ORIGINAL ARTICLE

LONG-TERM SURVIVAL OF THE TRANSPLANTED KIDNEY AND THE CLINICAL RELEVANCE OF DONOR-SPECIFIC ANTIBODIES

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Abstract: The aim of our study was to evaluate the relevance of donor-specific antibodies (DSA) as defined by solid-phase single-antigen (SA) assays for predicting long-term graft survival after kidney transplantation. Sera from 132 kidney transplant recipients were retrospectively tested before, 3, 6 and 12 months after transplantation. The incidence of rejection and graft survival was followed up for 7 years. We found 29 episodes of acute cellular rejection (CR), 21 cases of antibody-mediated rejection (AMR) and 18 graft failures due to immunological reasons. Pre-transplant DSA and DSA three months after transplantation correlated with an increased rate of AMR and impaired graft function. After the fourth year, recipients with persistent DSA were at a higher risk of graft failure (p = 0.0317). Antibody specificity was prevailingly to HLA class I antigens (66.6% DSA, 75% non-DSA). During the first year after transplantation, the number of patients with non-DSA decreased (30.3% to 10.7%), while, due to *de novo* production of antibodies, the number of DSA positive patients remained constant.

Conclusion: Detection of antibodies to HLA antigens using solid-phase assays, especially single-antigen bead technology before and three months after transplantation is predictive for increased incidence of antibody-mediated rejection and impaired long-term kidney graft survival.

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INTRODUCTION

Acute antibody-mediated rejection (AMR) is the main immunological cause of irreversible deterioration in graft function and impaired survival of the transplanted kidney.[1, 2] The detection and definition of HLA donor-specific antibodies (DSA) using solid-phase assays is now essential for the diagnosis of AMR. However, the role of pretransplant DSA and *de novo*-produced DSA (as defined by these techniques) in the assessment of long-term kidney graft survival is still uncertain. A number of studies have indicated worse graft survival in patients with pretransplant DSA and posttransplant *de novo*-produced DSA[3-6], even though they are contradicted by other reports.[7, 8] Intriguingly, two recent studies point out that non-donor-specific antibodies (non-DSA) might also have graft-damaging effects.[9, 10] The clinical relevance of various technical modifications of the single-antigen Luminex assay, which involves the role played by complement-activating vs. non-activating antibodies, is also under intense scrutiny.[11, 12] Given the different and often contradictory data in the literature, the goal of our retrospective study was to evaluate the clinical relevance of DSA as well as non-DSA (as defined using the single-antigen bead technique) for the prediction of long-term (7 years) graft survival after kidney transplantation.

MATERIAL AND METHODS

Our study included 132 kidney recipients who received kidneys from deceased donors in the Transplant centre

Table 1. Demographic characteristics of kidney transplant recipients

Patients		Total (n=132)	ACR[1] (n=29)	P value	AMR[2] (n=21)	P value
Gender	Male	82 (62%)	15 (18%)	NS	13 (16%)	NS
	Female	50 (38%)	14 (28%)		8 (16%)	
First Transplanted	PRA > 10%	15 (11%)	2 (13%)	NS	2 (13%)	NS
	PRA ≤ 10%	106 (81%)	25 (24%)		14 (13%)	
Retransplanted	PRA > 10%	4 (3%)	0 (0%)	NS	1 (25%)	NS
	PRA ≤ 10%	7 (5%)	2 (29%)		4 (57%)	
First Transplanted		121 (92%)	27 (22%)	NS	16 (13%)	P=0.0179
Retransplanted		11 (8%)	2 (18%)		5 (45%)	
ABDR Mismatch	> 4	33 (25%)	10 (30%)	NS	7 (31%)	NS
	≤ 4	98 (75%)	19 (19%)		14 (14%)	
Delayed graft function	Yes	19 (14%)	4 (21%)	NS	4 (21%)	NS
	No	113 (86%)	25 (22%)		17 (15%)	
ATN[3]	Yes	44 (33%)	12 (27%)	NS	9 (20%)	NS
	No	88 (67%)	17 (19%)		12 (14%)	
Induction Therapy	Yes	29 (22%)	5 (17%)	NS	4 (14%)	NS
	No	103 (78%)	24 (23%)		17 (17%)	

Legend: [1]ACR – cellular rejection, [2]AMR – antibody-mediated rejection, [3]ATN – acute tubular necrosis

IKEM between 2004 and 2005. All recipients were followed up for graft function, patient and kidney graft survival for 7 years after transplantation. The research was approved by the ethics committee of the institute and written informed consent was obtained from all patients before their inclusion in the study. The demographic characteristics of our cohort are shown in Table 1.

The detection and specification of HLA antibodies were performed before, 3, 6 and 12 months after transplantation. Patients with panel-reactive antibodies (PRA) > 50% before transplantation, as assessed using the complement-dependent lymphocytotoxic (CDC) assay, were treated with induction immunosuppressive therapy, including antithymocyte globulin (ATG) and corticosteroids. All kidney recipients had a negative CDC crossmatch test, performed with unseparated T and B cells (isolated from donor lymph nodes or the spleen) before transplantation. All pre- and post-transplantation sera were analysed retrospectively using the Luminex technique. After transplantation, 341 samples were analysed in total. The detection of HLA antibodies was performed using the LABScreen Mixed technique; positive sera from the LABScreen test were further tested for HLA specificity using single antigen (SA) beads (One Lambda Inc., Canoga Park, USA) according to the manufacturer's instructions on a Luminex 200 System. A cut-off for positivity of 1000 MFI and 2000 MFI was applied for class I and class II SA beads, respectively. Donor-specific antibodies were defined against HLA-A, -B and -DR antigens.

Cellular (CR) and antibody-mediated rejection (AMR) were diagnosed in graft biopsies according to the updated Banff classification (2008)[13]; biopsies were performed at the time points of kidney graft function deterioration. AMR was defined by morphological evidence of acute tissue injury, immunofluorescent detection of diffuse C4d deposits in peritubular capillaries (>10%) and the simultaneous presence of DSA. The standard immunosuppressive protocol after transplantation included calcineurin inhibitors, mycophenolate mofetil and corticosteroids. Statistical evaluation of data was performed using the Chi-square test, Fischer's exact test and Log-Rank test. Kaplan-Meier survival curves were drawn with GraphPad PRISM 7.0 (La Jolla, California, USA).

RESULTS

Incidence of rejection and graft loss

During the 7-year follow-up, 29 cases of acute cellular rejection (ACR) and 21 antibody-mediated (AMR) rejections were diagnosed. Eighteen kidney recipients (13.6%) experienced ACR and 10 (7.6%) AMR, while 11 developed AMR and ACR simultaneously (52% of all AMR episodes). AMR during the first year was observed in 17 (81%) cases; eight recipients developed AMR during the first month after transplantation and 7 during the next 2 months after transplantation. A trend for higher occurrence of AMR with an increasing number of HLA mismatches was observed. Recipients with fully HLA-A, -B and -DR-matched grafts remained free of AMR, while 35% of those with 6 HLA mismatches developed AMR (p = 0.049). No

Table 2. Pretransplant donor and non-donor-specific antibodies and correlation to the incidence of antibody-mediated rejection and graft failure

	Patients (n=132)	AMR[1] (n=21)	P value	Graft failure (n=18)	P value
Preformed DSA[2]	21 (16%)	10 (48%)	0.0001	7 (33%)	0.0224
DSA class I only	14 (11%)	5 (36%)	0.0497	5 (36%)	0.0245
DSA class I (& class II)	17 (13%)	7 (41%)	0.0067	6 (35%)	0.0134
DSA class II only	4 (3%)	3 (75%)	0.0127	1 (25%)	NS
DSA class II (& class I)	7 (5%)	5 (71%)	0.0011	2 (29%)	NS
DSA class I and II	3 (2%)	2 (67%)	NS	1 (33%)	NS
Preformed non DSA[3]	40 (30%)	4 (10%)	NS	3 (8%)	NS
Non-DSA class I only	30 (23%)	4 (13%)	NS	3 (10%)	NS
Non-DSA class II only	2 (1.5%)	0	NS	0	NS
Non-DSA class I & II	8 (6%)	0	NS	0	NS

Legend: [1]AMR – antibody-mediated rejection, [2]DSA – donor-specific antibodies, [3]Non-DSA – non-donor-specific antibodies

influence of gender, PRA levels, induction immunosuppressive therapy or other factors on the development of cellular and antibody-mediated rejection was found (Table 1). AMR occurred more frequently after retransplantation (p = 0.0179) and was associated with increased risk of graft failure: nine (43%) out of 21 recipients who experienced AMR lost their grafts, while 9 grafts (8%) failed in the remaining 111 recipients free of AMR (p = 0.0001).

Pretransplant HLA antibodies

Twenty-one (16%) recipients had preformed DSA before transplantation, while 40 (30%) had non-DSA. The majority of DSA specificities were against HLA class I antigens (66.7% class I; 14.3% class I & II); non-DSA were also more frequently specific to HLA class I antigens (75% class I, 20% I & II). The pretransplant presence of DSA significantly correlated with higher risk of AMR, both for class I- and class II-specific antibodies (p < 0.0001) (Table 2), while non-DSA were not associated with the occurrence of AMR. Concerning graft survival, DSA and especially DSA class I (also simultaneously with class II antibodies) were associated with a higher rate of long-term graft
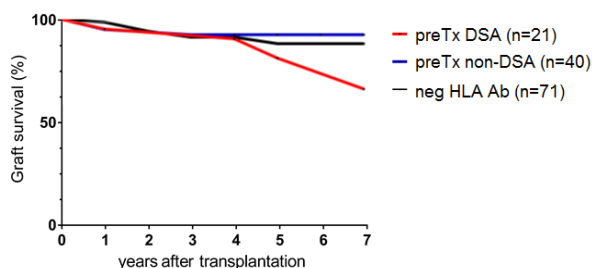
failure (p = 0.0224) (Table 2 and Fig.1a). Interestingly, graft survival was similar between DSA-positive and DSA-negative cohorts, with graft failures in the DSA-positive patients occurring more frequently only after the fourth year after transplantation. Comparison of individual specificities of DSA+ with DSA-negative recipients showed a trend of increased risk of graft failure in patients with DSA+ for class I (Log-Rank test p = 0.0106, Fig.1b). Pretransplant non-DSA did not influence graft survival (Fig.1a).
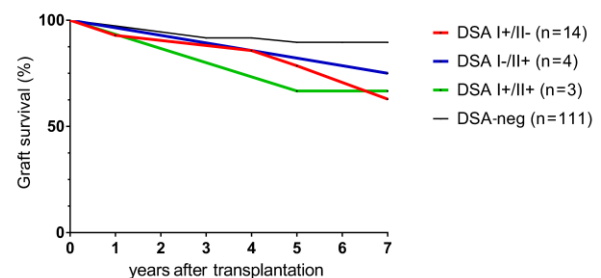

Figure 1b. Kaplan-Meier curves of graft survival in DSA-positive and DSA-negative kidney recipients. Increased risk of graft failure in DSA class I positive recipients (p = 0.0106).

Posttransplant HLA antibodies

During the first year after transplantation, 15 recipients lost their preformed DSA (71.4% pretransplant DSA-positive), and 21 recipients developed *de novo* DSA. Fifteen (71.4%) of those were pretransplant HLA antibody-negative, while 6 (28.6%) had pretransplant non-DSA. Six recipients (28.6%) had persistent pretransplant DSA at the end of the first year after transplantation. Conversely, non-DSA production decreased during the first year from 30.3% to 10.7%.

During the first 3 months after transplantation, the presence of DSA correlated with a higher risk of AMR (p = 0.0074) (Table 3) and graft failure (compared with

Figure 1a. Kaplan-Meier curves of graft survival in kidney recipients with pretransplant DSA, non-DSA and without HLA antibodies. Increased risk of graft loss in pretransplant DSA-positive patients (p = 0.0330).

Table 3. Donor and non-donor-specific antibodies after transplantation (3 months) and correlation with the incidence of antibody mediated rejection (AMR) and graft failure

	Patients (n=128)	AMR[1] (n=15)	P value	Graft failure (n=18)	P value
DSA[2]	18 (14%)	6 (33%)	0.0074	6 (33%)	0.0299
DSA class I only	13 (5 *de novo*)	3 (23%)	NS	4 (31%)	NS
DSA class II only	3 (2 *de novo*)	2 (67%)	NS	2 (67%)	NS
DSA class I and II	2 (1 *de novo*)	1 (50%)	NS	0	NS
Persistent DSA	10 (8%)	4 (40%)	0.0171	4 (40%)	0.0473
Lost pretransplant DSA	11 (9%)	5 (45%)	NS	3 (27%)	NS
De novo DSA	8 (6%)	2 (25%)	NS	2 (25%)	NS
Non-DSA[3]	37 (30%)	8 (22%)	0.0551	5 (14%)	NS
Non-DSA class I only	20 (16%)	4 (20%)	NS	4 (20%)	NS
Non-DSA class II only	5 (4%)	0	NS	0	NS
Non-DSA class I and II	12 (9%)	4 (33%)	NS	1 (8%)	NS

Legend: [1]AMR - antibody mediated rejection, [2]DSA – donor-specific antibodies, [3]Non-DSA – non donor-specific antibodies

DSA-negative recipients and those with non-DSA – Fig.2a). Interestingly, persistent DSA (pre- and post-transplant) were related to the highest risk for development of AMR (p = 0.0171), while no correlation was found between *de novo* DSA and the incidence of AMR. Surprisingly, persistent DSA at 3 months after transplantation were related to worse long-term (7 years) graft survival than *de novo* DSA at the same time point. *De novo* DSA played a role in graft survival during the first 4 years after transplantation (Fig.2b), but after the fourth year, recipients with pretransplant DSA were at a higher risk of graft failure. At 6 and 12 months after transplantation, the presence of antibodies (DSA and non-DSA) was not associated with AMR or graft failure (results not shown).

DISCUSSION

As indicated above, testing for donor-specific antibodies is now essential for the diagnosis of AMR after organ transplantation.[14] However, unresolved questions remain concerning the clinical relevance of antibodies (as defined using the SA technique) for predicting long-term allograft survival and the timing of posttransplant monitoring of antibodies.

In line with previous reports, an increased risk of graft failure in transplant recipients who experienced AMR was observed.[15] Retransplanted, high-risk kidney recipients and those with worse HLA matching with their respective donors were more prone to AMR. While all recipients included in the study were free of cytotoxic donor-specific antibodies before transplantation (CDC crossmatch negative), pretransplant DSA revealed retrospectively using the SA beads technique came up as a clear predictor for the development of AMR. This finding is in agreement with previous reports showing higher incidence of AMR in recipients with pretransplant DSA.[16-19]

Concerning graft survival, a new intriguing finding of our study was that impaired survival in DSA-positive recipients became apparent only 4 years after transplantation. Süsal et al. (2011) did not find worse
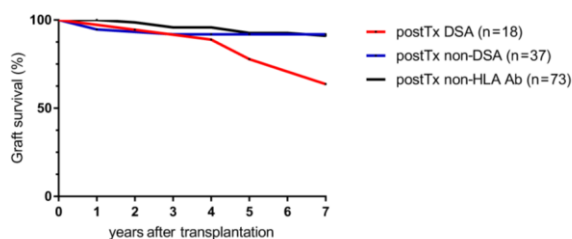

Figure 2a. Kaplan-Meier curves of kidney recipients with HLA-specific antibodies (DSA and non-DSA) and recipients without HLA antibodies. Increased risk of graft loss in DSA-positive patients (p = 0.0210).
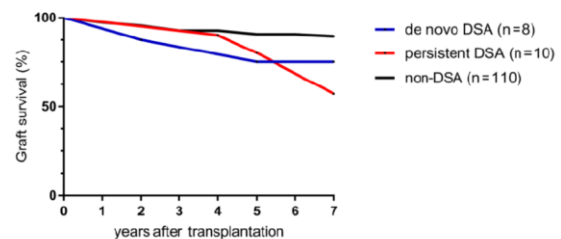

Figure 2b. Kaplan-Meier curves of graft survival in kidney recipients with persistent pretransplant DSA, de novo DSA and non-DSA detected 3 months after transplantation. Although de novo DSA played a main role in inferior graft survival during the first 4 years after transplantation, recipients with pretransplant DSA were at higher risk of graft failure after the fourth year (p = 0.0317).

allograft survival in recipients positive for DSA as defined using the SA technique; however, the follow-up speculate that the relatively late graft failure in patients with pretransplant DSA might be due to the slow damaging effect exerted by (low-level) DSA (20) and/or by accommodation of the transplanted kidney to antibody injury.[21] We found no correlation between non-DSA and the development of AMR[10], even though almost one third (28%) of the cohort with non-DSA developed *de novo* DSA after transplantation. In agreement with other studies, this may be a risk factor for graft survival.[22, 23]

As far as the relevance of the detection time point of antibodies, testing for DSA 3 months after transplantation is, in our estimation, important for predicting allograft outcomes. At 3 months, recipients with DSA had a higher risk for the development of AMR and worse long-term allograft survival. In our cohort, persistent DSA had a stronger impact on the incidence of AMR and graft survival in comparison with *de novo* DSA. Everly et al.[23] suggest that the production of *de novo* DSA occurs more often during the first year after transplantation and that these antibodies are associated with graft failure, which is also in line with our findings.

In conclusion, detection of antibodies to HLA antigens using SA bead technology before and three months after transplantation is predictive of impaired long-term graft survival. Patients with preformed and persisting DSA during the first three months after transplantation could be considered as a risk group and should be carefully monitored.

Acknowledgments

REFERENCES

1. Colvin RB, Smith RN. Antibody-mediated organ-allograft rejection. Nat Rev Immunol. 2005;5: 807-817.
2. Farkash EA, Colvin RB. Diagnostic challenges in chronic antibody-mediated rejection Nat Rev Nephrol. 2012;8: 255-257.
3. Loupy A, Suberbielle-Boissel C, Hill GS, Lefaucheur C, Anglicheau D, Zuber J, Martinez F, Thervet E, Méjean A, Charron D, Duong van Huyen JP, Bruneval P, Legendre C, Nochy D. Outcome of subclinical antibody-mediated rejection in kidney transplant recipients with preformed donor-specific antibodies. Am J Transplant. 2009;9: 2561-2570.
4. Otten HG, Verhaar MC, Borst HP, Hené RJ, van Zuilen AD. Pretransplant donor-specific HLA class-I and -II antibodies are associated with an increased risk for kidney graft failure. Am J Transplant. 2012;12:1618-1623.
5. Lefaucheur C1, Loupy A, Hill GS, Andrade J, Nochy D, Antoine C, Gautreau C, Charron D, Glotz D, Suberbielle-Boissel C. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. J Am Soc Nephrol. 2010;21:1398-1406.
6. Wiebe C, Gibson IW, Blydt-Hansen TD, Karpinski M, Ho J, Storsley LJ, Goldberg A, Birk PE, Rush DN, Nickerson PW. Evolution and clinical pathologic correlations of *de novo* donor-specific HLA antibody post kidney transplant. Am J Transplant. 2012;12:1157-1167.
7. Amico P, Hönger G, Mayr M, Steiger J, Hopfer H, Schaub S. Clinical relevance of pretransplant donor-specific HLA antibodies detected by single-antigen flow-beads. Transplantation. 2009;87 1681-1688.
8. Süsal C, Ovens J, Mahmoud K, Döhler B, Scherer S, Ruhenstroth A, Tran TH, Heinold A, Opelz G. 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.
9. Süsal C, Wettstein D, Döhler B, Morath C, Ruhenstroth A, Scherer S, Tran TH, Gombos P, Schemmer P, Wagner E, Fehr T, Živčić-Ćosić S, Balen S, Weimer R, Slavcev A, Bösmüller C, Norman DJ, Zeier M, Opelz G; Collaborative Transplant Study Report. Association of Kidney Graft Loss With *De Novo* Produced Donor-Specific and Non-Donor-Specific HLA Antibodies Detected by Single Antigen Testing. Transplantation. 2015;99:1976-1980.
10. Richter R, Süsal C, Köhler S, Qidan S, Schödel A, Holschuh L, Brzoska M, Asbe-Vollkopf A, Büttner S, Betz C, Herrmann E, Gauer S, Seifried E, Geiger H, Seidl C, Hauser I. Pretransplant human leukocyte antigen antibodies detected by single-antigen bead assay are a risk factor for long-term kidney graft loss even in absence of donor-specific antibodies. Transplant International. 2016;29:988-998.
11. Lefaucheur C, Viglietti D, Bentlejewski C, Duong van Huyen JP, Vernerey D, Aubert O, Verine J, Jouven X, Legendre C, Glotz D, Loupy A, Zeevi A. IgG Donor-Specific Anti-Human HLA Antibody Subclasses and Kidney Allograft Antibody-Mediated Injury. J Am Soc Nephrol. 2016;27:293-304.
12. Guidicelli G, Guerville F, Lepreux S, Wiebe C, Thaunat O, Dubois V, et al. Non-Complement-Binding *De Novo* Donor-Specific Anti-HLA Antibodies and Kidney Allograft Survival. J Am Soc Nephrol. 2016;27:615-625.
13. Solez K, Colvin RB, Racusen LC, Haas M, Sis B, Mengel M, Halloran PF, Baldwin W, Banfi G, Collins AB, Cosio F, David DS, Drachenberg C, Einecke G, Fogo AB, Gibson IW, Glotz D, Iskandar SS, Kraus E, Lerut E, Mannon RB, Mihatsch M, Nankivell BJ, Nickeleit V, Papadimitriou JC, Randhawa P, Regele H, Renaudin K, Roberts I, Seron D, Smith RN, Valente M. Banff 07 classification of renal allograft pathology: updates and future directions. Am J Transplant. 2008;8:753-760.
14. Eckels DD, Stehlik J, Kfoury AG. The detection and role of circulating antibodies in rejection. Curr Opin Organ Transplant. 2013;18:589-594.
15. Gosset C, Lefaucheur C, Glotz D. New insights in antibody-mediated rejection. Curr Opin Nephrol Hypertens. 2014;23:597-604.
16. Lefaucheur C, Loupy A, Hill GS, Andrade J, Nochy D, Antoine C, Gautreau C, Charron D, Glotz D, Suberbielle-Boissel C. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. J Am Soc Nephrol. 2010;21:1398-1406.
17. Willicombe M, Brookes P, Santos-Nunez E, Galliford J, Ballow A, Mclean A, Roufosse C, Cook HT, Dorling A, Warrens AN, Cairns T, Taube D. Outcome of patients with preformed donor-specific antibodies following alemtuzumab induction and tacrolimus monotherapy. Am J Transplant. 2011;11:470-477.
18. Caro-Oleas JL, González-Escribano MF, Gentil-Govantes MÁ, Acevedo MJ, González-Roncero FM, Blanco GB, Núñez-Roldán A.. Clinical relevance of anti-HLA donor-specific antibodies detected by Luminex assay in the development of rejection after renal transplantation. Transplantation. 2012;94:338-44.

19. Worthington JE, Martin S, Al-Husseini DM, Dyer PA, Johnson RW. Posttransplantation production of donor HLA-specific antibodies as a predictor of renal transplant outcome. Transplantation. 2003;75:1034-1040.
20. Einecke G, Sis B, Reeve J, Mengel M, Campbell PM, Hidalgo LG, Kaplan B, Halloran PF. Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. Am J Transplant. 2009;9:2520-2531.
21. Cai J, Terasaki PI. Humoral theory of transplantation: mechanism, prevention, and treatment. Hum Immunol. 2005;66:334-342.
22. Loupy A, Lefaucheur C, Vernerey D, Prugger C, Duong van Huyen JP, Mooney N, Suberbielle C, Frémeaux-Bacchi V, Méjean A, Desgrandchamps F, Anglicheau D, Nochy D, Charron D, Empana JP, Delahousse M, Legendre C, Glotz D, Hill GS, Zeevi A, Jouven X. Complement-binding anti-HLA antibodies and kidney-allograft survival. N Engl J Med. 2013;1215-1226.
23. Everly MJ, Rebellato LM, Haisch CE, Ozawa M, Parker K, Briley KP, Catrou PG, Bolin P, Kendrick WT, Kendrick SA, Harland RC, Terasaki PI. Incidence and impact of *de novo* donor-specific alloantibody in primary renal allografts. Transplantation. 2013;95:410-417.