SUPPLEMENTARY MATERIAL

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2. SUPPLEMENTARY TABLE

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A. CONTROL GROUP

The histopathological, immunological and clinical data of patients who received kidney transplantation at Edouard Herriot Hospital were screened by means of the departments' computer databases. Information from the three databases were integrated in order to identify 100 patients without any microvascular inflammation (g+cpt≤1) on biopsy and without any donor-specific antibodies (DSA) and matched with patients of the principal cohort regarding main baseline characteristics. A renal pathologist and a nephrologist reviewed the 100 specimens and 11 patients whose biopsies showed microvascular inflammation or were not adequate were excluded. Serum samples were retested using Single Antigen Flow Beads to ensure the absence of DSA. Two patients with DSA were excluded.

As in the principal cohort, patients received an ABO compatible transplantation with negative historical and current complement-dependent cytotoxicity cross matches. The end of follow-up was set to April 7th, 2014 (mean follow-up duration for the control group: 34.0 +/- 17.1 months).

B. EXTERNAL VALIDATION COHORT

All patients who experienced antibody-mediated rejection between January 1st 2005 and December 31st 2012 in the kidney transplantation department of the Pellegrin Hospital (University Hospital of Bordeaux, France) were included in the validation cohort. Patients received an ABO compatible transplantation with negative historical and current complement-dependent cytotoxicity cross matches. Clinical data were

obtained from the kidney transplantation department's computer database. The characteristics of the patients and their rejections are summarized in supplemental Table 2. The end of follow-up was set to April 7th, 2014 (mean follow-up duration for the cohort: 24.85 +/- 21.86 months). A nephrologist (AS) and a pathologist (MR) reviewed all biopsy specimens. Serum samples, which had been banked at time of biopsy for all patients, were analyzed in a blinded fashion for the presence of C3d-binding donor-specific anti-HLA antibodies by the same immunobiologist (VD) and in the same institution (Etablissement Français du Sang, Lyon) as the principal cohort.

C. DETECTION OF ANTI-HLA ANTIBODIES

Anti-HLA IgG-antibody assay:

Principle:

An aliquot of the beads is allowed to incubate with a small volume of test serum sample. The sensitized beads are then washed to remove unbound antibody. An anti-human IgG conjugated to phycoerythrin is then added. After another incubation, the test sample is diluted and analyzed on the Luminex platform. The signal intensity from each bead is compared to the signal intensity of negative control sera and negative control beads included in the bead preparation in order to determine if the bead is positive or negative for bound alloantibody.

Technique:

Briefly, pre wet wells to be used with 100-300µl of distilled water. After 2-5 minutes, remove water by gentle aspiration using the vacuum manifold. Add 40µl of LSA beads to each of the assigned wells, then add 10µl of patient serum or control serum and mix. Incubate for 30 minutes at room temperature in the dark, on a rotating

platform. After three washes with Wash Buffer, add 50µl of diluted conjugate to each well and incubate 30 minutes at room temperature. Add 130-150 µl of wash buffer to resuspend beads and collect data with the Luminex platform.

C3d-binding anti-HLA antibody assay:

Principle:

Sera are pretreated with Lifecodes serum cleaner®, a product designed to reduce the nonspecific binding of human serum components, and therefore the prozone phenomenon.

An aliquot of the beads is allowed to incubate with a small volume of test serum sample. The sensitized beads are then washed to remove unbound antibody. An anti-human C3d antibody conjugated to phycoerythrin is then added. After another incubation, the test sample is diluted and analyzed on the Luminex platform. The signal intensity from each bead is compared to the signal intensity of negative control sera and negative control beads included in the bead preparation to determine if the bead is positive or negative for bound alloantibody.

Technique:

Briefly, pre wet wells to be used with 100-300µl of distilled water. After 2-5 minutes, remove water by gentle aspiration using the vacuum manifold. Add 40µl of LSA beads to each of the assigned wells, then add 10µl of patient serum or control serum and mix. Incubate for 30 minutes at room temperature in the dark, on a rotating platform. After incubation, add 30µl of negative serum sample to each well including the negative control well. Incubate for 30 minutes at room temperature. After four

washes with Wash Buffer, add 50µl of conjugate to each well and incubate 30 minutes at room temperature. Add 100 µl of wash buffer and gently aspirate the plate, then perform one extra wash. Add 200µl of Wash Buffer to each well and mix to resuspend beads and collect data with the Luminex platform.

Interpretation of results:

Three adjusted values are calculated to determine if a bead is positive:

BCM = Raw MFI – background MFI (background MFI is the background noise due to bead variation, specific to each lot) calculated for each individual bead, cut-off value at 1500

BCR = BCM / MFI of the calculated control of its respective locus (lowest ranked antigen bead for that locus), cut-off value at 4

AD-BCR = BCR / relative amount of the antigens present on each bead, cut-off value = 5

The bead is considered positive if two or more of the adjusted values are above the cut-off values.

C1q-binding anti-HLA antibody assay:

Principle:

An aliquot of the beads is allowed to incubate in the presence of a fixed amount of recombinant C1q with a small volume of test serum which was first heat-inactivated to destroy CIq. Then an anti-human C1q antibody conjugated to phycoerythrin is added. After another incubation, the bead pellet is resuspended in PBS and analyzed on the Luminex platform. The signal intensity from each bead is compared to the

signal intensity of a negative control serum to determine if the bead is positive or negative for bound alloantibody.

Technique:

After heat inactivation at 56°C for 30 min and centrifugation, serum (5 µL) is spiked with 5 µL of a 1:5 dilution in HEPES buffer of human C1q and incubated with 5 µL class I or class II luminex beads for 20 min at room temperature. Then add to the bead pellet 5µl of PE-conjugated anti-human C1q antibody and incubate for another 20 min. Centrifuge, remove supernatant, resuspend the bead pellet in PBS and collect data with the Luminex instrument.

Interpretation of results:

Measured fluorescence intensity is normalized using the Baseline formula provided by the Fusion software (One Lambda): normalized MFI = [(MFI patient's serum for bead n) - (MFI patient's serum for negative control bead)] - [(MFI negative control serum for bead n) – [(MFI negative control serum for negative control bead)].

2. SUPPLEMENTARY TABLES

Table S1. Control group baseline characteristics

Variable	Control group (n=87)	Patients with antibody-mediated rejection (n=69)	P Value*
Gender, male, n (%)	60 (69)	42 (61)	0.29
Age, years	43.4 ± 13.7	39.2 ± 14.6	0.08
Blood group, n (%)			
Type A	46 (53)	38 (55)	0.78
Type B	10 (12)	6 (9)	0.56
Type O	28 (32)	24 (35)	0.73
Type AB	3 (3)	1 (1)	0.43
Retransplantation, n (%)	14 (16)	24 (35)	**0.007**
Deceased donor, n (%)	78 (90)	65 (94)	0.3
Donor age, (years)	43.7 ± 15.4	39.0 ± 17.6	0.1
Delayed graft function, n (%)	11 (13.2)	14 (20)	0.24
Banff scores*			
Microvascular inflammation†	0.22±0.44	3.5 ± 1.2	**<0.001**
Transplant glomerulopathy	0.09±0.32	1.1 ± 1.2	**<0.001**
Interstitial Inflammation and Tubulitis	1.7±1.9	2.6 ± 2.0	**<0.001**
Interstitial Fibrosis and Tubular Atrophy	1.42 ± 0.72	1.6 ± 0.8	0.11
Arteriosclerosis	0.7 ± 0.9	1.0 ± 1.1	0.16
Endarteritis (vasculitis)	0	0.25 ± 0.5	n/a
C4d deposition	0	1.56 ± 1.1	n/a
Follow-up post biopsy (months)	34.0 ± 17.1	21.6 ± 21.7	**<0.001**

Unless noted otherwise results are expressed as mean ± standard deviation. *$\chi 2$ tests for comparison of proportions and unpaired t test for comparison of continuous variables. *Banff scores (0: no significant lesion, 1: mild, 2: moderate, 3: severe), † Sum of the Banff scores for glomerulitis and capillaritis.

Table S2. Characteristics of the validation cohort

Variable	Patients with antibody-mediated rejection (n=39)	Patients with non-C3d-binding DSA (n=25)	Patients with C3d-binding DSA (n=14)	P Value*
Characteristics at the time of transplantation				
Recipient				
Gender, male, n (%)	22 (56)	13 (52)	9 (64)	0.46
Age, years	38.2 ± 12.8	40.5 ± 12.7	34.2 ± 12.5	0.14
Retransplantation, n (%)	16 (41)	9 (36)	7 (50)	0.39
Blood group, n (%)				
Type A	21 (54)	15 (60)	6 (43)	0.30
Type B	7 (18)	3 (12)	4 (28.5)	0.20
Type O	11 (28)	7 (28)	4 (28.5)	0.97
Type AB	0 (0)	0 (0)	0 (0)	n/a
Donor				
Age, years	36.2 ± 16.9	38.1 ± 18.1	33.0 ± 14.7	0.35
Deceased, n (%)	35 (90)	22 (88)	13 (93)	0.63
Transplantation				
Number of HLA A/B/DR mismatch	3.6 ± 1.2	3.8 ± 1.2	3.3 ± 1.1	0.18
Total ischemic time, minutes	1102 ± 663	1010 ± 609	1293 ± 771	0.21
Delayed graft function, n (%)	12 (31)	8 (32)	4 (29)	0.82
Characteristics of Antibody-Mediated Rejections				
Clinico-biological characteristics				
Time post-transplantation (days)	1904 ± 2255	1670 ± 2076	2324 ± 2572	0.39
Proteinuria (gram/day)	1.15 ± 1.78	0.53 ± 0.53	2.21 ± 2.6	**0.01**
Creatininemia (µmol/l)	255 ± 185	231 ± 174	300 ± 206	0.27
estimated GFR§ (ml/min/1.73 m^2)	34.3 ± 23.1	37.6 ± 25.4	28.0 ± 17.0	0.22
Biopsy for protocol	2 (5)	2 (8)	0 (0)	0.28
Subclinical antibody-mediated rejections, n (%)	2 (5)	2 (8)	0 (0)	0.28
Histological characteristics (Banff scores)**				
Microvascular inflammation†	2.33 ± 0.62	2.36 ± 0.70	2.14 ± 0.71	0.72
Transplant glomerulopathy	0.77 ± 1.01	0.64 ± 0.91	1.00 ± 1.18	0.30
Interstitial Inflammation and Tubulitis	1.62 ± 1.55	1.88 ± 1.51	1.14 ± 1.56	0.16
Interstitial Fibrosis and Tubular Atrophy	1.36 ± 0.93	1.36 ± 0.86	1.35 ± 1.08	0.99
Arteriosclerosis	0.79 ± 0.86	0.77 ± 0.81	0.82 ± 0.98	0.89
Endarteritis (vasculitis)	0.12 ± 0.54	0.05 ± 0.2	0.25 ± 0.87	0.30
Immunological characteristics				
Number of DSA				
Standard Single-Antigen Assay	3.05 ± 2.0	2.8 ± 2.0	3.4 ± 2.0	0.39
C3d binding Assay	n/a	n/a	1.4 ± 0.7	-
Classes of DSA				
Class I, n (%)	4 (10)	3 (12)	1 (7)	0.63
Class II, n (%)	15 (38)	9 (36)	6 (43)	0.67
Class I + II, n (%)	20 (51)	13 (52)	7 (50)	0.90
MFI of the highest DSA				
Standard Single-Antigen Assay	6495 ± 5021	5027 ± 4275	9317 ± 5557	**0.01**
C3d binding Assay	n/a	n/a	4607 ± 2756	-
Treatments				
Steroids pulses	31 (79)	20 (80)	11 (79)	0.91
Intravenous Immunoglobulins	33 (85)	22 (88)	11 (79)	0.43
Rituximab	34 (87)	22 (88)	12 (86)	0.84
Plasmapheresis	29 (74)	19 (76)	10 (71)	0.75
Bortezomib	3(8)	1(4)	2 (14)	0.25

Abbreviations: DSA: Donor-Specific antibodies, n/a: not adapted, MFI: Mean Fluorescence Intensity, GFR: Glomerular Filtration Rate. Unless noted otherwise results are expressed as mean ± standard deviation. *Comparison between patients with non-C3d-binding DSA and patients with C3d-binding-DSA ($\chi 2$ tests for comparison of proportions and unpaired t test for comparison of continuous variables). § Calculated with the Modification of Diet in Renal Disease formula. **Banff scores (0: no significant lesion, 1: mild, 2: moderate, 3: severe) † Sum of the Banff scores for glomerulitis and capillaritis.

Table S3. Histological lesions according to kidney allograft function at time of antibody-mediated rejection

Banff scores	eGFR < 30 ml/min/1.73m^2* (n=33)	eGFR ≥ 30 ml/min/1.73m^2* (n=36)	P Value†
Acute lesions			
Interstitial Inflammation and Tubulitis	2.9±2.1	2.3±1.9	0.16
Microvascular inflammation**	3.7±1.1	3.3±1.1	0.14
Chronic lesions			
Transplant glomerulopathy	1.0±1.1	1.0±1.1	0.58
Interstitial Fibrosis and Tubular Atrophy	1.81±0.8	1.44±0.73	**0.04**

Abbreviations: eGFR: estimated Glomerular Filtration Rate; *Calculated with the Modification of Diet in Renal Disease formula; **Sum of the Banff scores for glomerulitis and capillaritis; †unpaired t test.