Pre-transplant donor-specific anti-human leukocyte antigen antibodies are associated with high risk of delayed graft function after renal transplantation

Juha P. Peräsaari[1], Lauri E. Kyllönen[2], Kaija T. Salmela[2] and Jussi M. Merenmies[3]

[1]Clinical Laboratory, Finnish Red Cross Blood Service, Kivihaantie 7, 00310 Helsinki, Finland, [2]Department of Transplantation and Liver Surgery, Helsinki University Hospital, Helsinki, Finland and [3]Children's Hospital, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland

Correspondence and offprint requests to: Juha Peräsaari; E-mail: juha.perasaari@bloodservice.fi

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ABSTRACT

Background. Sensitive screening methods have revealed that many patients have donor-specific human leucocyte antigen antibodies (DSAs) prior to transplantation, regardless of negative crossmatch results. The clinical significance of pre-transplant (pre-Tx) DSAs for early graft function has remained unclear. Our aim was to examine the association of DSAs with delayed graft function (DGF).

Methods. Pre-Tx sera of 771 patients who received kidney transplants in our single-centre study were retrospectively screened. All transplantations were performed after negative complement-dependent cytotoxicity (CDC) crossmatch.

Results. DSAs were detected in 13% of the patients. The overall DGF rate in our study was 29%. Patients with DSAs had a higher incidence of DGF when compared with non-sensitized patients (48 and 26%, respectively; $P < 0.0001$). Third-party antibodies had no effect for DGF incidence (28%; $P = 0.6098$). The relative risk (RR) of DGF for patients with DSAs in the multivariate analysis was 2.039 (95% CI 1.246–3.335; $P = 0.0046$). Analyses of the cumulative mean fluorescent intensity (MFI) value of the DSAs revealed a rate of DGF more than two times higher in patients with a cumulative value of 3000–5000 MFI compared with a cumulative value of 1000–3000 (65 versus 31%; $P = 0.0351$). DSAs against any loci showed an elevated DGF incidence of 44–69% when compared with patients without DSA (27%).

Conclusions. The risk of DGF is twice as high in patients having pre-formed DSAs. Pre-Tx DSAs is a modifiable risk factor that can be obviated with careful organ allocation relying on careful pre-Tx analysis of non-accepted mismatches determined with sensitive solid phase methods.

Keywords: delayed graft function, dialysis, donor-specific antibody, graft survival, immunology, kidney transplantation

INTRODUCTION

In kidney transplantation (RTx) delayed graft function (DGF) is a post-transplant form of acute renal failure that is a significant clinical problem affecting the kidney allograft for the whole clinical course. There are several known patient-related risk factors for DGF. These include diabetes, prolonged cold ischaemia time (CIT), retransplantation and a high level of human leucocyte antigen (HLA) antibodies [1–4]. The effect of donor death by stroke/cerebrovascular accident, age, hemodynamic factors, terminal creatinine and estimated glomerular filtration rate are known donor-related factors predisposing to DGF [4–8]. Patients with DGF are at risk for overall decreased graft function, acute rejection and decreased graft survival [9]. Moreover, DGF leads to higher costs due to prolonged dialysis dependence and hospitalization. Acceptance of expanded criteria donors due to shortage of organs has increased the incidence of DGF [5, 10, 11].

DGF is considered a multifactorial complication and is a result of ischaemia-reperfusion injury, where both immune and non-immune factors play a role. It is known that presensitization is a risk factor for DGF [2]. This may be explained in part by longer CITs for sensitized recipients [12]. It has also been hypothesized but not confirmed that the potential immunological mechanism could be that a low level of DSA undetected in the crossmatch can cause an antibody-mediated rejection episode that is interpreted as DGF [13]. Clinically, this condition may be difficult to identify and thus remains unrecognized.

Recently developed solid-phase assays, especially bead-based technology (Luminex), enables identification of very low levels of HLA antibodies. This has made it possible to study the effect of well-defined antibodies to transplant outcomes. Several studies have demonstrated the role of preformed donor-specific antibodies for rejection and graft survival [14–16]. However, the clinical relevance of antibodies detected with single antigen beads is still controversial and some transplants may be denied based on overcautious risk estimations [17–19]. Understanding the role of DSAs in the context of negative complement-dependent cytotoxicity (CDC) crossmatch would be of great importance when aiming at extended graft survival. Only limited studies have been performed to understand the role of DSAs for early graft function and DGF. Earlier studies with need for dialysis within the first week as a definition for DGF have been unable to identify pre-RTx DSAs as a risk for DGF [20, 21]. We used the stricter definition of DGF by Halloran *et al.* [22], which considers better clinically relevant indicators of poor early graft function [23].

The purpose of this retrospective study was to examine the role of DSA status on DGF, an entity with only limited studies performed with modern sensitive antibody identification methods. We hypothesized that pre-existing DSAs, even with a negative prospective CDC crossmatch, possess an increased risk for DGF.

MATERIALS AND METHODS

Patients

This retrospective, single-centre study was carried out in accordance with a protocol approved by the Ethics Committee of Helsinki University Hospital. A total of 799 consecutive adult (≥16 years) patients received RTx during 2000–4 and were included after written informed consent. Fifteen patients with a living donor and 13 patients with surgical complication or arterial/venal thrombosis leading to early transplantectomy were excluded from the analysis, resulting in a total of 771 patients.

Immunosuppression

Primary immunosuppression consisted of triple therapy based on a calcineurin inhibitor (tacrolimus or cyclosporine), mycophenolate mofetil (MMF) and steroid. The trough level target for cyclosporine was 150–190 µg/L and for tacrolimus was 7–10 µg/L during the first month. Immunologically, high-risk patients received induction therapy (antithymocyte globulin, basiliximab or daclizumab). Calcineurin inhibitor was started prior to the transplant operation. Immunosuppression was not adjusted according to DGF status.

Diagnostic classifications

DGF was designated if one of the following criteria was fulfilled: (i) serum creatinine was >500 mol/L throughout the first perioperative week; (ii) more than one dialysis session during the first week after RTx and (iii) oliguria (<1 L/day) 2 days after transplantation [22]. The conventional DGF definition (need for dialysis during the first week after transplantation) was used to evaluate if the DGF definition has an impact on results. With the conventional definition the category slow graft function (SGF)

was used (impaired creatinine clearance without need for dialysis) [24]. Acute rejections were biopsy proven and determined according to Banff 1997 criteria. All biopsies were performed due to clinical indications, including DGF lasting over 14 days.

Donor HLA typing

The HLA typing of the donors was initially performed with a complement-mediated lymphocytotoxicity test (Biotest, Rockaway, NJ, USA) and low-resolution polymerase chain reaction with sequence-specific primer (One Lambda, Canoga Park, CA, USA). Additional typing with polymerase chain reaction with sequence-specific oligonucleotide (One Lambda) or sequencing (Atria Genetics, South San Francisco, CA, USA) was performed when the result was needed to confirm donor specificity of an antibody.

Crossmatches

CDC crossmatch was performed with gradient purified donor spleen cells [25]. Any cell death above background was considered positive. The test was performed at room temperature and at 37°C to eliminate autoantibodies, and it was considered positive if the positive result was detected at both temperatures. All patients transplanted in this study had negative CDC crossmatch results.

HLA antibodies

Serum samples of the patients were collected on the day of transplantation. The retrospective screening of HLA antibodies was performed with Luminex-based commercial kits (LABScreen® Mixed, One Lambda). Samples with detectable HLA antibodies were then analyzed with LABScreen® single antigen kits (One Lambda) to identify antibody specificities. All sera were tested for HLA class I (HLA-A,B,Cw) and class II (HLA-DR, DQ,DP) antibodies. Antibodies were assigned with HLA Fusion™ software (One Lambda). DSAs were assigned by comparing assigned antibodies to the serological equivalent of the donor's HLA type. The strength of identified donor-specific antibodies was determined by mean fluorescent intensity (MFI) values. Also, a low level of DSAs (<1000 MFI) was taken into account as suggested by the analysis software, so that the relevance of low level DSAs could be determined. The sum value of all DSAs directed against the donor was reported as a cumulative DSA. DSA identification was done retrospectively, so all transplants were performed relying on the CDC crossmatch only.

Statistical analysis

Categorical variables were analysed with the Fisher's exact test. Binary logistic regression analysis or Mann–Whitney U-test was used to compare continuous variables with the presence of DGF. All results with a P-value ≤0.05 were considered statistically significant. All presented P-values are uncorrected for possible multiple testing. Analyses were performed with SPSS statistics version 21.0 software (IBM, Armonk, NY, USA).

RESULTS

A total of 771 kidney transplants from 477 deceased donors were included in the study. The characteristics of the donors and patients are shown in Table 1. The mean age was 46.4 ± 13.7 years, the mean donor BMI was 25.01 ± 3.88 kg/m^2 and 53.0% were male. The mean age of recipients was 49.0 ± 12.2 years and 64% were male. All transplants were ABO compatible from deceased donors. Furthermore, 10% of the transplants were regrafts. The total incidence of DGF in our study was 29%. The incidence of DGF was higher for re-grafted patients (48 versus 27%, P = 0.0004) and for patients with DSAs (48 versus 27%, P < 0.0001).

Factors associated with DGF

The multivariate analysis with binary logistic regression analysis found five independent predictors for DGF (Table 2). The strongest statistical association was found for donor age [relative risk (RR) 1.037 (95% CI 1.022–1.053), P < 0.0001] and for CIT [RR 1.068 (95% CI 1.025–1.112), P = 0.0015]. The highest relative risk was found for DSAs [RR 2.039 (95% CI 1.246–3.335), P = 0.0046] and for patients with previous kidney transplant [RR 1.879 (95% CI 1.064–3.319), P = 0.0297].

Table 1. Characteristics of recipients with EF and DGF

	All (n = 771)	EF (n = 544)	DGF (n = 227)	P-value
Mean recipient age, years	49	48	50	**0.0348**
Mean donor age, years	46	44	51	**<0.0001**
Male recipients, n (%)	496 (64)	344 (63)	152 (67)	0.3646
Average HLA mismatch	2.17	2.18	2.17	0.8296
DSA positive, n (%)	103 (13)	54 (10)	49 (22)	**<0.0001**
Transplant number, n (%)				
First transplants	691 (90)	502 (92)	189 (83)	Reference
Retransplants	80 (10)	42 (8)	38 (17)	**0.0004**
CIT, hours	22.14	21.53	23.06	**<0.0001**

CIT, cold ischaemia time; EF, early function; DGF, delayed graft function; HLA, human leucocyte antigen; DSA, donor-specific HLA antibodies.
Significant P-values are in bold.

No association for early rejection with DGF was found [RR 0.8078 (95% CI 0.177–3.687), P = 0.7830].

HLA antibody status

Of the 771 patients, 265 (34%) had pre-existing HLA antibodies. Class I antibodies were detected in 225 (29%) patients and class II antibodies in 131 (17%). DSAs were detected in 103 (13%) patients. Class I DSAs alone were detected in 48 (6%) patients and class II DSAs in 36 (5%) and both class I and II DSAs were detected in 19 (2%) patients. Patients with re-transplantation had a higher DSA incidence of 53% (42/80) than patients with the first transplant [9% (61/691)] (P < 0.0001).

Association of antibody status to DGF

The incidence of DGF in non-sensitized and sensitized patients was 26% (132/506) and 36% (95/265), respectively (P = 0.0060). Patients with DSAs (n = 103) had a significantly higher prevalence of DGF when compared with patients without DSAs (n = 668) [48 and 27%, respectively (P < 0.0001)]. Antibodies against third-party antigens had no effect on DGF incidence (Figure 1A). The incidence of DGF was also dependent on the number of antigens detected as DSAs (Figure 1B). Patients with antibodies against a single donor antigen had a DGF rate of 43%, whereas patients with antibodies against two or three or more donor antigens had DGF rates of 57 and 53%, respectively (P < 0.0001). The presence of DSAs is a risk factor for DGF in univariate analysis [RR 1.765 (95% CI 1.394–2.235), P < 0.0001]. There was no difference in CIT between DSA− and DSA+ patients (22.2 versus 22.3 h, respectively) (P = 0.8205).

The impact of DSA loci

DSAs were detected against all classical HLA loci. When compared with the patients without DSAs (DGF rate 27%), DSAs against any loci showed an elevated DGF incidence of 44–69%. The risk of DGF was highest in patients with DSAs against DRB1 in the univariate analysis [RR 2.407 (95% CI 1.647–3.518), P = 0.0032; Table 3].

Association of DSA MFI values with DGF findings

To analyse the predictive value of the DSA MFI to the incidence of DGF, patients with DSAs were separated into three
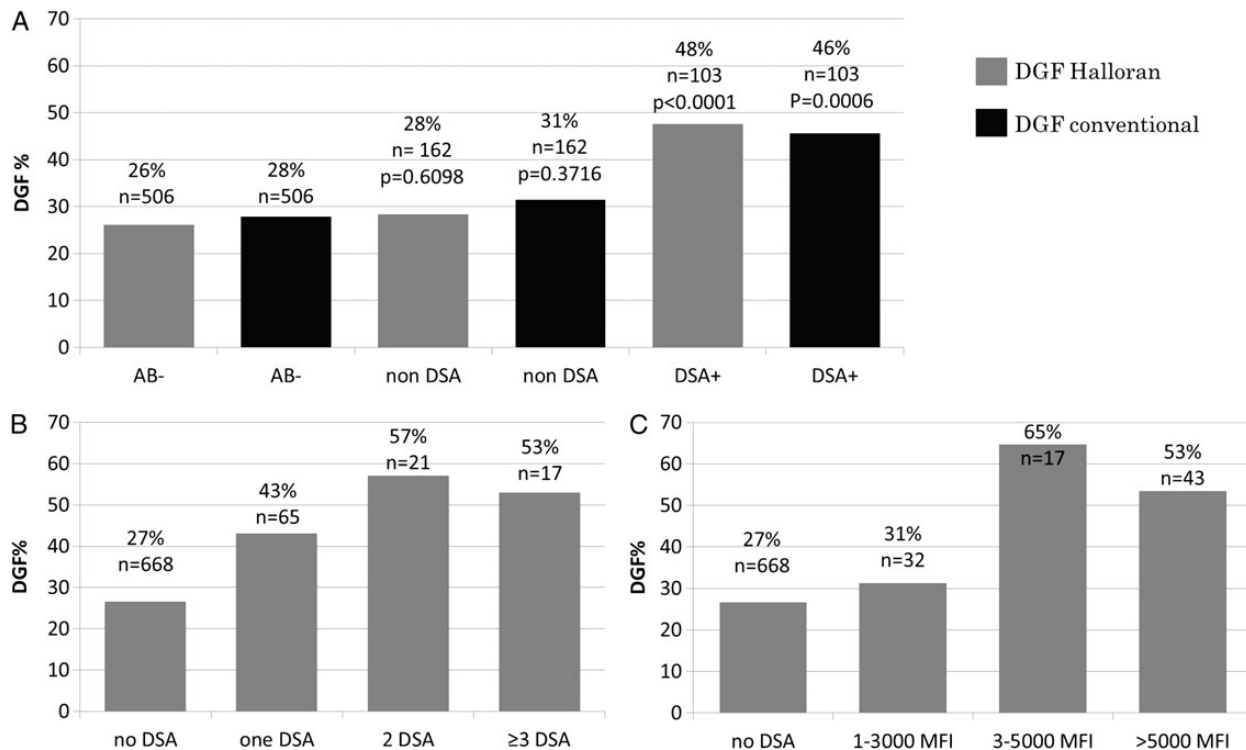
Table 2. Factors associating with DGF: results of the multivariate logistic regression analysis

Characteristics	DGF halloran				Conventional IGF versus DGF				Conventional IGF versus SGF + DGF			
	Wald	RR	95% CI	P-value	Wald	RR	95% CI	P-value	Wald	RR	95% CI	P-value
Recipient age, years	0.0306	1.001	0.987–1.016	0.8611	0.2188	0.996	0.981–1.012	0.6399	1.2120	0.992	0.979–1.006	0.2709
Recipient BMI, kg/m^2	3.1528	1.047	0.995–1.102	0.0758	5.3784	1.064	1.010–1.121	**0.0204**	8.8660	1.076	1.025–1.129	**0.0029**
Diabetes	0.0371	0.963	0.654–1.418	0.8473	0.1827	0.917	0.615–1.366	0.6690	0.2219	0.917	0.640–1.314	0.6376
Re-Tx	4.7251	1.879	1.064–3.319	**0.0297**	4.5011	1.883	1.049–3.378	**0.0339**	2.0041	1.503	0.855–2.643	0.1569
Rejection ≤1 week	0.0759	0.808	0.177–3.687	0.7830	2.0819	2.932	0.680–12.637	0.1491	1.3490	2.346	0.556–9.895	0.2454
Donor age, years	23.5774	1.037	1.022–1.053	**<0.0001**	30.3300	1.044	1.028–1.060	**<0.0001**	38.1912	1.044	1.030–1.058	**<0.0001**
Donor BMI, kg/m^2	4.5405	1.049	1.004–1.095	**0.0331**	5.9315	1.057	1.011–1.105	**0.0149**	3.5682	1.041	0.998–1.086	0.0589
CIT, hours	10.0416	1.068	1.025–1.112	**0.0015**	14.1251	1.085	1.040–1.132	**0.0002**	13.2695	1.075	1.034–1.117	**0.0003**
DSA	8.0462	2.039	1.246–3.335	**0.0046**	3.5829	1.653	0.982–2.782	0.0584	4.1837	1.668	1.022–2.723	**0.0408**

BMI, body mass index; Re-Tx, previous kidney transplant; CIT, cold ischaemia time; DSA, donor-specific antibody; DGF, delayed graft function; IGF, immediate graft function; SGF, slow graft function; RR, relative risk; CI, confidence interval.
Multivariate analysis with binary logistic regression analysis. Significant P-values are in bold.

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FIGURE 1: (**A**) Comparison of immunization status with DGF defined by the Halloran *et al.* criteria [22] and conventional criteria [24]. (**B**) Comparison of the number of DSAs with DGF incidence ($P < 0.0001$; Mann–Whitney U-test). (**C**) Comparison of the cumulative MFI value with DGF incidence ($P < 0.0001$; Mann–Whitney U-test). AB−, no detected antibodies; non-DSA, antibodies against a third party; DGF, delayed graft function; DSA, donor-specific antibody; MFI, mean fluorescent intensity.

Table 3. Comparison of DGF rate and relative risk of DSAs directed against different HLA antigens

DSA status	N	DGF, n (%)	RR	95% CI	P-value
No DSA	668	178 (27)			
HLA-A	22	10 (45)	1.569	0.979–2.513	0.0791
HLA-B	33	16 (48)	1.696	1.172–2.454	**0.0144**
HLA-C	26	13 (50)	1.741	1.166–2.599	**0.0200**
HLA-DRB1	13	9 (69)	2.407	1.647–3.518	**0.0032**
HLA-DRB3-5	18	8 (44)	1.528	0.901–2.592	0.1262
HLA-DQ	22	11 (50)	1.734	1.125–2.673	**0.0319**
HLA-DP	18	8 (44)	1.528	0.901–2.592	0.1262
Total HLA-DSA			1.785	1.406–2.266	**<0.0001**

HLA, human leucocyte antigen; DGF, delayed graft function; DSA, donor-specific antibody; RR, relative risk; CI, confidence interval.
Univariate analysis performed with two-tailed Fisher's exact test. Significant P-values are in bold.

cohorts according to their cumulative DSA MFI: patients with low DSAs (1000–3000 MFI), moderate DSAs (>3000–5000 MFI) and high DSAs (>5000 MFI). DGF status was correlated to the DSA findings. A significant correlation was found between DGF and the cumulative MFI value, revealing an incidence of DGF more than two times higher in patients with moderate DSAs when compared with low DSAs (65 and 31%, respectively; $P = 0.0351$). Also, the risk of DGF for patients with moderate DSAs was 2-fold higher [RR 1.9479 (95% CI 0.9822–3.8632), $P = 0.0563$]. No further increase in DGF incidence was observed in the category with high MFI (Figure 1C).

Graft outcomes

Four patients without kidney loss date were left out of the survival analysis. Overall 5-year graft survival was 90.5%. Because the frequency of DSAs was increased in patients with DGF, a Kaplan–Meier survival analysis was performed to analyse the effect of both DSAs and DGF on graft survival. Groups with DGF (DSA−DGF+ and DSA+DGF+) demonstrated the lowest 5-year graft survival (84.8 and 88.9%, respectively). In groups without DGF (DSA−DGF− and DSA+DGF−), survival was 92.1 and 93.3%, respectively. No additive risk was observed for DSAs in DGF+ patients (log-rank test, $P = 0.306$; Figure 2).

Acute reversible rejection or acute steroid-resistant rejection was detected in 119 patients. Rejections were detected on a median Day 20 after transplantation for patients with or without DGF. Patients with DGF had a rejection rate of 19%, whereas patients without DGF had rejection rate of 14% ($P = 0.1004$). Patients with DGF had a higher rejection rate if DSAs were detected, but this difference was not significant [27% (13/49) and 17% (30/178), respectively ($P = 0.1495$)]. Patients with DGF were more likely to undergo a biopsy during the first perioperative month (33 and 17%) as required by local protocol.

DISCUSSION

In this study we took a closer look to the role of sensitization and its relation to DGF. It is known that higher panel reactive
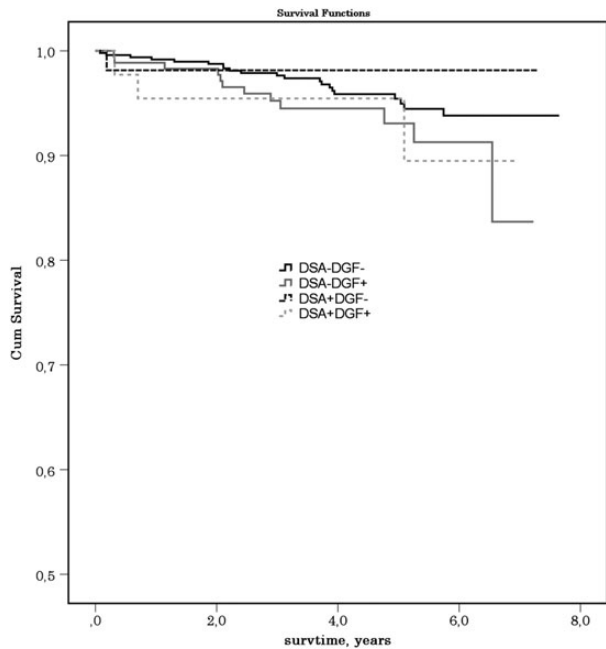
FIGURE 2: Graft survival for recipients with or without DSAs and with or without DGF (log-rank test, P = 0.306).

antibody values predispose to DGF, but evaluation of the strength and specificity of these antibodies has been scarce so far [20, 21]. This study demonstrates that HLA antibodies directed against allograft are associated with a higher risk for DGF compared with patients without HLA antibodies or with HLA antibodies against third-party antigens. DSAs were identified from 13% (103/771) of patients. This is in accordance with previous findings showing the same incidence of 13% for pre-RTx DSAs [20].

The total incidence of DGF in our study was 29% (227/771), which corresponds to earlier findings of 24–29% [2, 4, 12, 26]. According to our study, patients with DSAs had an incidence of DGF nearly twice as high as patients without third-party antibodies or non-DSA. This contradicts a previous study by Gupta et al. [20], where a DGF frequency of 15% was observed for patients with DSAs and 25% for patients without DSas or third-party antibodies [20]. Also, in a study by Thammanichanond et al. [21], no difference in DGF rate was observed in patients with or without DSAs. The contradicting results could be explained at least partially by two factors. First, the definition for DGF was different. We used the DGF definition of Halloran et al. [22], according to which at least two cycles of dialysis are required for the diagnosis. In addition, clearly elevated serum creatinine and oliguria were regarded as clinical indications for poor graft function and were taken into account for the definition of DGF [22]. In contrast, in previous studies a need for dialysis in the first week corresponded as a definition for DGF. However, simple need for dialysis after transplantation may not always indicate DGF and may originate from pre-RTx dialysis practices and fluid status or poor potassium balance after transplantation. Most importantly, the decision on the need of dialysis is based on the physician's individual interpretation of the post to

operative clinical status. The findings of Jayaram et al. [23] support this idea: only patients with DGF requiring more than one-time dialysis had an increased risk for death, lower renal function and higher incidence of acute rejections during the first perioperative year. The conventional definition of DGF introduces an additional category of SGF. In SGF, slow recovery of graft function is measured by an impaired decrease in serum creatinine. The difference between SGF and DGF is arbitrary. They both represent early graft dysfunction with the same risk factors and outcomes [27]. This category was not taken into account in the previous studies and may explain our contradicting results. Indeed, our analysis revealed that only when SGF was taken into account with the conventional definition was an association between DSAs and impaired graft function found in multivariate analysis. When studying early graft function relying on the conventional definition may be too simplistic to study this rather complex clinical condition.

The sensitivity of Luminex to identify very low level anti-HLA antibodies has raised the question regarding the cut-off value for clinically relevant antibody levels. In our study, we compared the cumulative MFI value of the DSAs to early graft function and found that the MFI value of the DSAs was a clearly significant factor, as shown with Mann–Whitney U-test (Figure 1). Comparison of the DSA MFI value categories revealed that the incidence of DGF was higher in the group with cumulative DSAs of 3000–5000 MFI versus 1000–3000 MFI (65 versus 31%), suggesting a cut-off value >3000 MFI for clinically relevant DSA in the context of early graft function. With the standard Luminex method, identification of complement-fixing antibodies is not possible. However, all transplants were performed against a negative CDC crossmatch. Thus, identified DSAs were most likely either low titre or non-complement binding. Modification of the assay to identify C1q binding antibodies has been reported to have a powerful predicting value of poor graft survival in RTx patients [28].

Traditionally the matching for organ allocation has been based on three loci—HLA-A, -B, and DRB1—with some variation between centres. The relevance of other HLA loci has been unclear since routine testing to identify antibodies against other loci has not been readily available. In our study, we found that the incidence of DGF was highest for patients with antibodies directed to donor DRB1 (69%), suggesting the importance of avoiding DRB1 DSAs. According to the univariate analysis, antibodies against HLA-B, HLA-C, DRB1 and DQB1 associated with DGF (P = 0.0144, 0.0200, 0.0032 and 0.0319, respectively) and the RR for DGF is highest for patients with DSAs directed against donor DRB1.

The importance of DGF for graft outcome has been shown in several previous studies. The risk for acute rejection is associated with a 38–44% relative increase for patients with DGF [2, 9]. Khalkhali et al. [29] showed that DGF poses an increased risk for death-censored graft loss (RR 6.087; P < 0.001).

In our study, we hypothesized that pre-existing DSAs, even with a negative prospective CDC crossmatch, poses a significant risk for DGF, and this might have been missed in other studies

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using need for dialysis as the only criterion for DGF. This information, when combined with the crossmatch result, would be of great importance when aiming for safe organ allocation with extended graft survival.

There are several limitations in our study. First, it is difficult to estimate the relevance of DSAs against certain loci, because DSAs were found against multiple loci in 37% of the DSA+ cases. Second, our study was not designed to study the capacity of complement binding of DSAs. Determing the effect of complement-binding DSAs on DGF will require further studies. Third, the criteria used for DGF have no time limit when graft function must be achieved. This allows patients with primary non-function of the graft to be included in the DGF group (these were excluded from the survival analysis).

In conclusion, the presence of DSAs detected by Luminex is a clear predisposing factor for DGF in our CDC crossmatch-negative recipients. However, it is not a major factor accounting for DGF, because the majority (78%) of the DGF was seen in our study in patients without DSAs. DSAs are a modifiable risk factor that can be considered with organ allocation for patients who are able to get a DSA-negative transplant. The presence of pretransplantation DSAs should not be used as an absolute contraindication for transplantation. Instead, it is a risk factor that needs to be considered critically in organ allocation schemes so that overall graft survival increases. However, transplants against low-level DSAs with good yet moderately decreased outcomes should be possible in highly sensitized patients.

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CONFLICT OF INTEREST STATEMENT

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of the presented manuscript or other conflicts of interest to disclose. The results presented in this article have not been published previously in whole or part, except in abstract format.

REFERENCES

1. Doshi MD, Garg N, Reese PP et al. Recipient risk factors associated with delayed graft function: a paired kidney analysis. Transplantation 2011; 91: 666–671
2. Humar A, Ramcharan T, Kandaswamy R et al. Risk factors for slow graft function after kidney transplants: a multivariate analysis. Clin Transplant 2002; 16: 425–429
3. Parekh J, Bostrom A, Feng S. Diabetes mellitus: a risk factor for delayed graft function after deceased donor kidney transplantation. Am J Transplant 2010; 10: 298–303
4. Patel SJ, Duhart BT, Jr, Krauss AG et al. Risk factors and consequences of delayed graft function in deceased donor renal transplant patients receiving antithymocyte globulin induction. Transplantation 2008; 86: 313–320
5. Hollmen ME, Kyllonen LE, Inkinen KA et al. Deceased donor neutrophil gelatinase-associated lipocalin and delayed graft function after kidney transplantation: a prospective study. Crit Care 2011; 15: R121
6. Koning OH, Ploeg RJ, van Bockel JH et al. Risk factors for delayed graft function in cadaveric kidney transplantation: a prospective study of renal function and graft survival after preservation with University of Wisconsin solution in multi-organ donors. European Multicenter Study Group. Transplantation 1997; 63: 1620–1628
7. Lebranchu Y, Halimi JM, Bock A et al. Delayed graft function: risk factors, consequences and parameters affecting outcome-results from MOST, a Multinational Observational Study. Transplant Proc 2005; 37: 345–347
8. Gavela Martinez E, Pallardo Mateu LM, Sancho Calabuig A et al. Delayed graft function after renal transplantation: an unresolved problem. Transplant Proc 2011; 43: 2171–2173
9. Yarlagadda SG, Coca SG, Formica RN, Jr et al. Association between delayed graft function and allograft and patient survival: a systematic review and meta-analysis. Nephrol Dial Transplant 2009; 24: 1039–1047
10. Ferrer F, Mota A, Alves R et al. Renal transplantation with expanded criteria donors: the experience of one Portuguese center. Transplant Proc 2009; 41: 791–793
11. Johnston TD, Thacker LR, Jeon H et al. Sensitivity of expanded-criteria donor kidneys to cold ischaemia time. Clin Transplant 2004; 18(Suppl 12): 28–32
12. McLaren AJ, Jassem W, Gray DW et al. Delayed graft function: risk factors and the relative effects of early function and acute rejection on long-term survival in cadaveric renal transplantation. Clin Transplant 1999; 13: 266–272
13. Peeters P, Terryn W, Vanholder R et al. Delayed graft function in renal transplantation. Curr Opin Crit Care 2004; 10: 489–498
14. Caro-Oleas JL, Gonzalez-Escribano MF, Gentil-Govantes MA et al. Influence of donor specific HLA antibodies detected by Luminex in kidney graft survival: a multivariate analysis. Hum Immunol 2013; 74: 545–549
15. Lefaucheur C, Loupy A, Hill GS et al. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. J Am Soc Nephrol 2010; 21: 1398–1406
16. Singh N, Djamali A, Lorentzen D et al. Pretransplant donor-specific antibodies detected by single-antigen bead flow cytometry are associated with inferior kidney transplant outcomes. Transplantation 2010; 90: 1079–1084
17. van den Berg-Loonen EM, Billen EV, Voorter CE et al. Clinical relevance of pretransplant donor-directed antibodies detected by single antigen beads in highly sensitized renal transplant patients. Transplantation 2008; 85: 1086–1090
18. Susal C, Ovens J, Mahmoud K et al. No association of kidney graft loss with human leukocyte antigen antibodies detected exclusively by sensitive Luminex single-antigen testing: a Collaborative Transplant Study report. Transplantation 2011; 91: 883–887
19. Middleton D, Jones J, Lowe D. Nothing's perfect: the art of defining HLA-specific antibodies. Transpl Immunol 2014; 30: 115–121
20. Gupta A, Iveson V, Varagunam M et al. Pretransplant donor-specific antibodies in cytotoxic negative crossmatch kidney transplants: are they relevant? Transplantation 2008; 85: 1200–1204
21. Thammanichanond D, Ingsathit A, Mongkolsuk T et al. Pre-transplant donor specific antibody and its clinical significance in kidney transplantation. Asian Pac J Allergy Immunol 2012; 30: 48–54
22. Halloran PF, Aprile MA, Farewell V et al. Early function as the principal correlate of graft survival. A multivariate analysis of 200 cadaveric renal transplants treated with a protocol incorporating antilymphocyte globulin and cyclosporine. Transplantation 1988; 46: 223–228

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23. Jayaram D, Kommareddi M, Sung RS et al. Delayed graft function requiring more than one-time dialysis treatment is associated with inferior clinical outcomes. Clin Transplant 2012; 26: E536–R543

24. Humar A, Johnson EM, Payne WD et al. Effect of initial slow graft function on renal allograft rejection and survival. Clin Transplant 1997; 11: 623–627

25. Amos DB, Bashir H, Boyle W et al. A simple micro cytotoxicity test. Transplantation 1969; 7: 220–223

26. Nagaraja P, Roberts GW, Stephens M et al. Influence of delayed graft function and acute rejection on outcomes after kidney transplantation from donors after cardiac death. Transplantation 2012; 94: 1218–1223

27. Lopez-Hoyos M, Fernandez-Fresnedo G, Rodrigo E et al. Effect of delayed graft function in hypersensitized kidney transplant recipients. Hum Immunol 2005; 66: 371–377

28. Loupy A, Lefaucheur C, Vernerey D et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. N Engl J Med 2013; 369: 1215–1226

29. Khalkhali HR, Ghafari A, Hajizadeh E et al. Risk factors of long-term graft loss in renal transplant recipients with chronic allograft dysfunction. Exp Clin Transplant 2010; 8: 277–282

ORIGINAL ARTICLE