figure 1A depicts the kidney allograft survival rate 5 years post-transplantation. After dividing the patients into groups based on donor characteristics (SCD or ECD) and recipient status (IGF or DGF), ECD
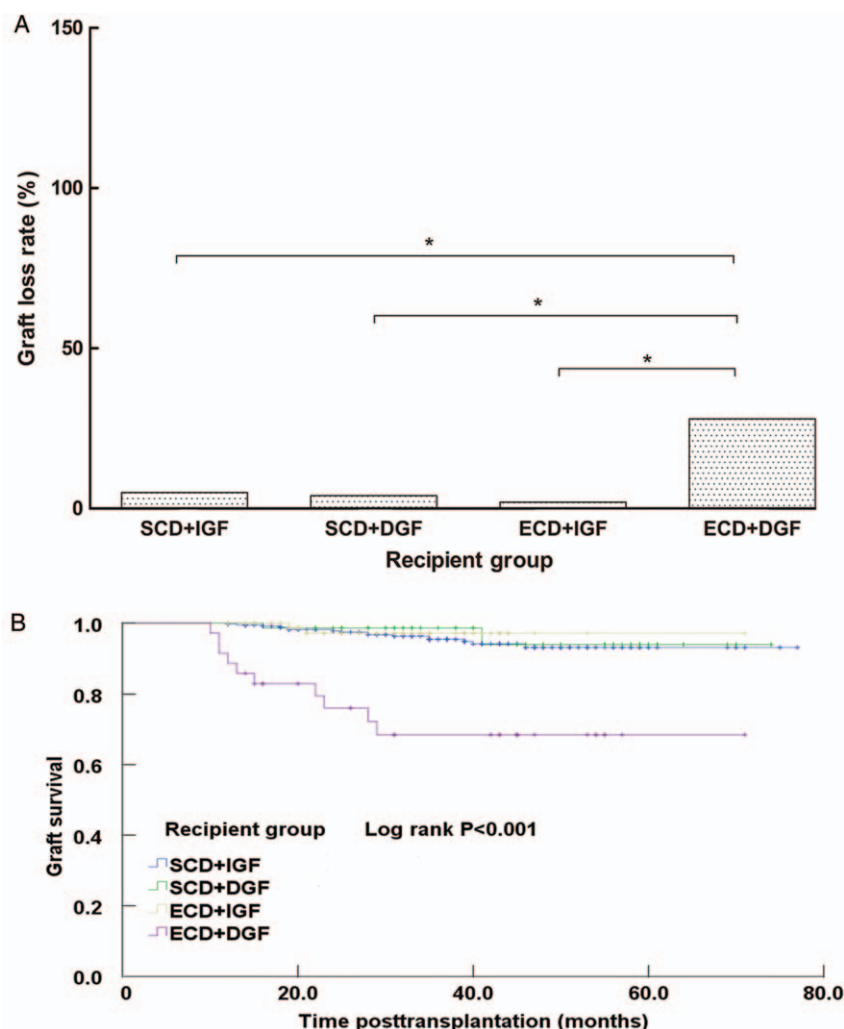
Figure 1: Kaplan-Meier estimates of graft survival after renal transplantation in ECD and SCD kidney recipients. (A) Kidney allograft survival rate at 5 years post-transplantation (SCD + IGF, n = 349; SCD + DGF, n = 72; ECD + IGF, n = 85; ECD + DGF, n = 35). (B) Kaplan-Meier curves of kidney allograft survival by donor type (SCD + ECD) and recipient status on day 7 post-transplantation (IGF + DGF). *P < 0.05. DGF: Delayed graft function; ECD: Expanded-criteria donors; IGF: Immediate graft function; SCD: Standard-criteria donor.

+ DGF recipients exhibited higher graft loss rate (28.6%) than ECD + IGF (4.6%), SCD + IGF (4.2%), and SCD + DGF (4.6%) recipients. Comparing recipients of SCD kidneys with and without DGF, the 5-year graft survival rate was not significantly different (95.8% *vs.* 95.4%; P = 0.580). However, for ECD recipients, comparing those with and without DGF, the 5-year graft survival rate was significantly different (71.4% *vs.* 97.6%; P = 0.001). Figure 1B shows the Kaplan-Meier curves of kidney allograft survival by donor type (SCD + ECD) and recipient status at day 7 post-transplantation (IGF + DGF) (log-rank test, P < 0.001).

Table 4 shows the associations among donor and recipient characteristics, transplant characteristics, and immunological parameters associated with graft loss, after dividing patients into three groups (all recipients, patients receiving SCD transplants, patients receiving ECD transplants). Table 5 shows the identified baseline independent predictors of graft loss.

Based on Cox analysis of all recipients, the major determinant independently associated with graft failure was WIT >18 min (HR = 1.336; 95% CI = 1.005–5.428; P = 0.049); induction therapy with ATG was an independent protective factor for graft survival (HR = 0.308; 95% = 0.130–0.728; P = 0.007), and this was adjusted for WIT, cold ischemia time, induction therapy, and recipient status (DGF).

When we performed Cox analysis for patients receiving SCD transplants, induction therapy with ATG was a protective factor independently associated with graft survival (HR = 0.351; 95% CI = 0.109–0.930; P = 0.049); WIT (>18 min) was a risk factor for graft survival (HR = 1.941; 95% CI = 1.625–6.909; P = 0.033), and this was adjusted for donor proteinuria level (3+), WIT (>18 min), induction therapy (ATG), and human leukocyte antigen mismatch level.

When we performed Cox analysis for patients receiving ECD transplants, induction therapy with ATG was still a protective factor for graft survival (HR = 0.162; 95% CI = 0.026–0.952; P = 0.012). However, DGF was the major determinant independently associated with graft failure (HR = 1.885; 95% CI = 1.305–7.630; P = 0.024),

Table 4: Cox proportional hazards regression model for the predictors of graft survival in univariate analyses.

Items	All recipients			SCD			ECD		
	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P
Male donor	0.918	0.303–2.783	0.879	1.366	0.302–6.171	0.685	0.474	0.081–2.761	0.406
Donor BMI	1.085	0.978–1.204	0.122	1.120	1.001–1.253	0.168	0.936	0.733–1.195	0.595
Donor cause of death	0.941	0.737–1.202	0.627	1.095	0.796–1.506	0.578	0.795	0.504–1.254	0.324
Donor proteinuria level (3+)	0.941	0.737–1.202	0.627	0.380	0.152–0.952	0.059	1.090	0.617–1.925	0.766
Terminal serum creatinine	1.002	0.998–1.005	0.408	1.000	0.995–1.006	0.925	1.003	0.998–1.008	0.317
Warm ischemia time (>18min)	3.624	1.730–7.589	0.065	2.908	1.245–7.368	0.098	4.043	1.588–15.773	0.058
Cold ischemia time	1.090	1.007–1.180	0.032	1.040	0.866–1.249	0.677	1.099	0.969–1.246	0.143
Induction therapy (ATG)	0.377	0.177–0.802	0.011	0.446	0.170–1.168	0.099	0.226	0.063–0.805	0.022
PRA (positive or negative)	1.875	0.247–14.223	0.543	0.772	0.099–6.001	0.804	3.246	0.986–12.724	0.628
HLA mismatches level	1.302	0.795–2.133	0.294	1.721	0.983–3.013	0.057	0.605	0.170–2.151	0.438
Recipient age	0.998	0.966–1.031	0.885	1.021	0.980–1.065	0.317	0.949	0.899–1.003	0.162
Male recipient	2.002	0.846–4.735	0.114	1.262	0.470–3.392	0.644	6.471	0.805–52.003	0.79
Model of dialysis (HD)	1.534	0.607–3.874	0.365	2.912	1.062–7.981	0.138	0.989	0.745–2.541	0.258
Duration of dialysis before transplantation	0.996	0.973–1.019	0.716	0.998	0.971–1.026	0.888	0.989	0.944–1.037	0.657
Rejection	1.251	0.925–2.354	0.098	1.145	0.879 -3.487	0.125	1.125	0.958–4.288	0.091
Infection	0.974	0.852–2.365	0.421	0.945	0.889–2.589	0.358	0.987	0.854–3.254	0.541
Recipient status (DGF)	3.196	1.513–6.751	0.002	0.905	0.257–3.190	0.876	16.600	3.410–80.807	0.001
Donor type (ECD)	2.351	1.107–4.993	0.026						

SCD: Standard criteria donor; ECD: Expanded criteria donor; OR: Odds ratio; CI: Confidence interval; BMI: Body mass index; ATG: Anti-thymocyte globulin; DGF: Delayed graft function; HLA: Human leukocyte antigen; PRA: Panel active antibody; HD: Hemodialysis.

Table 5: Cox proportional hazards regression model for the parameters of graft survival in the multivariate analysis.

Items	All recipients			SCD			ECD		
	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P
Donor proteinuria level (3+)				0.989–1.010	0.899	1.019			
Warm ischemia time (>18 min)	1.336	1.005–5.428	0.049	1.941	1.625–6.909	0.033	1.662	1.132–3.883	0.013
Cold ischemia time	0.979	0.800–1.197	0.833						
Induction therapy (ATG)	0.308	0.130–0.728	0.007	0.351	0.109–0.930	0.049	0.162	0.026–0.952	0.012
HLA mismatches level				1.634	0.809–3.301	0.171			
Recipient statue (DGF)	1.511	0.546–4.179	0.427				1.885	1.305–7.630	0.024

The multivariate Cox model was obtained by entering risk factors from the univariate model that achieved $P \leq 0.10$ as the thresholds in a single multivariate proportional hazards model. For all recipients, it was adjusted for the following parameters: WIT, CIT, induction therapy, recipient statue (DGF). For patient receiving SCD transplants, it was adjusted for the following parameters: Donor proteinuria level (3+), Warm ischemia time (>18 min), induction therapy (ATG), HLA mismatches level. For patient receiving ECD transplants, it was adjusted for the following parameters: induction therapy (ATG), warm ischemia time (>18 min), recipient statue (DGF). SCD: Standard criteria donor; ECD: Expanded criteria donor; OR: Odds ratio; CI: Confidence interval; ATG: Anti-thymocyte globulin; DGF: Delayed graft function; HLA: Human leukocyte antigen.

in addition to WIT (>18 min) (HR = 1.662; 95% CI = 1.132–3.883; $P = 0.013$), and this was adjusted induction therapy (ATG), WIT (>18 min), and recipient statue (DGF).

Higher levels of donor proteinuria are associated with higher incidences of de novo proteinuria after renal transplantation and graft loss

According to clinical test results, proteinuria could be divided into four levels, specifically 0, 1+, 2+, and 3+. After dividing patients into groups based on donor characteristics (SCD or ECD) and recipient status (IGF or DGF), including SCD + IGF, SCD + DGF, ECD + IGF, and ECD + DGF, the percentages in each group based on donor proteinuria levels were as follows: level 0: 57%, 56%, 48%, and 37%, respectively; level 1+: 26%, 28%, 32%, and 36%, respectively; levels 2+: 10%, 8%, 12%, and 17%, respectively; level 3+: 7%, 8%, 8%, and 20% [Figure 2A]. Donors in the ECD + DGF group had an especially higher proportion of proteinuria of 3+ compared with that in other groups. Figure 2B and 2C shows the estimated glomerular filtration rates (eGFRs) and probability of proteinuria in each group during the 5-year follow-up. Patients in the ECD + DGF group showed a significant decrease in eGFR and a significant increase in the probability of proteinuria beginning at 36 months of follow-up, and the ECD + DGF group had an increased incidence of graft loss [Figure 1A].

ATG is a protective factor against DGF

We next stratified the recipients according to donor type (ECD or SCD) and induction regimen (ATG or basilix-
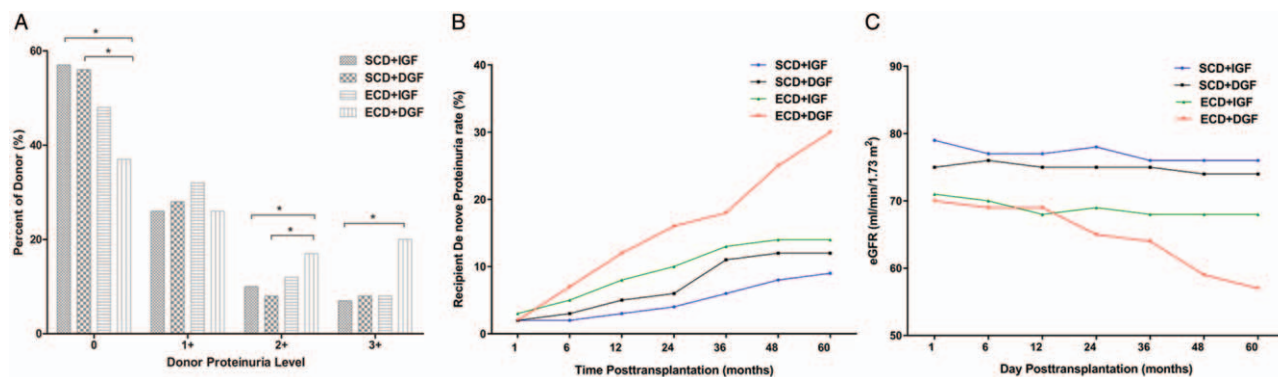
Figure 2: Distribution of donor proteinuria in each group and the probability of *de novo* proteinuria in each group as well as glomerular filtration rate tendency. (A) Patients were divided into groups based on donor characteristics (SCD or ECD) and recipient status (IGF or DGF) as follows: SCD + IGF, $n = 349$; SCD + DGF, $n = 72$; ECD + IGF, $n = 85$; ECD + DGF, $n = 35$. The proportions of patients in each group based on donor proteinuria scores are shown. Urine protein classification standards are <0.2 g/L: 0; urine protein is 0.2 to 1.0 g/L: 1+; 1.0 to 2.0 g/L: 2 +; ≥ 2 g/L: 3+. (B) Probability of *de novo* proteinuria in each group during the 5-year follow-up. (C) Estimated glomerular filtration rate trend in each group during the 5-year follow-up. [*]$P < 0.05$. DGF: Delayed graft function; ECD: Expanded-criteria donor; IGF: Immediate graft function; SCD: Standard-criteria donor.
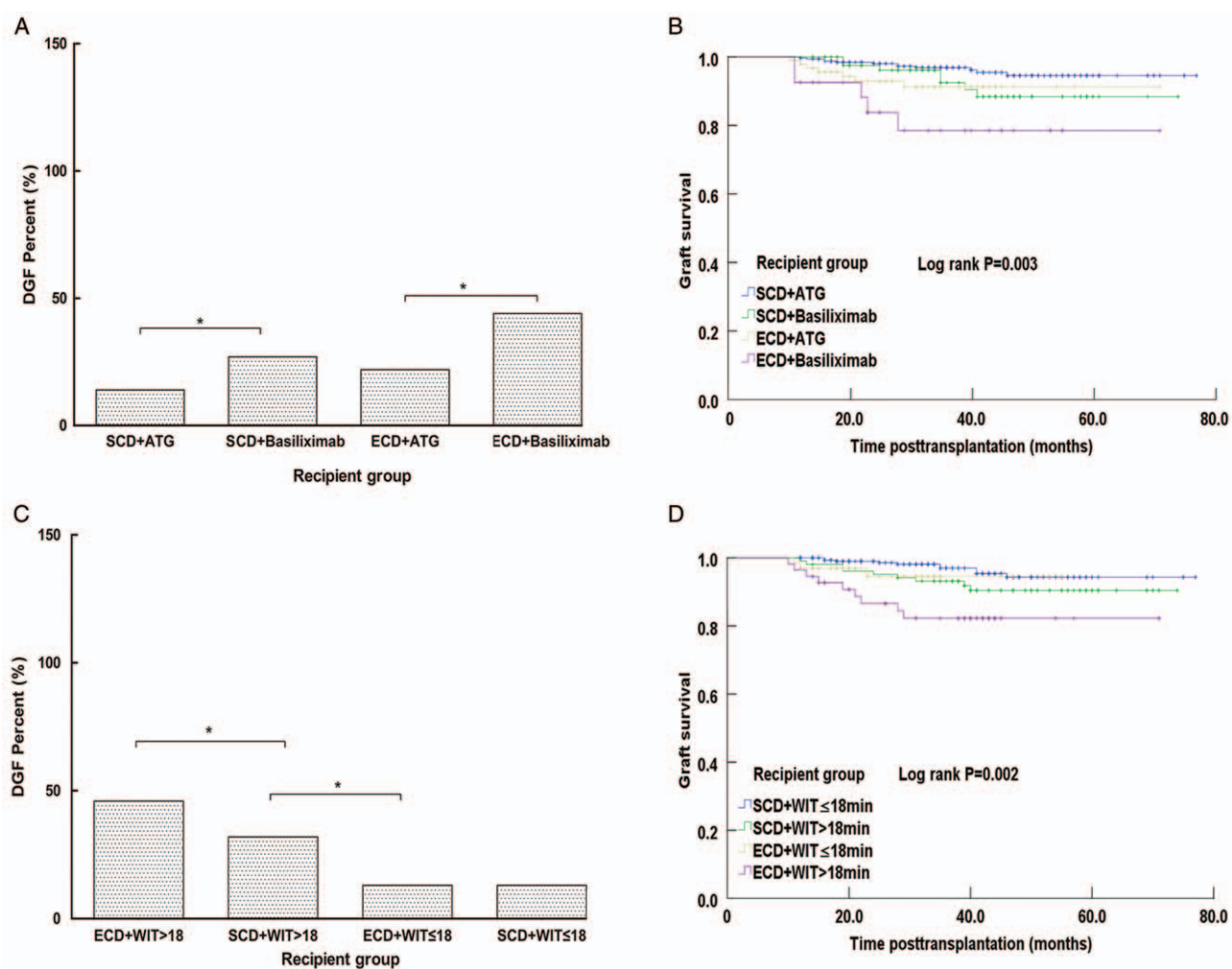
Figure 3: Recipients stratified into four groups according to donor type (ECD or SCD) and induction regimen (ATG or basiliximab): incidence of DGF (A) and graft loss (B). (SCD + ATG, $n = 332$, SCD + basiliximab, $n = 89$, ECD + ATG, $n = 93$, ECD + basiliximab, $n = 27$). Recipients stratified into four groups according to donor type (ECD or SCD) and WIT (≤ 18 or >18 min): incidence DGF (C) and graft loss (D). (SCD + WIT ≤ 18 min, $n = 318$, SCD + WIT >18 min, $n = 103$, ECD + WIT ≤ 18 min, $n = 65$, ECD + WIT >18 min, $n = 55$). [*]$P < 0.05$. ATG: Antithymocyte globulin; DGF: Delayed graft function; ECD: Expanded-criteria donors; IGF: Immediate graft function; SCD: Standard-criteria donor; WIT: Warm ischemia time.

imab) into four groups including patients receiving SCD transplants with ATG (SCD + ATG, $n = 332$), patients receiving SCD transplants with basiliximab (SCD + basiliximab, $n = 89$), patients receiving ECD transplants with ATG (ECD + ATG, $n = 93$), and patients receiving ECD transplants with basiliximab (ECD + basiliximab, $n = 27$). The rates of DGF were 14%, 27%, 22%, and 44%, respectively [Figure 3A], and the rates of graft loss

were 3.6%, 7.8%, 7.5%, and 18.5%, respectively [Figure 3B]. The DGF rates between the SCD + ATG and SCD + basiliximab groups ($P = 0.006$) and between the ECD + ATG and ECD + basiliximab groups ($P = 0.043$) were significantly different. Further, the ECD + basiliximab group had a lower graft survival rate than the other groups (log-rank test, $P = 0.003$).

WIT is a risk factor for DGF and graft loss

We next stratified the recipients according to donor type (SCD or ECD) and WIT (≤ 18 or >18 min) into four groups including patients receiving SCD transplants with WIT ≤ 18 min (SCD + WIT ≤ 18 min, $n = 318$), patients receiving SCD transplants with WIT >18 min (SCD + WIT >18 min, $n = 103$), patients receiving ECD transplants with WIT ≤ 18 min (ECD + WIT ≤ 18 min, $n = 65$), and patients receiving ECD transplants with WIT >18 min (ECD + WIT >18 min, $n = 55$). The rates of DGF were 13.5%, 28.2%, 13.8%, and 47.3%, respectively [Figure 3C], whereas the rates of graft loss were 3.1%, 8.7%, 4.6%, and 16.4%, respectively [Figure 3D]. The DGF rates in the ECD + WIT >18 min and SCD + WIT >18 min groups were especially higher than those in the other groups, and the difference between the SCD + WIT >18 min and ECD + WIT ≤ 18 min groups was statistically significant ($P = 0.023$). No significant difference between the ECD + WIT ≤ 18 min and SCD + WIT >18 min groups was observed ($P = 0.539$); however, the graft survival rate between these groups was significantly different (log-rank test, $P = 0.002$).

Discussion

In this retrospective, multicenter, observation cohort study, we found that DGF is an independent risk factor for graft survival in ECD recipients, but not SCD recipients. For donors with WITs >18 min, the incidence of DGF was significantly increased and the graft survival rate was decreased. Further, we demonstrated for the first time that recipient induction therapy with ATG decreases the rate of DGF and prolongs graft survival.

DGF was associated with graft loss in recipients receiving ECD, but not SCD, kidneys. The use of ECD kidneys has alleviated the pressures of organ shortages but has also increased the incidence of DGF. Understanding the effect of DGF on long-term outcomes will help to manage kidney transplant patients; however, the effect of DGF on graft loss is uncertain based on the published literature. Lim et al[11] recently showed that recipients of DCD kidneys with DGF had a higher incidence of death-censored graft loss compared with patients with IGF. A systematic review involving 151,194 kidney transplant recipients also showed that DGF has an adverse effect on graft outcomes.[12] In contrast, Boom et al[13] revealed that DGF affects renal function but not graft survival. Weber et al[15] confirmed that although the incidence of DGF in DCD kidney recipients is higher than of recipients of kidney donation after brain death, there is no significant difference in the long-term outcomes between the two graft types. In our study, we demonstrated that DGF is only an independent risk factor for recipients of ECD, but not

SCD kidneys. This result explains why different researchers have obtained different results on the association between DGF and graft survival.

The etiology of DGF was hypothesized as follows: nephron loss results in ischemia-reperfusion injury during the procedure, which causes a cascade of molecular events, leading to apoptosis, inflammation, and endothelial injury.[5] In this study, recipients of ECD kidneys had a higher rate of DGF than individuals receiving SCD kidneys, and DGF was an independent risk factor for graft survival in recipients with ECD kidneys. There are several reasons for this result. First, ECD kidneys are more sensitive to ischemia-reperfusion than SCD kidneys; therefore, ECD kidneys could lose more nephrons during the procedure, leading to increased incidence of DGF. Second, the capability of self-repair in ECD kidneys is diminished compared with that in SCD kidneys. After transplantation, part of the nephrons might never recover, whereas the remaining nephrons might be more sensitive to drug toxicity; the eGFR after transplantation was found to be gradually decreased [Figure 2C], and this could be the reason why the graft survival rate of ECD recipients is significantly lower than that of SCD recipients.[23,25] Third, the relationship between acute kidney injury (AKI) and chronic kidney disease (CKD) has been studied for several decades, and these are closely associated and interconnected.[26-29] AKI might contribute to the development and progression of CKD, and CKD is known to sensitize patients to AKI. We observed that ECDs had a higher level of proteinuria than SCDs [Figure 2A] and that ECDs had a higher incidence of hypertension history, which might suggest that most ECDs had a history of CKD. AKI, occurring in donors with CKD, will lead to transplanted kidneys with more severe injury and difficult recoveries; moreover, patients receiving ECD kidneys have a greater possibility of de novo proteinuria [Figure 2B], indicating that these patients are more likely to develop CKD after kidney transplantation. All of these reasons could indicate why patients receiving ECD transplants with DGF had a lower survival rate.

Recently, a study has shown that every additional hour of cold ischemia increases the risk of graft loss.[14] Tennankore et al[30] found that prolonged WIT was associated with graft failure and mortality post-transplantation. In this study, WIT was recorded from the termination of life support to the hypothermic perfusion of the graft. Recipients of ECD kidneys with WIT >18 min had an especially high incidence of DGF (47%), and the recipient graft loss rate was nearly 16.4% over 5 years. However, for recipients of ECD kidneys with WIT <18 min, the DGF rate was lower than that for recipients of SCD kidneys with WIT >18 min (13.8% vs. 28.1%; $P = 0.023$) and the graft loss rate was similar (4.6% vs. 8.7%). Specifically, the DGF and graft loss rates were not significantly different between the ECD + WIT ≤ 18 min and SCD + WIT ≤ 18 min groups. These results revealed that recipients of ECD kidneys could experience considerable graft survival rates if WIT is controlled within reasonable limits; further, for recipients of ECD kidneys with WIT >18 min, we should strengthen post-operative management to maintain allograft functions for as long as possible.

Several studies have revealed the association between ATG and DGF,[31-33] and induction therapy with ATG compared with other regimens significantly decreased the incidence of DGF. More recently, Chapal et al[34] revealed that the risk of DGF was reduced 1.73-folds in patients with ATG. Other studies also revealed the same association between ATG and DGF.[35-38] Our results showed that recipient induction therapy with ATG resulted in a lower incidence of DGF, compared with that with basiliximab; moreover, for recipients of ECD kidneys, the Cox proportional hazards regression model for the parameter of graft survival, based on multivariate analysis, showed that ATG was a protective factor for long-term allograft survival. In general, we recommend that for recipients of ECD kidneys, induction therapy with ATG is a better option.

In conclusion, our study demonstrated that DGF is an independent risk factor associated with long-term allograft survival in recipients of ECD kidneys, but not SCD kidneys. Further, a donor WIT >18 min will not only increase the incidence of DGF but also decrease allograft survival time. Further research is needed to verify our studies.

Funding

Conflicts of interest

None.

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