

Antibody-mediated vascular rejection of kidney allografts: a population-based study



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Summary

Background Rejection of allografts has always been the major obstacle to transplantation success. We aimed to improve characterisation of different kidney-allograft rejection phenotypes, identify how each one is associated with anti-HLA antibodies, and investigate their distinct prognoses.

Methods Patients who underwent ABO-compatible kidney transplantations in Necker Hospital and Saint-Louis Hospital (Paris, France) between Jan 1, 1998, and Dec 31, 2008, were included in our population-based study. We assessed patients who provided biopsy samples for acute allograft rejection, which was defined as the association of deterioration in function and histopathological lesions. The main outcome was kidney allograft loss—ie, return to dialysis. To investigate distinct rejection patterns, we retrospectively assessed rejection episodes with review of graft histology, C4d in allograft biopsies, and donor-specific anti-HLA antibodies.

Findings 2079 patients were included in the main analyses, of whom 302 (15%) had acute biopsy-proven rejection. We identified four distinct patterns of kidney allograft rejection: T cell-mediated vascular rejection (26 patients [9%]), antibody-mediated vascular rejection (64 [21%]), T cell-mediated rejection without vasculitis (139 [46%]), and antibody-mediated rejection without vasculitis (73 [24%]). Risk of graft loss was 9·07 times (95 CI 3·62–19·7) higher in antibody-mediated vascular rejection than in T cell-mediated rejection without vasculitis ($p < 0·0001$), compared with an increase of 2·93 times (1·1–7·9; $P = 0·0237$) in antibody-mediated rejection without vasculitis and no significant rise in T cell-mediated vascular rejection (hazard ratio [HR] 1·5, 95% CI 0·33–7·6; $p = 0·60$).

Interpretation We have identified a type of kidney rejection not presently included in classifications: antibody-mediated vascular rejection. Recognition of this distinct phenotype could lead to the development of new treatment strategies that could salvage many kidney allografts.

Funding None.

Introduction

Kidney transplantation is the treatment of choice for patients with end-stage renal disease, improving survival and quality of life and lowering costs compared with dialysis.^{1–3} The alloimmune response induced by transplantation from a donor who differs genetically from the kidney recipient has always been the major obstacle to graft success. When not controlled, this response will destroy the graft,⁴ leading to increased morbidity, mortality, and costs.

In the past decade, the view of acute allograft rejection has changed greatly. Although rejection was initially thought to be a T cell-mediated process, humoral mechanisms have progressively been recognised and are now acknowledged to lead to high rates of graft loss in HLA-incompatible kidney transplants.^{5,6} International classification presently includes two acute rejection phenotypes: T cell-mediated rejection and antibody-mediated acute rejection.^{7,8} However, rejection phenotypes and outcomes are more complex in clinical practice than in theory. Phenotypes might be difficult to identify or missed entirely, with direct negative effects on therapeutic management.

Vascular rejection of kidney allografts—defined by endarteritis, which is infiltration of immune cells beneath the endothelium—has been thought to be a T cell-mediated process.⁴ Since the late 1990s, vascular rejection has been reported as a severe clinical disorder that does not respond to usual treatments directed at T cells.⁴ More recently, clinical findings have suggested its association with alloantibodies,^{9–12} challenging the notion of a unique T cell-mediated rejection process in vascular rejection. Importantly, these findings have been extended to transplantation of hearts,¹³ composite tissues,¹⁴ and small bowels,¹⁵ and are reinforced by murine models showing that alloantibodies interact with vessels,^{16,17} pointing towards a general idea of vascular damage and immune-mediated arteriosclerosis.¹⁸

We thus aimed to redefine rejection patterns by addressing their distinct clinical, histological, and immunological phenotypes, and their prognoses. In view of new immunosuppressive drugs available, such information would be particularly useful for adaptation of treatment strategies to rejection type, which might improve long-term prognoses of kidney allografts. We postulated that vascular rejection could be associated

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with anti-HLA antibodies, which might have important clinical implications for graft survival.

Methods

Participants

Patients who underwent kidney transplantation in Necker Hospital and Saint-Louis Hospital (Paris, France) between Jan 1, 1998, and Dec 31, 2008, were included in our population-based study. Patients were followed up until March 31, 2010. We used an additional independent validation sample of patients who underwent kidney transplantation in Foch Hospital (Suresnes, France) between Jan 1, 2004, and Jan 31, 2010 (appendix).

All transplants were ABO compatible (ie, donors and recipients had the same blood type). Negative IgG T-cell and B-cell complement-dependent cytotoxicity cross-matching was necessary for all recipients. We excluded patients with primary non-functioning grafts due to surgical vascular or urological problems.

The study was approved by the institutional review boards of the two hospitals in Paris. Use of data from Foch Hospital is based on agreement between centres that participate in the national database system

(appendix). All patients provided written informed consent.

Procedures

We took clinical data for donors and recipients from two validated computer databases (Données Informatiques Validées en Transplantation and Agence de la Biomédecine), in which data are prospectively entered at specific points for each patient (day 0, 6 months, and 1 year post-transplantation) and follow-up is updated annually thereafter (appendix). All data were retrieved from the database on March 31, 2010. Data from Foch Hospital were retrieved on Dec 22, 2011.

We assessed all patients who provided an indication biopsy sample (for deterioration in function, proteinuria, or impaired function) as part of standard care between Jan 1, 1998, and March 31, 2010, for acute clinical rejection episodes. Acute rejection was defined as the association of deterioration in function and histopathological lesions of allograft rejection, according to consensus rules represented by the international Banff classification criteria in use at the time.^{7,19,20}

Patients with rejection episodes deemed to be T cell-mediated were given methylprednisolone pulses (500 mg/day for 3 days). Patients whose rejection was not controlled by this regimen received additional rabbit antithymocyte globulin (1.5 mg/kg/day for 5 days) or muromonab-CD3 (5 mg/day for 5 days). Patients who had episodes of antibody-mediated rejection were initially given methylprednisolone pulses (500 mg/day for 3 days) and intravenous immune globulin (2 g/kg, repeated every 3 weeks for three rounds). After January, 2004, all patients with antibody-mediated rejection received four plasmaphereses and two weekly doses of rituximab (375 mg/m²) as additional treatment.²¹ The main outcome was kidney allograft loss, which was defined as the return of patient to dialysis.

Patients with rejection episodes were retrospectively reassessed between March, 2010, and December, 2010, with review of histology and immunochemistry for C4d in allograft biopsies and identification of circulating donor-specific anti-HLA antibodies on sera saved at time of biopsies. All graft biopsies of patients with biopsy-proven acute rejection were reviewed by two renal pathologists (DN, GSH) masked to clinical information. All biopsies were scored and graded from 0 to 3 according to the updated Banff criteria^{7,8} for several histological factors: glomerular inflammation, tubulitis, interstitial inflammation, endarteritis (vasculitis), peritubular capillary inflammation, transplant glomerulopathy, interstitial fibrosis, tubular atrophy, arteriosclerosis, and hyaline arteriolar thickening. The microcirculation inflammation score was defined as the sum of glomerular and peritubular capillary inflammation, and the tubular and interstitial score by the sum of interstitial inflammation and tubulitis. C4d staining was done by immunochemistry on paraffin sections

For Données Informatiques Validées en Transplantation see <http://divat.fr>

For Agence de la Biomédecine see <http://www.agence-biomedecine.fr>

See Online for appendix

	Kidney recipients without rejection (n=1777)	Kidney recipients with rejection (n=302)	p value*
Recipient age (years)	49 (13) [A1]	45 (10)	<0.0001
Men	1225 (69%)	148 (49%)	<0.0001
Retransplantation	212 (12%)	122 (40%)	<0.0001
Time since dialysis (months)	51 (54)	53 (62)	0.56
Donor age (years)	51 (16)	50 (12)	0.29
Male donor	970 (55%)	169 (56%)	0.66
Deceased donor	1444 (81%)	239 (79%)	0.38
Cardiovascular cause of donor death	622 (43%)	98 (41%)	0.39
Cold ischaemia time (h)	18.8 (10.3)	17.8 (8.1)	0.09
Delayed graft function	906 (51%)	177 (59%)	0.02
Cause of nephropathy			
Diabetes	211 (12%)	18 (6%)	0.003
Vascular	164 (9%)	15 (5%)	0.02
Glomerulopathy	433 (24%)	125 (41%)	<0.0001
Congenital	291 (16%)	88 (29%)	0.0009
Other	70 (4%)	14 (5%)	0.53
Interstitial nephropathy	171 (10%)	35 (12%)	0.29
Unknown	437 (25%)	7 (2%)	<0.0001
Immunology			
HLA A+B mismatch	2.1 (1.1) [A1]	1.2 (0.4)	<0.0001
HLA DR mismatch	0.8 (0.7)	1.1 (0.3)	<0.0001
Blood group†			
Type A	747 (42%)	122 (40%)	0.41
Type B	176 (10%)	49 (16%)	0.0027
Type O	730 (41%)	104 (34%)	0.0134
Type AB	84 (5%)	27 (9%)	0.0058

Data are mean (SD) or n (%). NS=non-significant. * χ^2 tests for comparison of proportions and unpaired t test for comparison of continuous variables †Data missing for 40 patients without rejection. [A1: Rounded for consistency.]

Table 1: Baseline characteristics

with human C4d polyclonal antibody (Biomedica Gruppe, Vienna, Austria).

Presence of circulating donor-specific anti-HLA-A, anti-HLA-B, anti-HLA-DR, and anti-HLA-DQ antibodies at the time of biopsy was retrospectively analysed with single-antigen flow bead assays (One Lambda, Canoga Park, CA, USA) on the Luminex platform. All beads showing a normalised mean intensity of fluorescence higher than 500 arbitrary units were judged positive, as previously described.⁶ For each patient, we recorded the number, specificities, and mean intensity of fluorescence of all donor-specific anti-HLA antibodies detected. We defined the maximum mean intensity of fluorescence of donor-specific anti-HLA antibodies as the highest ranked donor-specific bead. We categorised concentration of donor-specific antibody at time of rejection as absence (0), presence (1, 2, or 3) according to thresholds previously defined.⁶

HLA typing of transplant recipients was done by molecular biology (Innolipa HLA Typing Kit, Innogenetics, Ghent, Belgium). For all donors, tissue typing was done with the microlymphocytotoxicity technique with One Lambda INC tissue-typing trays and was controlled by molecular biology.

Statistical analysis

We provide mean (SD) values for descriptive analyses of continuous variables, with the exception of mean intensity of fluorescence, for which we use mean (SE) because of its wide distribution. We compared means and proportions of identified rejection phenotypes with Student's *t* test, ANOVA, or χ^2 test (Fisher's exact test when appropriate).

We addressed the hypothesis that vascular rejection with donor-specific antibodies is an independent event with unsupervised methods such as hierarchical cluster analysis and principal component analysis on the basis of combined histological lesions, C4d staining, and concentrations of donor-specific anti-HLA antibodies. We used Kaplan-Meier analysis to assess kidney allograft survival after rejection. We compared kidney allograft survival with the log-rank test across rejection phenotypes. A Bonferroni correction for several tests was used when we did two-by-two survival analysis comparison. The time of origin was time of acute rejection and the event of interest was a graft loss. In case of death with a functioning graft, we censored graft survival at time of death.²²

To identify factors associated with risk of graft failure after antibody-mediated vascular rejection, we did univariate Cox analyses with variables such as baseline characteristics (donor or recipient age, cold ischaemia time, donor sex, donor's cause of death, type of nephropathy, and HLA mismatches) and characteristics of rejection (histopathology, immunology, and type of antirejection treatment used). All variables with a *p* value of 0.20 or less were then included in one multivariate Cox model. The proportionality assumption of the Cox model was verified with the log-graphic method.

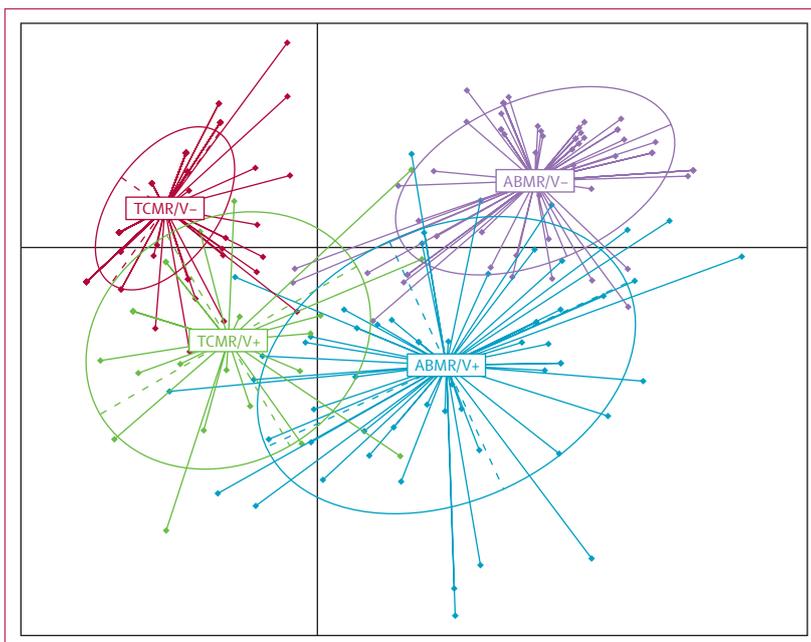


Figure 1: Identification of four distinct rejection patterns according to clinical, histological, and immunological variables

The unsupervised principal component analysis examined kidney recipients with acute biopsy-proven rejection with seven variables: glomerulitis, peritubular capillaritis, donor-specific anti-HLA antibodies, C4d deposition, interstitial inflammation, tubulitis, and endarteritis. The horizontal axis opposes cellular rejection (interstitial inflammation and tubulitis) and antibody-mediated rejection (donor-specific anti-HLA antibodies, glomerulitis, peritubular capillaritis and C4d), as recognised by the international Banff classification. The vertical axis defines the presence or absence of lesions of endarteritis (appendix).

All these statistical analyses were subsequently replicated and confirmed in the independent validation sample. We did hierarchical cluster analysis and dendrograms with the *hcluster* module of the *amap* package (version 0.8.7) of R (version 2.10.1). We did the principal components analysis with the *dudi.pca* module of the *ade4* package (version 1.5-1) of R (version 2.10.1).²³ We used Stata (version 11.0) for descriptive and survival analyses. All tests were two-sided and we used an α of 0.05, unless otherwise stated.

Role of the funding source

There was no funding source for this study. CL and AL had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

2079 patients received a kidney allograft in Necker Hospital or Saint-Louis Hospital, of whom 302 (15%) had acute biopsy-proven rejection. Table 1 shows the characteristics of recipients at time of renal transplantation who subsequently did and did not have acute allograft rejection. Acute biopsy-proven rejection occurred at a median of 3.1 months post-transplant (IQR 1.0–11.3). 790 patients provided 952 indication biopsy samples. 147 samples (15%) showed acute tubular necrosis, 64 (7%) borderline lesions, 76 (8%) calcineurin inhibitor toxic effects, 68 (7%) recurrent disease, 40 (4%)

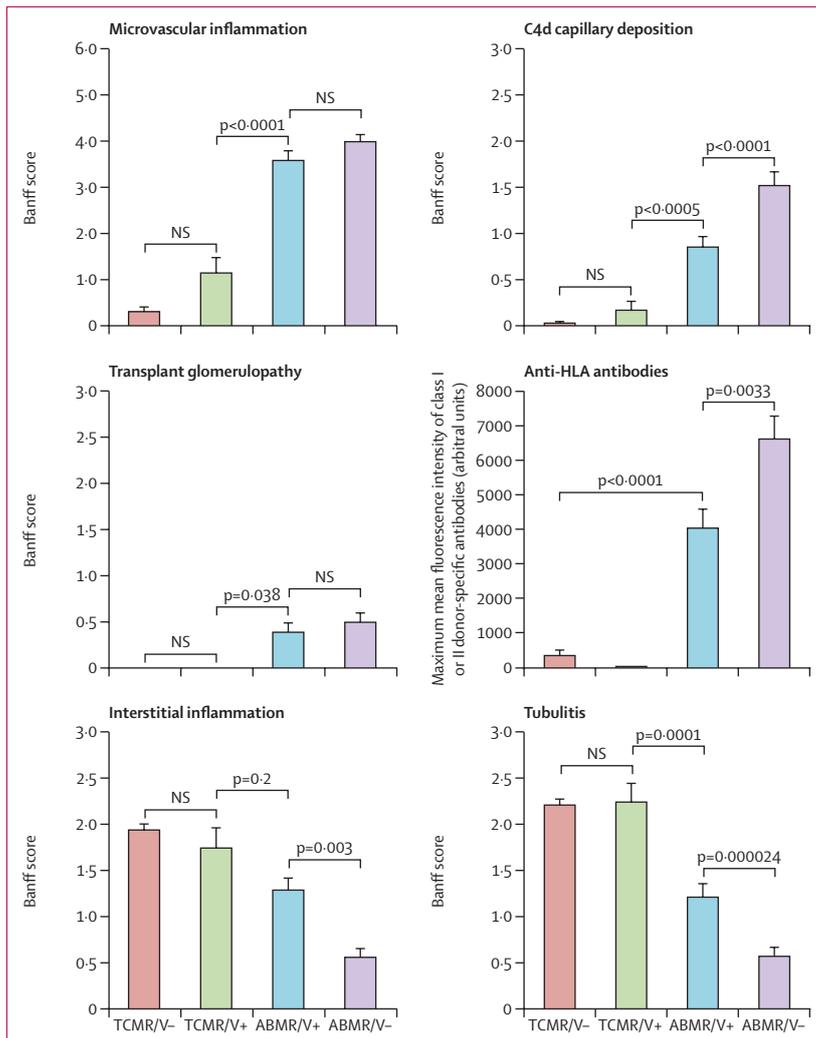


Figure 2: Comparison of morphological and immunological variables in the four rejection patterns
 Bars represent SD. NS=not significant. TCMR/V-=T cell-mediated rejection without vasculitis.
 TCMR/V+=T cell-mediated vascular rejection. ABMR/V+=antibody-mediated vascular rejection.
 ABMR/V-=antibody-mediated rejection without vasculitis.

BK virus nephropathy, 124 (13%) interstitial fibrosis and tubular atrophy, and 44 (5%) transplant glomerulopathy, and 87 (9%) had other diagnoses. Median follow-up after transplantation was 43.7 months (27.2–64.1) in patients with acute rejection and 49.5 months (27.4–72.0) in those who did not experience rejection. 123 (7%) of the 1777 patients who did not experience rejection had graft loss, as did 40 (13%) of the 302 who had rejection.

We retrospectively identified four distinct rejection patterns: T cell-mediated vascular rejection (26 patients [9%]), antibody-mediated vascular rejection (64 [21%]), T cell-mediated rejection without vasculitis (139 [46%]), and antibody-mediated rejection without vasculitis (73 [24%]; figure 1). Median time between kidney transplantation and rejection was 1.6 months (IQR 0.2–4.4) in T cell-mediated vascular rejection, 1.1 months (0.4–4.4) in antibody-mediated vascular rejection,

3.3 months (3.0–12.0) in T cell-mediated rejection without vasculitis, and 7.0 months (1.1–18.0) in antibody-mediated rejection without vasculitis.

Antibody-mediated rejection both with and without vasculitis was associated with microvascular inflammation, transplant glomerulopathy, and anti-HLA antibodies (figure 2). 20 (31%) of the 64 patients with antibody-mediated vascular rejection and 26 (36%) of the 73 patients with antibody-mediated rejection without vasculitis had circulating class I immunodominant donor-specific anti-HLA antibodies. 44 patients (69%) with antibody-mediated vascular rejection and 47 (64%) with antibody-mediated rejection without vasculitis had circulating class II immunodominant donor-specific anti-HLA antibodies. Of patients with antibody-mediated vascular rejection, 33 patients (52%) had endarteritis graded as v1 with Banff criteria, 19 (30%) had v2, and 12 (19%) had v3. Of those with T cell-mediated vascular rejection, 16 (62%) had v1 endarteritis, 5 (19%) had v2, and 5 (19%) had v3. Additionally, 56 patients (88%) with antibody-mediated vascular rejection, 73 (100%) with antibody-mediated rejection without vasculitis, and 4 (15%) with T cell-mediated vascular rejection had glomerular and peritubular capillary inflammation; 46 (72%), 27 (37%), 134 (96%), and 23 (88%) had interstitial inflammation; and 40 (63%), 21 (29%), 137 (99%) and 23 (88%) had tubulitis (appendix). C4d staining was positive in 36 patients (56%) with antibody-mediated vascular rejection, of whom 27 (75%) had intimal staining for C4d in arteries and arterioles (appendix). C4d staining was positive in 45 patients (62%) with antibody-mediated rejection without vasculitis, 6 (4%) with T cell-mediated rejection without vasculitis, and 2 (8%) with T cell-mediated vascular rejection.

Importantly, some patients with antibody-mediated vascular rejection were misclassified at time of biopsy as having T cell-mediated rejection (table 2), and received inappropriate treatment. Notably, the 42 patients with antibody-mediated vascular rejection treated with a T cell-mediated rejection strategy had a significantly greater risk of graft loss than did the 22 who received appropriate treatment (log rank $p=0.035$; appendix).

The Kaplan-Meier estimate of graft survival after T cell-mediated rejection without vasculitis (97.7% at 24 months; 93.2% at 72 months) was similar to that in patients after T cell-mediated vascular rejection (95.6% at 24 months; 91.3% at 72 months; figure 3). Graft survival after antibody-mediated rejection without vasculitis (93.8% at 24 months; 82.6% at 72 months) was significantly lower than after T cell-mediated rejection without vasculitis ($p=0.0237$). Patients with antibody-mediated vascular rejection had the poorest graft survival (82.5% at 24 months; and 50.3% to 72 months) when compared with those with T cell-mediated rejection without vasculitis ($p<0.0001$; figure 3). Cox univariate analysis showed that risk of graft loss was 9.07 times

(95% CI 3.62–19.7) higher in antibody-mediated vascular rejection than in T cell-mediated rejection without vasculitis ($p < 0.0001$). By comparison, antibody-mediated rejection without vasculitis was associated with an increase of 2.93 times (1.1–7.9; $p = 0.0237$). T cell-mediated vascular rejection was not associated with an increased risk of graft loss compared with T cell-mediated rejection without vasculitis (hazard ratio 1.5, 95% CI 0.33–7.6; $p = 0.60$).

In univariate analysis, none of the clinical factors investigated were associated with graft loss after antibody-mediated vascular rejection (data not shown; all $p > 0.20$). Transplant glomerulopathy ($p = 0.03$), microcirculation inflammation ($p = 0.18$), interstitial inflammation and tubulitis score ($p = 0.09$), endarteritis scores ($p = 0.07$), concentration of donor-specific anti-HLA antibodies at time of biopsy ($p = 0.06$), and antibody-targeting regimen ($p = 0.10$) were potential prognostic factors. When entered in one multivariate Cox regression analysis, a high interstitial inflammation and tubulitis score, high grade of endarteritis lesions, and the concentration of circulating donor-specific anti-HLA antibodies in the biopsy at time of rejection were independent predictors of graft loss (table 3). Conversely, the use of an antibody-targeting strategy was independently associated with a reduced risk of graft loss compared with non-antibody directed treatment strategies (table 3).

The principal component analysis of the independent validation cohort ($n = 672$) confirmed that antibody-mediated vascular rejection with donor-specific anti-HLA antibodies had a distinct morphological and immunological phenotype compared with other rejection phenotypes (appendix). Additionally, antibody-mediated vascular rejection with donor-specific anti-HLA antibodies had a distinct phenotype characterised by the highest risk of graft loss (log-rank test $p < 0.0001$; appendix).

Discussion

We have defined four relevant clinical phenotypes of rejection after kidney transplantation. With contemporary immunological and histopathological techniques, we have shown that the previously unrecognised phenotype of antibody-mediated vascular rejection is characterised by endarteritis, associated with circulating donor-specific anti-HLA antibodies, and has the poorest graft survival. Our data suggest that antibody-depleting strategies could be beneficial in the long term in patients with donor-specific anti-HLA antibody-associated vascular lesions.

Lesions of vascular rejection have long interested transplant groups because of their presentation and severity.^{24,25} Vascular rejection has been considered to be a severe disorder that does not respond to traditional treatment for T cell-mediated rejection and so necessitates potent antilymphocyte-antibody treatment (muromonab-CD3 or antithymocyte globulin).²⁶ Endarteritic lesions form part of the Banff criteria only for diagnosis of T cell-mediated rejection. Only cases with transmural arteritis or arterial fibrinoid change, or both, and necrosis

	TCMR/V- (n=139)	TCMR/V+ (n=26)	ABMR/V+ (n=64)	ABMR/V- (n=73)
Traditional approach				
T cell-mediated rejection	139 (100%)	24 (92%)	29 (45%)	0
Antibody-mediated rejection	0	2 (8%)	35 (55%)	73 (100%)
Treatment strategy				
Steroids	139 (100%)	19 (73%)	18 (28%)	0
Steroids plus muromonab-CD3 or rabbit antithymocyte globulin	0	5 (19%)	11 (17%)	0
Steroids and intravenous immune globulin	0	2 (8%)	13 (20%)*	29 (40%)
Steroids, plasmapheresis, intravenous immune globulin, and rituximab	0	0	22 (34%)	44 (60%)

Data are n (%). TCMR/V- = T cell-mediated rejection without vasculitis. TCMR/V+ = T cell-mediated vascular rejection. ABMR/V+ = antibody-mediated vascular rejection. ABMR/V- = antibody-mediated rejection without vasculitis. *These patients were classified as having antibody-mediated rejection, but were not treated as such; steroids and intravenous immune globulin was practice at that time in the referring centre.

Table 2: Comparison between initial diagnosis of rejection made by international classification and the diagnosis of the new approach

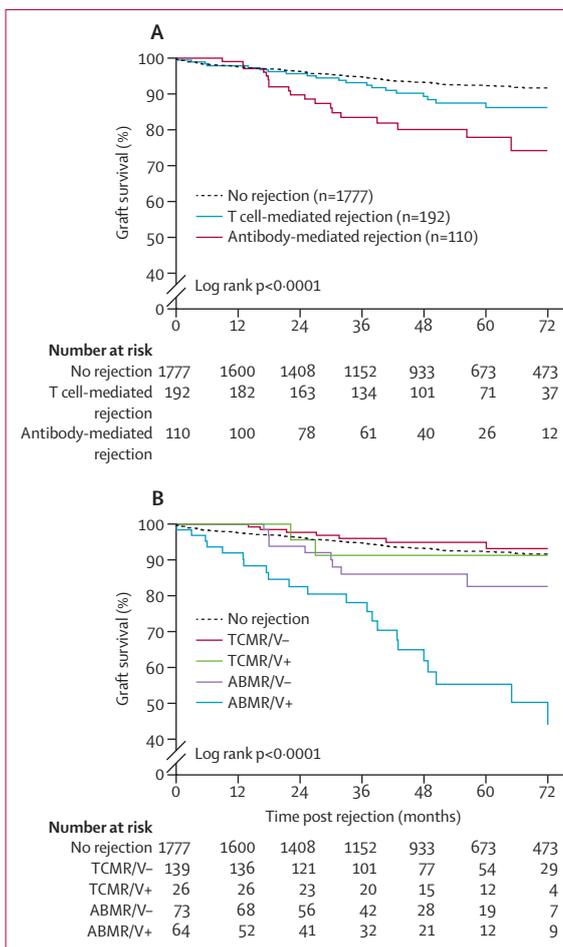


Figure 3: Kaplan-Meier curves for kidney graft survival by acute rejection phenotype

Initial diagnoses as per (A) Banff classifications and (B) our new approach. Graft survival in patients without rejection is purely illustrative; graft survival in these individuals starts at time of transplantation.

	Number of patients	Number of events	Hazard ratio (95% CI)	p value
Interstitial inflammation and tubulitis score*				
≤3	32	7	1	..
>3	32	14	4.33 (1.5-12.1)	0.005
Endarteritis score				
<3	52	15	1	..
3	12	6	5.17 (1.8-14.6)	0.002
DSA_{max} MFI				
<3000	41	9	1	..
>3000	23	12	3.88 (1.5-9.8)	0.004
Treatment strategy				
Steroids and intravenous immune globulin	13	7	1	..
Steroids plus muromonab-CD3 or rabbit antithymocyte globulin	29	11	0.4 (0.2-1.3)	0.1
Steroids, plasmapheresis, intravenous immune globulin, and rituximab	22	3	0.16 (0.04-0.66)	0.01

Hazard ratios were estimated in a single Cox proportional hazards model. DSA_{max} MFI=maximum mean intensity of fluorescence of donor-specific anti-HLA antibodies. *Interstitial inflammation and tubulitis score was defined as the sum of interstitial inflammation and tubulitis, and was graded from 0 to 6.

Table 3: Multivariate analysis of factors associated with graft loss in patients with antibody-mediated vascular rejection

Panel: Research in context

Systematic review

We searched PubMed for reports in any language published before April 30, 2012, with the search terms "kidney allograft rejection" and "antibody mediated rejection". We identified 691 reports overall, of which eight were results of the Banff working group and three were reviews. We did another search with the search terms "vascular rejection", "vasculitis/endarteritis", and "Banff allograft rejection classification". We identified 37 reports overall, of which eight were reviews. Previous studies focusing on vascular rejection of kidney allografts are limited by small samples and absence of contemporary techniques that are increasingly used worldwide for graft-rejection phenotyping. Additionally, little is known about the association of vascular rejection with donor-specific anti-HLA antibodies.

Interpretation

With a population-based approach, we have identified a type of allograft rejection that is not represented by the present classification of renal rejection. This type of rejection—antibody-mediated vascular rejection—is characterised by the association of circulating antibodies with arteritis lesions of allograft arteries and carries the highest risk of graft loss of all rejection phenotypes. If antibody-mediated vascular rejection becomes widely recognised, anti-HLA antibody-targeting strategies could reduce risk of graft loss. Overall, our results support the notion of immunological development of arteriosclerosis in people, which has been established in murine models.

of medial smooth muscle cells are deemed to be grade III T cell-mediated rejection or antibody-mediated rejection with the Banff criteria.^{7,8} Patients with v1 or v2 lesions are judged to have T cell-mediated rejection. Our study now permits the division of all cases of endarteritis into two separate profiles of rejection on the basis of donor-specific anti-HLA antibody status.

Antibody-mediated vascular rejection is a separate form of acute rejection, with a distinct prognosis. Our approach has allowed this rejection type to be epidemiologically

established. In the past decade, several reports have suggested that anti-HLA antibodies can be associated with endarteritis lesions.⁹⁻¹² Our study thus reinforces the idea that lesions of endarteritis form part of the range of general endothelial inflammatory changes mediated by anti-HLA antibodies. Importantly, our investigation provides the basis for the application of antibody-mediated transplant arteriopathy developed in mice¹⁷ into a human model represented by kidney transplantation. This idea extends beyond the specialty of renal transplantation to reports of immunological damage to arteries in transplantation of hearts,¹³ composite tissues,¹⁴ and small bowels.¹⁵ Generally, our findings reinforce those from previous studies,¹⁸ supporting the notion of immunological development of arteriosclerosis and atherogenesis in people, which has already been established in murine models.¹⁶

Overall, our study presents a contemporary picture of acute allograft rejection on the basis of techniques that are becoming standard in organ transplantation worldwide. Our principal component analysis allowed us to hierarchically rank the variables that characterise the different rejection profiles. Thus, we could establish that microcirculation inflammation and anti-HLA donor-specific antibodies on the one hand, and the presence of endarteritis lesions on the other, allow effective discrimination. Not surprisingly, C4d is not a principal discriminating factor in the diagnosis of rejection. Although several groups agree that C4d staining is specific when it occurs, it is not a sensitive indicator of humoral rejection activity.^{9,27-29}

The importance of precise identification of type of kidney allograft rejection is primarily therapeutic, because it enables assessment of treatment effectiveness. The resistance of antibody-mediated rejection to treatments traditionally given for T cell-mediated rejection has been recognised since the 1990s,³⁰ and the improvement in diagnostic criteria for antibody-mediated rejection has led to advances in treatment.^{21,31-34} The guiding principle of treatment in antibody-mediated rejection is diminution of donor-specific anti-HLA antibody concentration and production.³⁵ Plasmapheresis, intravenous immune globulin, rituximab, and more recently the proteasome inhibitor bortezomib³⁶ are used in such regimens. Our findings support the view that interventions directed towards a decrease in donor-specific anti-HLA antibody concentration and production could be useful. Further studies will be necessary to define which treatments are most effective in reversal of antibody-mediated vascular rejection; the observational design of our study does not allow us to make any formal therapeutic conclusions about the superiority of one treatment.

Our study has some limitations. It was not designed to provide mechanistic insights or pathways linking anti-HLA antibodies with vascular cell biology. Additionally, few graft losses were reported in some subgroups, indicating that we might not have had precision in our point estimates.

In conclusion, advances in HLA testing techniques, together with the ability to better characterise kidney allograft rejection histologically, have allowed different profiles of allograft rejection to be elucidated. Our study has identified a clinically relevant profile of rejection linking vascular rejection lesions in the allograft with circulating donor-specific anti-HLA antibodies (panel). The prognosis and course of this type of rejection is substantially different from other types of rejection, with antibody-mediated vascular rejection having the poorest outcome. Because rejection is still the leading cause of kidney allograft loss, we hope that the recognition of this distinct type of rejection will lead to development of new treatment strategies that could salvage many kidney allografts.

Contributors

CL, AL, DA, MD, J-PE, DG, CL, and XJ designed the study. CL, AL, JPD-V-H, CS, JV, DN, PB, DC, MD, and GSH collected data. AL, DV, JPD-V-H, CS, DN, PB, DC, J-PE, and XJ analysed data. AL, DV, J-PE, and XJ prepared figures. CL, AL, DV, DA, TB, J-PE, GSH, DG, CL, and XJ interpreted data. CL, AL, DV, DA, PB, J-PE, GSH, DG, CL, and XJ wrote the report.

Conflicts of interest

We declare that we have no conflicts of interest.

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