ORIGINAL ARTICLE

Complement-Binding Anti-HLA Antibodies and Kidney-Allograft Survival

Alexandre Loupy, M.D., Ph.D., Carmen Lefaucheur, M.D., Ph.D., Dewi Vernerey, M.P.H., Christof Prugger, M.D., Jean-Paul Duong van Huyen, M.D., Ph.D., Nuala Mooney, Ph.D., Caroline Suberbielle, M.D., Ph.D., Véronique Frémeaux-Bacchi, M.D., Ph.D., Arnaud Méjean, M.D., François Desgrandchamps, M.D., Dany Anglicheau, M.D., Ph.D., Dominique Nochy, M.D., Dominique Charron, M.D., Ph.D., Jean-Philippe Empana, M.D., Ph.D., Michel Delahousse, M.D., Christophe Legendre, M.D., Denis Glotz, M.D., Ph.D., Gary S. Hill, M.D.,* Adriana Zeevi, Ph.D., and Xavier Jouven, M.D., Ph.D.

ABSTRACT

BACKGROUND

Anti-HLA antibodies hamper successful transplantation, and activation of the complement cascade is involved in antibody-mediated rejection. We investigated whether the complement-binding capacity of anti-HLA antibodies plays a role in kidney-allograft failure.

METHODS

We enrolled patients who received kidney allografts at two transplantation centers in Paris between January 1, 2005, and January 1, 2011, in a population-based study. Patients were screened for the presence of circulating donor-specific anti-HLA antibodies and their complement-binding capacity. Graft injury phenotype and the time to kidney-allograft loss were assessed.

RESULTS

The primary analysis included 1016 patients. Patients with complement-binding donor-specific anti-HLA antibodies after transplantation had the lowest 5-year rate of graft survival (54%), as compared with patients with non–complement-binding donor-specific anti-HLA antibodies (93%) and patients without donor-specific anti-HLA antibodies (94%) (P<0.001 for both comparisons). The presence of complement-binding donor-specific anti-HLA antibodies after transplantation was associated with a risk of graft loss that was more than quadrupled (hazard ratio, 4.78; 95% confidence interval [CI], 2.69 to 8.49) when adjusted for clinical, functional, histologic, and immunologic factors. These antibodies were also associated with an increased rate of antibody-mediated rejection, a more severe graft injury phenotype with more extensive microvascular inflammation, and increased deposition of complement fraction C4d within graft capillaries. Adding complement-binding donor-specific anti-HLA antibodies to a traditional risk model improved the stratification of patients at risk for graft failure (continuous net reclassification improvement, 0.75; 95% CI, 0.54 to 0.97).

CONCLUSIONS

Assessment of the complement-binding capacity of donor-specific anti-HLA antibodies appears to be useful in identifying patients at high risk for kidney-allograft loss.

From the Paris Translational Research Center for Organ Transplantation (A.L., C. Lefaucheur, J.-P.D.H., M.D., D.G., C. Legendre, V.F.-B., X.J.), INSERM Unité 970 (A.L., C. Lefaucheur, D.V., C.P., J.-P.D.H., J.-P.E., X.J.), Department of Kidney Transplantation, Hôpital Necker, Université Paris Descartes, and Assistance Publique–Hôpitaux de Paris (AP-HP) (A.L., D.A., C. Legendre), the Departments of Nephrology and Kidney Transplantation (C. Lefaucheur, D.G.), Immunology and Histocompatibility (C.S., D.C.), and Urology (F.D.), Hôpital Saint-Louis, Paris Diderot University, AP-HP, and the Departments of Pathology (J.-P.D.H., D.N., G.S.H.), Immunology (V.F.-B.), and Urology (A.M.), Georges Pompidou European Hospital, INSERM Unité 940 (N.M., D.G.) — all in Paris; the Department of Nephrology and Kidney Transplantation, Foch Hospital, Suresnes, France (M.D.); and the Department of Transplantation Pathology, University of Pittsburgh Medical Center, Pittsburgh (A.Z.). Address reprint requests to Dr. Loupy at alexandreloupy@gmail.com.

Drs. Loupy and Lefaucheur and Drs. Zeevi and Jouven contributed equally to this article.

*Deceased.

N Engl J Med 2013;369:1215-26.
DOI: 10.1056/NEJMoa1302506

Despite considerable advances in transplantation, the induced alloimmune response remains a major determinant of late kidney-allograft loss.[1-3] In the United States and Europe, thousands of kidney transplants fail each year, and kidney-allograft failure is a major cause of end-stage renal disease, leading to increased morbidity, mortality, and costs.[4,5]

One of the most important advances in transplantation medicine has been the recognition that anti-HLA antibodies are destructive.[6-10] Various studies over the past decade have indicated that the alloimmune response, mediated by anti-HLA antibodies, plays a key role in the failure of kidney allografts; this concept has been extended to heart, lung, and composite tissue transplants.[6] Although anti-HLA antibodies are considered to be harmful, there is a wide spectrum of graft injury related to these antibodies, ranging from no recognizable damage to florid rejection.[11,12] Such a varied effect underscores the need to define distinct graft phenotypes and outcomes according to the presence or absence and characteristics of donor-specific anti-HLA antibodies after transplantation.

Since the pioneering discovery in 1969 that anti-HLA antibodies are lymphocytotoxic,[13] activation of the complement cascade has been considered to be a key component of antibody-mediated allograft rejection, and C4d deposition in renal capillaries has been considered the footprint of antibody-mediated allograft damage.[14-16]

The capacity of anti-HLA antibodies to bind complement fraction C1q, which is the first step in activation of the classic complement cascade, determines the cytotoxic potential of these antibodies, and an assessment of their complement-binding capacity may be useful both for risk stratification and for diagnosis of antibody-mediated rejection. Small studies have suggested that the C1q-binding properties of donor-specific anti-HLA antibodies may be specifically related to antibody-mediated rejection; these findings provide support for the general principle that complement-binding donor-specific anti-HLA antibodies have a role in the pathogenesis of antibody-mediated rejection.[17-24] We hypothesized that the complement-binding properties of donor-specific anti-HLA antibodies detected after transplantation are involved in kidney-allograft failure.

We conducted a study to define the full spectrum of kidney-allograft injury according to the C1q-binding properties of donor-specific anti-HLA antibodies in a large population-based study and to determine whether assessment for the presence of C1q-binding donor-specific anti-HLA antibodies after transplantation might improve risk stratification for kidney-allograft loss.

Methods

Study Population

We enrolled all consecutive patients who underwent kidney transplantation at Necker Hospital and Saint-Louis Hospital (Paris) between January 1, 2005, and January 1, 2011, in this population-based study. Patients were followed until April 15, 2012. We also included an external-validation cohort comprising patients who underwent kidney transplantation at Foch Hospital (Suresnes, France) between January 1, 2004, and January 31, 2010 (see the Methods section in the Supplementary Appendix, available with the full text of this article at NEJM.org). The study was approved by the institutional review boards of Necker Hospital, Saint-Louis Hospital, and Foch Hospital. Written informed consent was obtained from all patients. One Lambda donated reagents but was not otherwise involved in either the conduct of the study or the preparation of the manuscript.

The transplantation allocation system was identical for the three centers and followed the rules of the French national agency for organ procurement (Agence de la Biomédecine). All transplants were compatible with the ABO blood group. A negative result of cross-matching for IgG T-cell and B-cell complement-dependent cytotoxicity was required for all recipients.

Clinical Data

Clinical data on the donors and recipients in the derivation cohort (at Necker and Saint-Louis Hospitals) and the validation cohort (at Foch Hospital) were obtained from two national registries, Données Informatiques Validées en Transplantation (Necker Hospital) and Agence de la Biomédecine (Saint-Louis and Foch Hospitals). Anonymized data from these registries are prospectively entered at specific time points for each patient (on day 0 and 6 months and 1 year after transplantation) and are updated annually thereafter[25,26] (see the Methods section in the Supplementary Appen-

dix). The derivation-cohort data were obtained from the database on April 15, 2012, whereas the validation-cohort data were obtained on December 19, 2012. We documented all cases of acute clinical rejection, defined by deterioration in graft function, proteinuria, or impaired function and histopathological evidence of rejection, according to the consensus rules of the international Banff classification criteria,[27,28]

Immunosuppression protocols and treatment of allograft-rejection episodes after transplantation were similar among the centers.[29,30] The protocols and treatments are described in the Methods section in the Supplementary Appendix.

HISTOLOGIC AND IMMUNOCHEMICAL TESTS

We used specimens from protocol-specified graft biopsies performed 1 year after transplantation in 845 patients without any acute clinical rejection episodes diagnosed in the first year after transplantation, as well as specimens from biopsies performed in 171 patients with acute allograft rejection during the first year after transplantation. All graft-biopsy specimens were scored and graded from 0 to 3 according to the updated Banff criteria[27,28] by three trained pathologists who were unaware of the patient's status with respect to the presence of donor-specific anti-HLA antibodies, C1q-binding status, and clinical course (see the Methods section in the Supplementary Appendix). C4d staining was performed by means of immunochemical analysis on paraffin sections with the use of polyclonal human anti-C4d antibodies (Biomedica Gruppe).

DETECTION AND CHARACTERIZATION OF DONOR-SPECIFIC ANTIBODIES

All patients were tested for the presence of circulating donor-specific anti-HLA antibodies in banked serum samples (at the Jean Dausset Histocompatibility Laboratory, Paris) obtained at the time of transplantation (day 0) and in serum samples obtained at the time of the biopsy (1 year after transplantation or during an episode of acute rejection in the first year after transplantation). The presence of circulating donor-specific anti-HLA-A, -B, -Cw, -DR, -DQ, and -DP antibodies was retrospectively determined with the use of single-antigen flow bead assays (One Lambda) on a Luminex platform.

Serum samples from patients with circulating donor-specific anti-HLA antibodies were analyzed in a blinded fashion at the University of Pittsburgh for the presence of C1q-binding donor-specific anti-HLA antibodies with the use of single-antigen flow bead assays according to the manufacturer's protocol (C1qScreenTM, One Lambda).[17,18,21] For details, see the Methods section in the Supplementary Appendix.

STATISTICAL ANALYSIS

We used means and standard deviations for the description of continuous variables, with the exception of mean fluorescence intensity, for which we used the mean and standard error. We compared means and proportions using Student's t-test and the chi-square test (or Fisher's exact test if appropriate). Survival was analyzed from the time of transplantation to a maximum of 7 years, with kidney-graft loss as the event of interest. Data on graft survival were censored at the time of death.[31] Rates of kidney-allograft survival were plotted on Kaplan–Meier curves and compared according to anti-HLA antibody status with the use of the log-rank test. Cox proportional-hazards models were used to quantify hazard ratios and 95% confidence intervals for kidney-graft loss.

The association of clinical, histologic, functional, and immunologic factors with graft loss was assessed in separate univariate and multivariate Cox regression analyses. The factors identified in these analyses were thereafter included in a final multivariable model with stepwise backward elimination.

The predictive value that C1q-binding status added to a reference risk model (including independent predictors of the final multivariable model plus circulating donor-specific anti-HLA antibodies after transplantation) was evaluated with the use of the C-statistic. This analysis was repeated 1000 times with the use of bootstrap samples to derive 95% confidence intervals for the difference in the C-statistic between models. We calculated the continuous net reclassification improvement and the integrated discrimination improvement associated with the addition of C1q to the reference model.[32,33] Results for complement-binding donor-specific anti-HLA antibodies and kidney-allograft survival were replicated in the independent validation sample. Analyses were conducted with the use of SAS software, version 9.2 (SAS Institute), and R software (version 2.10.1).

All tests were two-sided, and P values less than 0.05 were considered to indicate statistical significance.

RESULTS

BASELINE CHARACTERISTICS OF THE KIDNEY-ALLOGRAFT RECIPIENTS

In total, 1016 patients undergoing renal transplantation (695 at Necker Hospital and 321 at Saint-Louis Hospital) were included in the main analysis. Three distinct populations were identified after transplantation, according to the presence or absence of donor-specific anti-HLA antibodies and complement-binding capacity: 700 patients without circulating donor-specific anti-HLA antibodies, 239 patients with non–complement-binding donor-specific anti-HLA antibodies, and 77 patients with complement-binding donor-specific anti-HLA antibodies. Table 1 shows the characteristics of the donors and recipients at the time of renal transplantation.

KIDNEY-ALLOGRAFT INJURY

In the first year after transplantation, acute clinical rejection developed in 171 patients: 96 patients had T-cell–mediated rejection (56%) and 75 had antibody-mediated rejection (44%). T-cell–mediated rejection occurred in 14 of 77 patients with donor-specific anti-HLA antibodies plus C1q-binding capacity (18%), in 30 of 239 patients with donor-specific anti-HLA antibodies without C1q-binding capacity (13%), and in 52 of 700 patients without donor-specific anti-HLA antibodies (7%) (P<0.001). Antibody-mediated rejection occurred in 37 patients with C1q-binding donor-specific anti-HLA antibodies (48%) and 38 patients with non–C1q-binding donor-specific anti-HLA antibodies (16%) (P<0.001).

Among the patients with C1q-binding donor-specific anti-HLA antibodies, 67 had microvascular inflammation (87%), 28 had tubular and interstitial inflammation scores of 2 or higher (on a scale of 0 to 3, with higher scores indicating more severe abnormality) (36%), 18 had endarteritis (23%), 17 had transplant glomerulopathy (22%), 30 had moderate-to-severe arteriosclerosis (39%), 23 had moderate-to-severe atrophy-scarring lesions (interstitial fibrosis and tubular atrophy) (30%), and 47 had C4d deposition in peritubular capillaries (61%). Patients with C1q-binding donor-specific anti-HLA antibodies had more extensive microvascular inflammation and transplant glomerulopathy and higher scores for graft peritubular capillary C4d deposition than both patients with non–C1q-binding donor-specific anti-HLA antibodies and patients without donor-specific anti-HLA antibodies (Fig. 1). Stratified analyses revealed that these increases applied to samples from protocol-specified biopsies, performed at 1 year, and samples from biopsies performed during an acute-rejection episode in the first year (Fig. S1 in the Supplementary Appendix).

Patients with C1q-binding donor-specific anti-HLA antibodies had a lower estimated glomerular filtration rate (GFR) at 1 year (42±22 ml per minute per 1.73 m^2 of body-surface area) than did patients with non–C1q-binding donor-specific anti-HLA antibodies (51±20 ml per minute per 1.73 m^2) and patients without donor-specific anti-HLA antibodies (54±19 ml per minute per 1.73 m^2) (P<0.001).

KIDNEY-ALLOGRAFT SURVIVAL

The median follow-up after transplantation was 4.8 years (range, 0.2 to 7.0). The median follow-up times were 3.9 years (range, 0.4 to 7.0) in patients with C1q-binding donor-specific anti-HLA antibodies and 4.3 years (range, 0.2 to 7.0) in patients with non–C1q-binding donor-specific anti-HLA antibodies.

Figure 2A shows kidney-allograft survival according to donor-specific anti-HLA antibody status after transplantation. Patients with donor-specific anti-HLA antibodies had significantly worse graft survival than patients without donor-specific anti-HLA antibodies (5-year graft survival after transplantation, 83% vs. 94%; P<0.001 by the log-rank test). When patients with donor-specific anti-HLA antibodies after transplantation were subsequently categorized according to complement-binding capacity, patients with C1q-binding donor-specific anti-HLA antibodies had the poorest 5-year graft survival after transplantation (54%), as compared with patients with non–C1q-binding donor-specific anti-HLA antibodies and patients without donor-specific anti-HLA antibodies (93% and 94%, respectively; P<0.001 for both comparisons) (Fig. 2B). The risk of graft loss according to the donor-specific anti-HLA antibodies–C1q status at day 0 and the status after transplantation revealed that patients with C1q-binding donor-specific anti-HLA antibodies after transplantation had the highest risk of graft loss (Fig. 2C).

Table 1. Baseline Characteristics of the Study Population, According to the Presence or Absence of Donor-Specific Anti-HLA Antibodies and C1q Binding after Transplantation.*

Characteristic	All Patients (N=1016)	Patients without Donor-Specific Antibodies (N=700)	Patients with Donor-Specific Antibodies		P Value†
			Without C1q Binding (N=239)	With C1q Binding (N=77)	
Recipients					
Age — yr	47.6±13	47.8±13	46.5±13	48.3±13	0.29
Male sex — no. (%)‡	599 (59)	436 (62)§	124 (52)	39 (51)	0.85
Retransplantation — no. (%)‡	181 (18)	78 (11)¶	70 (29)	33 (43)	0.03
Time since dialysis — yr‖	4.9±4.7	4.4±4¶	5.6±5	6.5±6	0.26
Donors					
Age — yr	51.0±16	51.3±16	50.1±17	51.8±16	0.44
Male sex — no. (%)‡	559 (55)	387 (55)	129 (54)	43 (56)	0.77
Deceased — no. (%)‡	835 (82)	550 (79)¶	213 (89)	72 (94)	0.26
Cold-ischemia time — hr	17.0±9.6	16.6±10§	18.1±9	20.3±9	0.06
Immunologic characteristics					
HLA A/B/DR mismatch	3.2±1.5	3.1±1.5¶	3.5±1.4	3.3±1.4	0.18
Recipient blood type — no.‡					0.80
A	461	312	115	34	
B	94	58	27	9	
O	424	305	87	32	
AB	37	25	10	2	
Characteristics of anti-HLA antibodies at time of transplantation					
Anti-HLA antibodies — no. (%)	345 (34)	132 (19)¶	153 (64)	60 (78)	<0.001
Donor-specific anti-HLA antibodies — no. (%)	196 (19)	10 (1)¶	128 (54)	58 (75)	<0.001
HLA class of donor-specific anti-HLA antibodies — no.					0.84
I	125	8	81	36	
II	146	6	98	42	
I and II	82	5	57	20	
C1q-binding donor-specific anti-HLA antibodies — no. (%)	45 (4)	0	22 (9)	23 (30)	<0.001

* Plus–minus values are means ±SD.
† P values are for the comparison between non–C1q-binding donor-specific anti-HLA antibodies and C1q-binding donor-specific anti-HLA antibodies.
‡ Chi-square tests were used for the comparison of categorical variables, and the unpaired t-test was used for the comparison of continuous variables.
§ P<0.01 for the comparison between no donor-specific anti-HLA antibodies and donor-specific anti-HLA antibodies.
¶ P<0.001 for the comparison between no donor-specific anti-HLA antibodies and donor-specific anti-HLA antibodies.
‖ Time since dialysis was determined for 874 patients overall: 593 patients who had no donor-specific anti-HLA antibodies, 208 patients who had non–C1q-binding donor-specific anti-HLA antibodies, and 73 patients who had C1q-binding donor-specific anti-HLA antibodies.

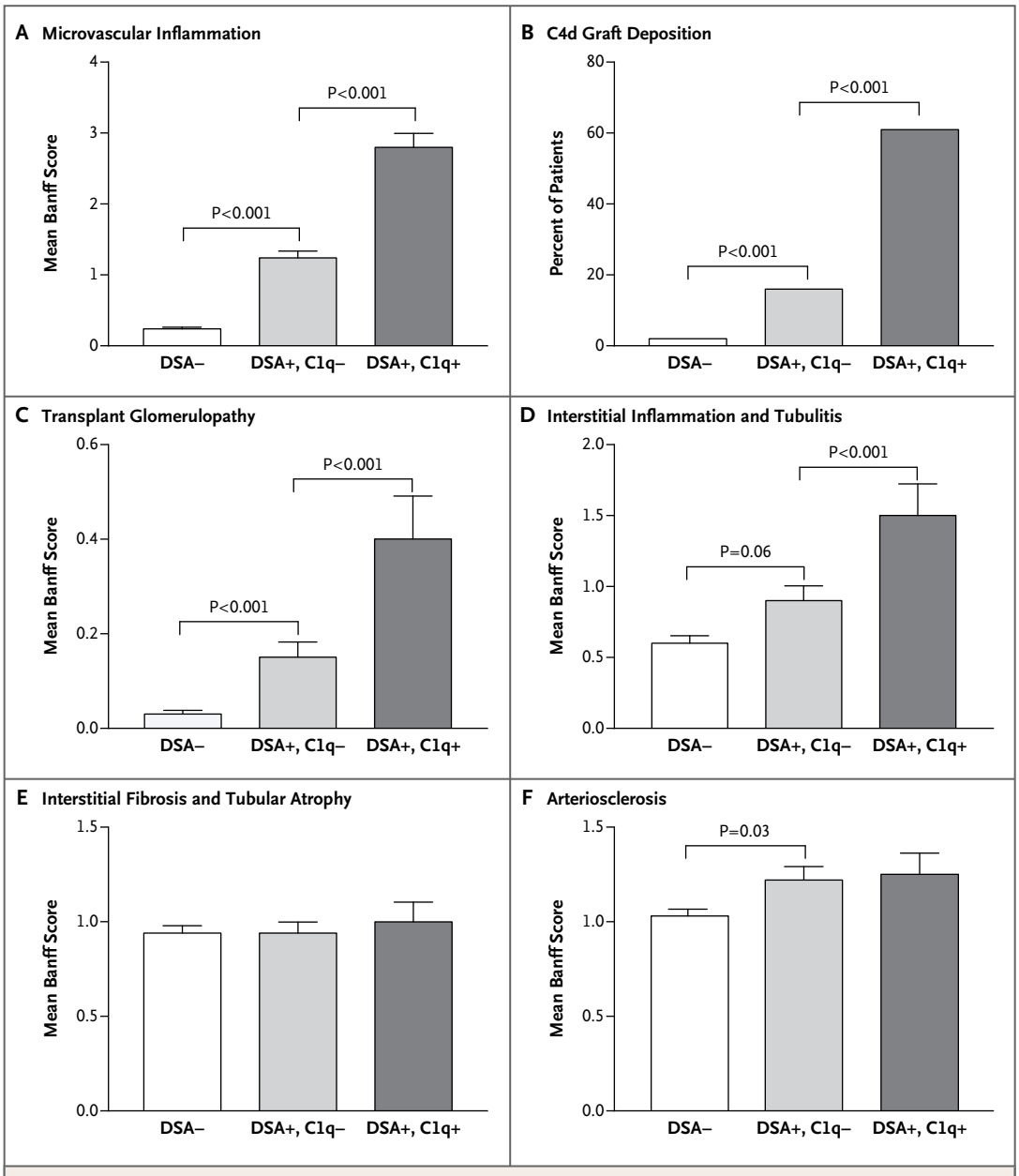


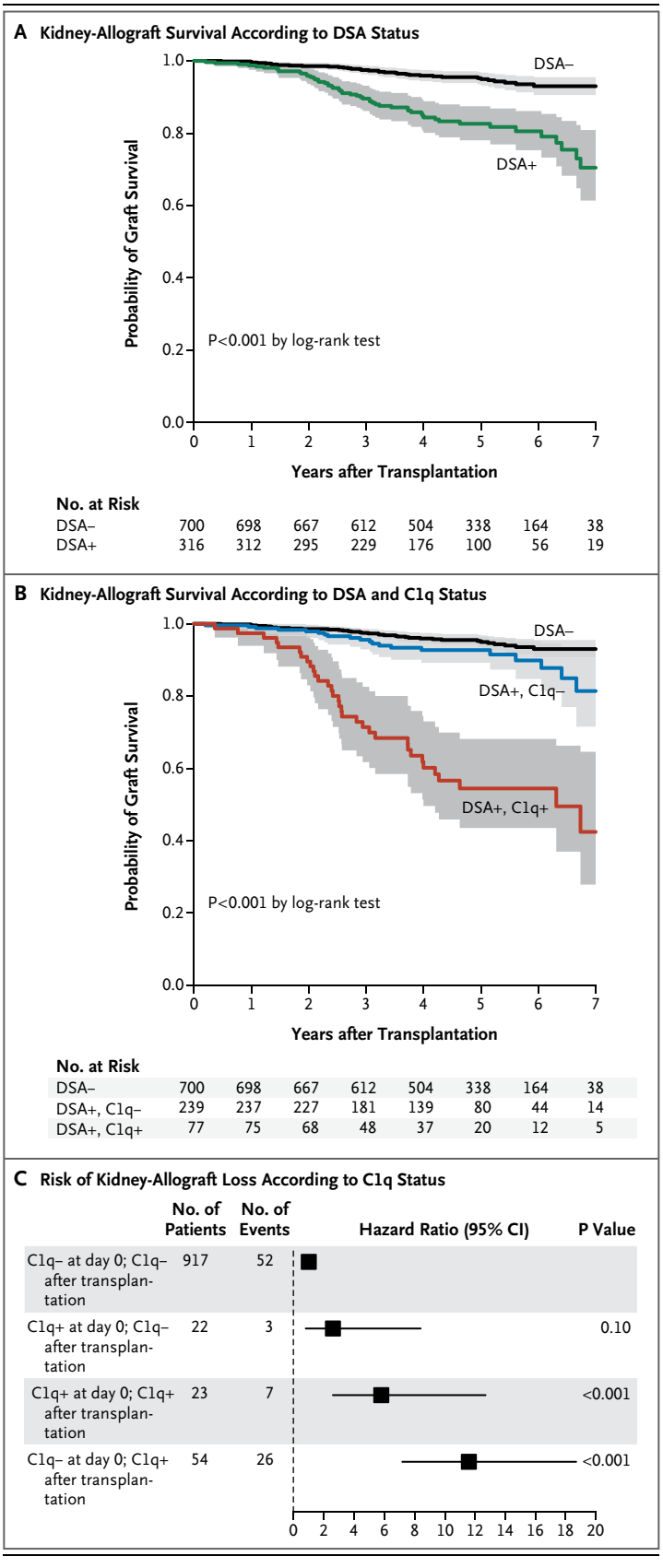
Figure 1. Phenotypic Features of Kidney-Allograft Injury, According to Anti-HLA Antibody Status after Transplantation.
Data are based on 1016 kidney-allograft biopsies performed in the first year after transplantation (845 at 1 year after transplantation and 171 during acute rejection in the first year). The score for microvascular inflammation (Panel A) is the sum of the Banff scores for glomerulitis and peritubular capillaritis. Each of these scores ranges from 0 to 3, with higher scores indicating more severe abnormality. The T bars indicate standard errors. DSA denotes donor-specific anti-HLA antibody.

DETERMINANTS OF KIDNEY-ALLOGRAFT LOSS

The association of clinical, functional, histologic, and immunologic factors with graft loss in univariate and backward-elimination multivariate Cox regression analysis is shown in Tables 2 and 3. The following independent predictors of graft loss were identified: low estimated GFR at 1 year (hazard ratio, 12.49; 95% confidence interval [CI], 5.56 to 28.06; P<0.001), interstitial fibrosis and tubular atrophy (hazard ratio, 2.22; 95% CI, 1.41 to 3.49; P=0.005), glomerular and peritubular inflammation and transplant glomerulopathy (hazard ratio, 2.26; 95% CI, 1.31 to 3.89; P=0.003), and the presence of complement-binding donor-

Figure 2. Kaplan–Meier Curves for Kidney-Graft Survival, According to Donor-Specific Anti-HLA Antibody Status after Transplantation.

Panel A shows the classic approach to determining the probability of graft survival, which is based on the presence or absence of donor-specific anti-HLA antibodies. Panel B shows our approach, which is based on the presence or absence of donor-specific anti-HLA antibodies and their C1q-binding capacity. Panel C shows the risk of graft loss according to C1q status at day 0 and after transplantation.



specific anti-HLA antibodies after transplantation (hazard ratio, 4.78; 95% CI, 2.69 to 8.49; P<0.001). Complement-binding donor-specific anti-HLA antibodies remained independently associated with the risk of kidney-allograft loss after adjustment for the mean fluorescence intensity of donor-specific anti-HLA antibodies (hazard ratio, 4.48; 95% CI, 2.23 to 8.98; P<0.001) (Table S1 in the Supplementary Appendix). The Kaplan–Meier curves for graft survival stratified according to status with respect to donor-specific anti-HLA antibodies, C1q status, and mean fluorescence intensity of donor-specific anti-HLA antibodies showed that patients with C1q-binding donor-specific anti-HLA antibodies had similar graft survival, regardless of whether the mean fluorescence intensity was low (<6000 arbitrary units) or high (≥6000 arbitrary units, P=0.70) (Fig. S2 in the Supplementary Appendix).

SENSITIVITY ANALYSIS

In the sensitivity analysis, we assessed the robustness of our study results by investigating associations separately in each study center and according to kidney function and the timing of biopsies. First, at both centers, patients with complement-binding donor-specific anti-HLA antibodies had the lowest rate of graft survival (Fig. S3 in the Supplementary Appendix). Second, complement-binding donor-specific anti-HLA antibodies after transplantation remained independently associated with graft loss whether they were detected in specimens from protocol-specified biopsies performed at 1 year (hazard ratio, 5.7; 95% CI, 1.7 to 19.4; P=0.005) or in specimens from biopsies performed during acute rejection in the first year (hazard ratio, 4.6; 95% CI, 1.8 to 11.2; P=0.001). Furthermore, the addition of an acute-rejection variable to the final multivariate model did not modify the significant predictors of graft loss. Third, C1q-binding donor-specific anti-HLA

Table 2. Clinical, Functional, Histologic, and Immunologic Factors Associated with Kidney-Graft Loss (Univariate Analysis).*

Variable	No. of Patients	Hazard Ratio (95% CI)	P Value
Clinical factors			
Age per 1-yr increment			
Donor	1016	1.02 (1.01–1.04)	0.001
Recipient	1016	1.01 (1.00–1.03)	0.10
Cold-ischemia time per 1-min increment	1016	1.001 (1.000–1.001)	0.001
Donor type			
Living	181	1.00	
Deceased	835	3.76 (1.52–9.26)	0.004
Donor sex			
Male	559	1.00	
Female	457	1.45 (0.96–2.21)	0.08
Recipient sex			
Male	599	1.00	
Female	417	0.96 (0.63–1.48)	0.87
Graft rank			
First transplant	835	1.00	
Subsequent transplant	181	1.92 (1.22–3.04)	0.005
No. of HLA mismatches	1016	1.04 (0.91–1.20)	0.56
Functional factors			
Estimated GFR at 1 yr†			
≥60 ml/min/1.73 m^2	313	1.00	
≥30 and <60 ml/min/1.73 m^2	579	2.87 (1.28–6.44)	
<30 ml/min/1.73 m^2	111	21.96 (9.86–48.90)	<0.001
Histologic factors‡			
Interstitial fibrosis and tubular atrophy			
Low score: 0 or 1	743	1.00	
High score: ≥2	273	2.55 (1.68–3.87)	<0.001
Arteriosclerosis			
Low score: 0 or 1	653	1.00	
High score: ≥2	363	1.99 (1.31–3.02)	0.001
Interstitial inflammation and tubulitis			
Low score: 0 or 1	832	1.00	
High score: ≥2	184	1.68 (1.06–2.68)	0.028
Glomerular and peritubular inflammation and transplant glomerulopathy			
Low score: 0	820	1.00	
High score: ≥1	196	4.80 (3.16–7.29)	<0.001
Endarteritis			
Low score: 0	949	1.00	
High score: ≥1	64	2.81 (1.56–5.07)	<0.001
C4d graft deposition			
Low score: 0	919	1.00	
High score: ≥1	97	5.91 (3.78–9.24)	<0.001

Table 2. (Continued.)

Variable	No. of Patients	Hazard Ratio (95% CI)	P Value
Immunologic factors			
Donor-specific antibodies at time of transplantation			
No	820	1.00	
Yes	196	3.53 (2.30–5.40)	<0.001
C1q-binding donor-specific antibodies at time of transplantation			
No	971	1.00	
Yes	45	2.95 (1.53–5.70)	0.001
Donor-specific antibodies after transplantation			
No	700	1.00	
Yes	316	3.90 (2.54–5.98)	<0.001
C1q-binding donor-specific antibodies after transplantation			
No	939	1.00	
Yes	77	9.23 (5.99–14.23)	<0.001

* CI denotes confidence interval.
† The estimated glomerular filtration rate (GFR) was calculated with the use of the Modification of Diet in Renal Disease formula.
‡ Banff scores range from 0 to 3, with higher scores indicating more severe abnormality.

antibodies were associated with an increased risk of graft loss in each category of estimated GFR as defined by the National Kidney Foundation[34] (Fig. S4 in the Supplementary Appendix).

PREDICTION OF KIDNEY-ALLOGRAFT LOSS

The inclusion of complement-binding donor-specific anti-HLA antibodies in the reference model significantly improved its discrimination capacity (i.e., its capacity to discriminate between patients with graft loss and those without graft loss) since the C-statistic increased from 0.81 to 0.85 (bootstrap mean difference, 0.013; 95% CI, 0.012 to 0.015), and the integrated discrimination improvement was 0.03 (P=0.008). Similarly, the addition of complement-binding donor-specific anti-HLA antibodies to the reference model adequately reclassified patients at lower risk for graft loss and those at higher risk, as shown by a continuous net reclassification improvement of 0.75 (95% CI, 0.54 to 0.97).

EXTERNAL VALIDATION

The external-validation cohort was composed of 643 patients; their baseline characteristics are detailed in Table S2 in the Supplementary Appendix. The median follow-up after transplantation was 3.4 years (range, 0.2 to 5.0). The Kaplan–Meier estimate of graft survival confirmed that patients with complement-binding donor-specific anti-HLA antibodies had the highest risk of graft loss as compared with patients with non–complement-binding donor-specific anti-HLA antibodies and patients without donor-specific anti-HLA antibodies (P<0.001) (Fig. S5 in the Supplementary Appendix).

DISCUSSION

In a cohort of 1016 carefully phenotyped kidney-transplant recipients, we observed that the presence of complement-binding donor-specific anti-HLA antibodies detected in the first year after transplantation was an independent predictor of kidney-allograft loss more than 5 years after transplantation and significantly improved individual risk stratification for graft failure. Patients with complement-binding donor-specific anti-HLA antibodies after transplantation had a graft injury phenotype characterized by microvascular inflammation and complement split-product C4d deposition. As compared with a traditional approach

Table 3. Clinical, Functional, Histologic, and Immunologic Factors Associated with Kidney-Graft Loss (Multivariate Analysis).*

Variable	No. of Patients	No. of Events	Hazard Ratio (95% CI)	P Value
Estimated GFR at 1 yr				
≥60 ml/min/1.73 m²	313	7	1.00	
≥30 and <60 ml/min/1.73 m²	579	36	2.45 (1.09–5.53)	
<30 ml/min/1.73 m²	111	42	12.49 (5.56–28.06)	<0.001
Interstitial fibrosis and tubular atrophy†				
Low score: 0 or 1	738	45	1.00	
High score: 2 or 3	265	40	2.22 (1.41–3.49)	0.005
Glomerular and peritubular inflammation and transplant glomerulopathy				
No	809	42	1.00	
Yes	194	43	2.26 (1.31–3.89)	0.003
C1q-binding donor-specific anti-HLA antibodies after transplantation				
No	928	52	1.00	
Yes	75	33	4.78 (2.69–8.49)	<0.001

* Risk factors were identified with the use of backward elimination, with a P value of 0.05 or lower for retention in the model.
† Banff scores range from 0 to 3, with higher scores indicating more severe abnormality.

to predicting graft loss, based only on the presence of donor-specific anti-HLA antibodies after transplantation as a risk factor for graft loss, the approach we used, which integrates the capacity of donor-specific anti-HLA antibodies to bind complement, identified an additional group of patients with an increased risk of graft loss.

It has been known for at least 40 years that cytotoxic anti-HLA antibodies are associated with graft rejection.[13] However, the complement-dependent cytotoxicity assay used to identify these clinically deleterious antibodies lacks sensitivity and specificity and cannot be used on a large scale in transplantation follow-up because of the limited reserves of donor cells. Of the three complement-dependent pathways, the classical pathway involves antibody–C1q fixation. Antibody binding to an antigen and subsequent C1q binding initiate activation of the complement cascade.[35]

A sensitive detection method for C1q-binding anti-HLA antibodies yield findings in addition to those of the single-antigen flow bead test. Although the mean fluorescence intensity of donor-specific anti-HLA antibodies is widely used in clinical practice for risk stratification, our study showed that C1q-binding donor-specific anti-HLA antibodies remained strongly associated with the risk of graft loss regardless of the mean fluorescence intensity of the antibodies. Our results may have implications for other transplanted organs such as the heart, lungs, and small bowel, since accumulating evidence from small studies supports the deleterious effect of C1q-binding donor-specific anti-HLA antibodies on these other transplanted organs.[18-21]

From a prognostic perspective, our results provide support for the finding that C1q and C4d do not provide equivalent predictive information. C1q testing may help identify patients at risk, despite C4d negativity. We and others[36,37] have found that C4d, although specific, lacks sensitivity as an indicator of humoral rejection. Assessment for complement-binding donor-specific anti-HLA antibodies may provide an early indication of the potential for complement-mediated injury, without the functional requirement for progression through the pathway to C4d deposition. Detection of complement-binding donor-specific anti-HLA antibodies may indicate which of the antibodies present have the capacity to activate the complement cascade,

potentially but not inevitably leading to C4d deposition and complement-mediated damage or antibody-mediated injury in vivo. Such findings do not rule out a possible role for complement-independent mechanisms in allograft injury mediated by complement-binding donor-specific anti-HLA antibodies.[12]

There are therapeutic implications of identifying critical pathologic pathways responsible for kidney-allograft loss. Since promising therapeutic agents targeting complement (e.g., a C5 inhibitor [eculizumab] or a C1 inhibitor) are increasingly used in patients undergoing transplantation,[38-40] the present study may provide a basis for future clinical trials. One limitation of our study was that it was not designed to provide kinetics of the capacity of anti-HLA antibodies to bind complement or the effect of treatment on these antibodies. This would require further investigations.

In conclusion, we systematically evaluated immunologic characteristics before and after transplantation in a population-based sample of kidney-allograft recipients, incorporating the full spectrum of graft phenotypes. We found that the presence of complement-binding anti-HLA donor-specific antibodies after transplantation is strongly associated with graft injury and loss and that incorporation of this risk factor improves risk stratification for graft loss.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the members of the Laboratory of Excellence, Transplantex for helpful discussions.

REFERENCES

1. Nankivell BJ, Kuypers DR. Diagnosis and prevention of chronic kidney allograft loss. Lancet 2011;378:1428-37.
2. Gaston RS, Cecka JM, Kasiske BL, et al. Evidence for antibody-mediated injury as a major determinant of late kidney allograft failure. Transplantation 2010;90:68-74.
3. Sellarés J, de Freitas DG, Mengel M, et al. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. Am J Transplant 2012;12:388-99.
4. Organ Procurement and Transplantation Network and Scientific Registry of Transplant Recipients 2010 data report. Am J Transplant 2012;12:Suppl 1:1-156.
5. Rapport 2011 de l'activité de prélèvement et de greffe de l'Agence de la Biomédecine (http://www.agence-biomedecine.fr/Rapport-annuel-2011).
6. Terasaki PI, Ozawa M. Predicting kidney graft failure by HLA antibodies: a prospective trial. Am J Transplant 2004;4:438-43.
7. Hourmant M, Cesbron-Gautier A, Terasaki PI, et al. Frequency and clinical implications of development of donor-specific and non-donor-specific HLA antibodies after kidney transplantation. J Am Soc Nephrol 2005;16:2804-12.
8. Lachmann N, Terasaki PI, Budde K, et al. Anti-human leukocyte antigen and donor-specific antibodies detected by Luminex posttransplant serve as biomarkers for chronic rejection of renal allografts. Transplantation 2009;87:1505-13.
9. Lefaucheur C, Loupy A, Hill GS, et al. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. J Am Soc Nephrol 2010;21:1398-406.
10. Nankivell BJ, Alexander SI. Rejection of the kidney allograft. N Engl J Med 2010;363:1451-62.
11. Racusen LC, Haas M. Antibody-mediated rejection in renal allografts: lessons from pathology. Clin J Am Soc Nephrol 2006;1:415-20.
12. Colvin RB. Antibody-mediated renal allograft rejection: diagnosis and pathogenesis. J Am Soc Nephrol 2007;18:1046-56.
13. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. N Engl J Med 1969;280:735-9.
14. Feucht HE, Felber E, Gokel MJ, et al. Vascular deposition of complement-split products in kidney allografts with cell-mediated rejection. Clin Exp Immunol 1991;86:464-70.
15. Collins AB, Schneeberger EE, Pascual MA, et al. Complement activation in acute humoral renal allograft rejection: diagnostic significance of C4d deposits in peritubular capillaries. J Am Soc Nephrol 1999;10:2208-14.
16. Böhmig GA, Exner M, Habicht A, et al. Capillary C4d deposition in kidney allografts: a specific marker of alloantibody-dependent graft injury. J Am Soc Nephrol 2002;13:1091-9.
17. Chen G, Sequeira F, Tyan DB. Novel C1q assay reveals a clinically relevant subset of human leukocyte antigen antibodies independent of immunoglobulin G strength on single antigen beads. Hum Immunol 2011;72:849-58.
18. Chin C, Chen G, Sequeria F, et al. Clinical usefulness of a novel C1q assay to detect immunoglobulin G antibodies capable of fixing complement in sensitized pediatric heart transplant patients. J Heart Lung Transplant 2011;30:158-63.
19. Yabu JM, Higgins JP, Chen G, Sequeira F, Busque S, Tyan DB. C1q-fixing human leukocyte antigen antibodies are specific for predicting transplant glomerulopathy and late graft failure after kidney transplantation. Transplantation 2011;91:342-7.
20. Sutherland SM, Chen G, Sequeira FA, Lou CD, Alexander SR, Tyan DB. Complement-fixing donor-specific antibodies identified by a novel C1q assay are associated with allograft loss. Pediatr Transplant 2012;16:12-7.
21. Zeevi A, Marrari M, Feingold B, Webber S, Duquesnoy RJ. Human leukocyte antigen epitope analysis to assess complement- and non-complement-binding donor-specific antibody repertoire in a pediatric heart transplant recipient. Hum Immunol 2012;73:48-51.
22. Otten HG, Verhaar MC, Borst HP, Hene 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;12:1618-23.
23. Lachmann N, Todorova K, Schulze H, Schonemann C. Systematic comparison of four cell- and Luminex-based methods for assessment of complement-activating HLA antibodies. Transplantation 2013;95:694-700.
24. Freitas MC, Rebellato LM, Ozawa M, et al. The role of immunoglobulin-G subclasses and C1q in de novo HLA-DQ donor-specific antibody kidney transplantation outcomes. Transplantation 2013;15:113-9.
25. Cristal. Agence Biomédecine (http://www.sipg.sante.fr/portail/)
26. Données Informatiques VAlidées en Transplantation (DIVAT) software (http://www.divat.fr/).
27. Racusen LC, Colvin RB, Solez K, et al. Antibody-mediated rejection criteria — an addition to the Banff 97 classification of renal allograft rejection. Am J Transplant 2003;3:708-14.
28. Solez K, Colvin RB, Racusen LC, et al. Banff 07 classification of renal allograft pathology: updates and future directions. Am J Transplant 2008;8:753-60.

29. Lefaucheur C, Nochy D, Andrade J, et al. Comparison of combination Plasmapheresis/IVIg/anti-CD20 versus high-dose IVIg in the treatment of antibody-mediated rejection. Am J Transplant 2009;9:1099-107.
30. Loupy A, Suberbielle-Boissel C, Zuber J, et al. Combined posttransplant prophylactic IVIg/anti-CD 20/plasmapheresis in kidney recipients with preformed donor-specific antibodies: a pilot study. Transplantation 2010;89:1403-10.
31. Lamb KE, Lodhi S, Meier-Kriesche HU. Long-term renal allograft survival in the United States: a critical reappraisal. Am J Transplant 2011;11:450-62.
32. Pencina MJ, D'Agostino RB. Overall C as a measure of discrimination in survival analysis: model specific population value and confidence interval estimation. Stat Med 2004;23:2109-23.
33. Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med 2008;27:157-72.
34. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis 2002;39:Suppl 1:S1-S266.
35. Colvin RB, Smith RN. Antibody-mediated organ-allograft rejection. Nat Rev Immunol 2005;5:807-17.
36. Sis B, Jhangri GS, Bunnag S, Allanach K, Kaplan B, Halloran PF. Endothelial gene expression in kidney transplants with alloantibody indicates antibody-mediated damage despite lack of C4d staining. Am J Transplant 2009;9:2312-23.
37. Loupy A, Hill GS, Suberbielle C, et al. Significance of C4d Banff scores in early protocol biopsies of kidney transplant recipients with preformed donor-specific antibodies (DSA). Am J Transplant 2011; 11:56-65.
38. Stegall MD, Diwan T, Raghavaiah S, et al. Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. Am J Transplant 2011;11:2405-13.
39. A pilot study to evaluate the use of C1 esterase inhibitor (human) in patients with acute antibody-mediated rejection. ClinicalTrials.gov (http://clinicaltrials.gov/ct2/show/NCT01147302).
40. Safety & tolerability of Berinert (C1 inhibitor) therapy to prevent rejection. ClinicalTrials.gov (http://clinicaltrials.gov/ct2/show/NCT01134510).