ORIGINAL ARTICLE

C1q binding is not an independent risk factor for kidney allograft loss after an acute antibody-mediated rejection episode: a retrospective cohort study

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SUMMARY

After kidney transplantation, C4d is an incomplete marker of acute antibody-mediated rejection (AMR) and C1q-binding donor-specific antibodies (DSA) have been associated with allograft survival. However, the impact on allograft survival of C1q+ DSA after clinical AMR has not been studied yet. We analysed retrospectively in clinical AMR C4d staining and C1q-binding impact on allograft survival. We compared clinical, histological and serological features of C4d− and C4d+ AMR, C1q+ and C1q− DSA AMR and analysed C4d and C1q-binding impact on allograft survival. Among 500 for-cause kidney allograft biopsies, 48 fulfilled AMR criteria. C4d+ AMR [$N = 18$ (37.5%)] have significantly higher number class I DSA ($P = 0.02$), higher microvascular score ($P = 0.02$) and more transplant glomerulopathy ($P = 0.04$). C1q+ AMR [$N = 20$ (44%)] presented with significantly more class I and class II DSA ($P = 0.005$ and 0.04) and C4d+ staining ($P = 0.01$). Graft losses were significantly higher in the C4d+ group ($P = 0.04$) but similar in C1q groups. C4d+ but not C1q+ binding was an independent risk factor for graft loss [HR = 2.65; (1.11–6.34); $P = 0.028$]. In our cohort of clinical AMR, C4d+ staining but not C1q+ binding is an independent risk factor for graft loss. Allograft loss and patient survival were similar in C1q+ and C1q− AMR.

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Key words
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Introduction

Recognition of the role of anti-HLA donor-specific antibodies (DSA) after kidney transplantation was limited to hyperacute rejection until the 1980s, when antibody-mediated vasculitis and early graft failure was related to donor-specific humoral presensitization [1]. Nowadays, DSA duty in acute and chronic allograft-mediated rejection is well recognized [2] and is the important cause of short-term and long-term injury leading to allograft loss after kidney transplantation [3–6].

Until the last Banff conference, acute antibody-mediated rejection (AMR) criteria included microvascular inflammation (peritubular capillaritis and/or glomerulitis and/or arteritis), anti-HLA DSA detection and C4d staining, a complement split product binding covalently to endothelial cells and basement membranes [7,8]. C4d had been considered the most specific feature, as microvascular inflammation can be seen in acute calcineurin inhibitor nephrotoxicity and recurrent thrombotic microangiopathy [9]. However, molecular approaches rapidly highlighted the lack of C4d staining to diagnose AMR [10–12]. AMR is C4d− in up to 50% of cases, and delay after transplant is variable [9,12,13]. The reliability of the prognostication of C4d− AMR appeared close to that of C4d+ AMR but worse than that of acute T-cell-mediated rejection (ACR) [9]. Since the last Banff conference, C4d+ staining is no longer a mandatory criterion to diagnose AMR [14]. In the context of early clinical AMR, C4d positivity and AMR morphology have been reported to be independent risk factors for graft loss [15]. So more, C4d positivity was associated with graft loss independently of the morphologic presentation [15]. However, the capability of C4d positivity to predict allograft loss after AMR remains controversial [16–18].

Recently, the C1q-binding properties of DSA at the time of kidney allograft transplant have been associated with an increased rate of AMR, a more severe graft injury phenotype presenting with more extensive microvascular inflammation and increased deposition of complement fraction C4d within capillaries on protocol biopsies [19]. C1q-binding in *de novo* DSA have also been associated with allograft survival and occurrence of AMR episodes [20–22]. In context of de novo DSA, C1q-binding capability is associated with AMR; however, impact on allograft survival has been reported negative [16,23].

We propose to analyse in a cohort of clinical AMR after kidney transplant the capacity of C4d staining and C1q binding to predict allograft survival. Our objectives were to describe and compare clinical, histological and serological features of C4d− and C4d+ AMR and C1q+ and C1q− DSA-associated AMR and specifically analyse C4d staining and C1q-binding impact on allograft survival.

Patients and methods

We reviewed all for-cause kidney allograft biopsies performed between January 2005 and January 2012 in two French transplantation centres. We selected patients who displayed for the first-time morphologic evidence of acute tissue injury consistent with acute AMR associated with anti-HLA DSA. ABO-incompatible kidney transplants were excluded. Inclusion of patients was consecutive during the study time precluding the selection bias.

All biopsies were looked over by three pathologists independently (A.M., D.D. and I.B.). Histological injury was classified according to the Banff '14 update of Banff '97 classification [haematoxylin and eosin (HE), periodic acid Schiff (PAS), Masson trichrome and Jones stained sections] [7]. C4d staining was performed on cryosections by immunofluorescence using monoclonal anti-C4d antibody (Quidel, Santa Clara, CA) and/or on paraffin sections using immunohistochemistry (clonal anti-human C4d antibody, DB Biotech, DB 107 RTU, Popradzka, Slovak Republic). According to the Banff classification, more than 50% (diffuse – C4d3) and 10–50% (focal – C4d2) of peritubular capillary staining by immunofluorescence from cortical or medullary area defined positive C4d [7]. Using immunohistochemistry, C4d positivity was considered as soon as C4d staining was more than 1% (C4d1, C4d2 or C4d3) [7].

Recipients' serum samples picked out before kidney transplant, at the time of AMR, and 12 months after AMR were retrospectively analysed using Luminex assays technology. Specificities of HLA class I (A and B) and class II (DR and DQ) IgG antibodies were determined with LABScreen single-antigen HLA class I (97 beads) and class II (92 beads) detection tests (One Lambda Inc., Canoga Park CA, USA) according to the manufacturer's instructions. Presence and specificity of antibodies were then tested using a LabScan 100, and the mean fluorescence intensity (MFI) of each sample with each bead was evaluated. A baseline value of MFI >500 was considered positive. Immunodominant DSA (iDSA) was defined as DSA with the highest MFI. All analyses were performed in one laboratory (Jean Dausset Histocompatibility Laboratory, Paris). Serum samples at the time of the AMR

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episode were also tested for the presence of C1q-binding DSA with the use of single-antigen flow bead assays according to the manufacturer's protocol (C1qscreenTM, One Lambda) [19].

Demographic, clinical, biochemical, pathology, treatment and follow-up data were collected. Acute T-cell-mediated rejection was defined accordingly to the Banff '14 update of Banff '97 classification and included borderline lesions. Delayed graft function was defined as the need for dialysis treatment within the first week of transplant. We used the MDRD formula to estimate glomerular filtration rate (eGFR) [24]. Standard antirejection therapy included methylprednisolone, intravenous immunoglobulins (IVIG) or plasmapheresis. AMR was considered resistant if eGFR did not return to within 15% of the baseline within eight weeks after the initiation of antirejection therapy. The primary outcome was graft loss defined as persistent decline in eGFR to <15 ml/min/1.73 m^2 or return to dialysis. C1q-binding capacity and C4d staining were analysed blinded to the allograft status (allograft loss or not). Patients who were lost to follow-up, who died with a functioning graft or who did not reach the outcome were eliminated as of their last follow-up.

A descriptive analysis was performed using means ± standard deviations (SD) or median (IQR) for continuous variables. For categorical variables, absolute numbers and percentages were computed. Comparison of baseline characteristics between the C4d+ and C4d− groups and C1q+ and C1q− groups were based on the chi-square test or the Fisher test for categorical variables, as appropriate, and the parametric Student's t-test or nonparametric Mann–Whitney *U*-test for continuous data, depending on the value distribution. The primary endpoint was the graft survival rate, considering the following event: graft loss. Patient survival rates were determined using the Kaplan–Meier method. The prognostic significance of potential variables was first determined by means of univariate survival analysis (log rank test): all variables yielding *P*-values less than 0.15 were then integrated into a multivariate analysis to adjust for possible confounders, using a Cox proportional hazard regression. The Cox regression used a backward stepwise selection method with a significance criterion set at 0.10 for inclusion in the model, nonsignificant variables being removed at each step of the selection. Two-by-two correlation and interactions between explicative variables were tested to avoid over-fitting. Association of each variable with the outcome was estimated with hazard ratios and 95% confidence interval (CI). Calibration of the multivariate model that indicates the gap between observed and predicted value was tested using the slope of the observed probabilities on predicted event probabilities. Statistical significance was assumed at $P < 0.05$, and all reported *P*-values were two-sided. All statistical analyses were performed with STATA v 12.0 (StataCorp, College Station TX, USA).

All procedures performed were in accordance with the ethical standards of our institution and with the 1964 Helsinki Declaration and its later amendments. All patients were informed at the time of transplantation that their clinical data would be used for research purposes and signed a written informed consent.

Results

Whole cohort

We reviewed all for-cause kidney allograft biopsies performed between January 2005 and January 2012. Among 500 cases, 48 patients displayed first-time morphologic evidence of acute tissue injury consistent with acute AMR and were associated with anti-HLA DSA. Recipients at the time of transplant are depicted in Table 1. Forty-four (92%) donors were deceased, and 12 (25%) patients had a repeat transplant. Among the 37 sera available before transplant, 24 (65%) were DSA positive. Among those, one patient underwent desensitization immediately following transplantation including high-dose intravenous immunoglobulin and plasmapheresis. Immunosuppressive treatment included induction therapy in 43 (90%) patients and calcineurin inhibitors in 47 (98%) patients. Mean baseline eGFR was 49 (±24) ml/min/1.73 m^2. Nine (19%) patients had a previous episode of acute T-cell-mediated rejection (ACR).

AMR episodes (Table 2) occurred 22 (2–51) months after kidney transplant [27 (56.3%) after 1 year], and the leading cause of allograft biopsy was acute kidney injury [$N = 40$ (83%)]. Median eGFR at the time of biopsy was 25 (13–32) ml/min/1 73 m^2. At the time of AMR, the number of DSA was 2 (1–3), the MFI sum was 8621 (2885–20.702), and the highest MFI was 6757 (2265–11.822). Class I and class II DSA with complement fixing ability (C1q) were detected in 9 (20%) and 14 (31%) patients, respectively. Peritubular capillaritis (ptc) and glomerular (g) inflammation were depicted in 46 (96%) and 40 (83%) biopsies, respectively, and median microvascular injury score (g + ptc) was 3 (2, 3). The C4d+ group included 18 (37.5%) biopsies from 18 patients. The C4d− group included 30 (62.5%) biopsies from 30 patients.

C4d+ and C4d− AMR episodes

Next, we compared C4d-positive ($N = 18$ [37.5%]) and C4d-negative ($N = 30$ [62.5%]) AMR episodes. Demographic recipients' and donors' characteristics were similar in both groups (Tables 1 and 2). The number of repeat transplants was comparable ($P = 1.00$). Before transplantation, seven (70%) patients presented with DSA in the C4d+ group and 17 (63%) in the C4d− group ($P = 1.00$). The number of patients with class II DSA was significantly higher in the C4d− group ($N = 15$ [88%] vs. $N = 3$ [43%]; $P = 0.04$). The C4d+ group received significantly more antithymocyte globulin than the C4d− group ($P = 0.04$). Maintenance immunosuppressive therapy was comparable in both groups ($P = 1.00$) as were baseline eGFR ($P = 0.75$) and the number of ACR before an AMR episode ($N = 2$ [11%] in the C4d+ group vs. $N = 7$ [23%] in the C4d− group; $P = 0.45$).

Median delay between transplant and AMR was similar between both groups [29 (0–76) months vs. 15 (2–38) months; $P = 0.28$] as were the DSA numbers at the

Table 1. Kidney transplant and patient characteristics.

Variables	Whole cohort $N = 48$ (100%)	C4d positive $N = 18$ (38%)	C4d negative $N = 30$ (62%)	*P*-value*
Recipients				
Age (years), mean, SD	46 ± 14	42 ± 13	48 ± 14	0.14
Gender, male, *N* (%)	26 (54)	11 (31)	15 (50)	0.45
End-stage kidney disease				
Glomerular disease, *N* (%)	14 (29)	5 (28)	9 (30)	0.11
Hypertension, *N* (%)	5 (10.5)	1 (5.5)	4 (13)	
Diabetes, *N* (%)	3 (6)	1 (5.5)	2 (7)	
Hereditary, *N* (%)	5 (10.5)	2 (11)	3 (10)	
Unknown, *N* (%)	11 (23)	6 (33)	5 (17)	
Others, *N* (%)	10 (21)	3 (17)	7 (23)	
Repeat transplant, *N* (%)	12 (25)	4 (22)	8 (27)	1.00
Pretransplant anti-HLA antibodies				
Available, *N* (%)	37 (77)	10 (56)	27 (90)	0.01
Total DSA (class I and class II), *N* (%)	24 (65)	7 (70)	17 (63)	1.00
DSA class I, *N* (%)	14 (38)	6 (86)	8 (47)	0.17
Number, median (IQR)	0 (0–1)	1 (0–1.75)	0 (0–1)	0.09
Sum of MFI, median (IQR)	0 (0–1724)	861 (0–4884)	0 (0–1418)	0.16
Class I iDSA, *N* (%)	9 (37)	5 (50)	4 (15)	0.04
DSA class II, *N* (%)	18 (49)	3 (43)	15 (88)	0.04
Number, median (IQR)	0 (0–1)	0 (0–0.75)	1 (0–1)	0.36
Sum of MFI, median (IQR)	0 (0–3897)	0 (0–654)	875 (0–4039)	0.28
Class II iDSA, *N* (%)	15 (63)	2 (20)	13 (48)	0.15
Transplant characteristics				
Donor age (years), mean, SD	49 ± 19	45 ± 14	52 ± 21	0.18
Deceased donor, *N* (%)	44 (92)	16 (89)	28 (93)	0.59
Delayed graft function, *N* (%)	19 (36)	5 (28)	14 (47)	0.20
Cold ischaemia time (h), mean, SD	19 ± 8	18 ± 8	21 ± 9	0.27
Induction immunosuppression, *N* (%)	43 (90)	16 (89)	27 (90)	1.00
Interleukine receptor-2 blockers, *N* (%)	27 (60)	6 (35)	21 (75)	0.01
Antithymocyte globulin, *N* (%)	18 (40)	10 (59)	8 (29)	0.04
Maintenance immunosuppression				
Calcineurin inhibitors, *N* (%)	45 (98)	18 (100)	27 (96)	1.00
Others, *N* (%)	1 (2)	0	1 (4)	1.00
Acute T-cell-mediated rejection, *N* (%)	9 (19)	2 (11)	7 (23)	0.45
Baseline eGFR, ml/min/1.73 m^2, mean, SD	49 ± 24	47 ± 11	50 ± 28	0.75

DSA, donor-specific antibodies; iDSA, immunodominant DSA; eGFR, estimated glomerular filtration rate.

**P*-value between the two groups C4d+ and C4d− by Student's t-test or Mann–Whitney test for continuous variables and by chi-square test or Fisher's test for categorical variables, as appropriate.

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Table 2. Acute antibody-mediated rejection episode characteristics.

Variables	Whole cohort N = *48* (100%)	C4d positive *N* = 18 (38%)	C4d negative *N* = 30 (62%)	*P*-value*
Cause of biopsy				
Delayed graft function, *N* (%)	5 (10)	1 (6)	4 (14)	0.02
Acute kidney injury, *N* (%)	40 (83)	15 (83)	25 (83)	
Proteinuria, *N* (%)	3 (6)	2 (11)	1 (4)	
Clinical presentation				
eGFR, ml/min/1,73 m^2, median (IQR)	25 (13–32)	28 (12–34)	23 (14–32)	0.83
Proteinuria >1 g/l *N* (%)	11 (25)	3 (18.8)	8 (28.6)	0.72
Time to rejection, months, median (IQR)	22 (1–51)	29 (0–76)	15 (2–38)	0.28
>12 months *N* (%)	27 (56)	11 (61)	16 (53)	0.60
Anti-HLA DSA				
Both DSA class I and class II, *N* (%)	48 (100)	18 (100)	30 (100)	1.00
DSA class I, *N* (%)	30 (63)	13 (76)	17 (57)	0.36
Number, median (IQR)	1 (0–1)	1 (1–2)	1 (0–1)	0.02
Sum of MFI, median (IQR)	891 (0–4243)	4230 (411–13 270)	587 (0–1785)	0.02
Class I iDSA, *N* (%)	11 (23)	7 (41)	4 (14)	0.07
DSA class II, *N* (%)	42 (88)	14 (82)	28 (93)	0.18
Number, median (IQR)	1 (1–2)	1 (1–3)	1 (1–2)	0.58
Sum of MFI, median (IQR)	4472 (1369–13 555)	4472 (540–17 543)	4506 (1856–11 141)	0.62
Class II iDSA, *N* (%)	36 (75)	10 (59)	26 (87)	0.03
Both C1q class I and class II DSA, *N* (%)	20 (44)	11 (65)	9 (30)	0.03
C1q class I DSA, *N* (%)	9 (20)	6 (40)	3 (10)	0.04
Number, median (IQR)	0 (0–0)	0 (0–1)	0 (0.0)	0.02
MFI max, median (IQR)	0 (0–0)	0 (0–742)	0 (0–0)	0.01
Sum of MFI, median (IQR)	0 (0–0)	0 (0–742)	0 (0–0)	0.01
C1q Class II DSA, *N* (%)	14 (31)	7 (47)	7 (23)	0.11
Number, median (IQR)	0 (0–1)	0 (0–1)	0 (0–0)	0.06
MFI max, median (IQR)	0 (0–2799)	0 (0–17 000)	0 (0–0)	0.07
Sum of MFI, median (IQR)	0 (0–2799)	0 (0–11 229)	0 (0–0)	0.09
Pathology				
Acute tubular injury, *N* (%)	22 (46)	6 (30)	16 (50)	0.25
Peritubular capillary inflammation (ptc), *N* (%)	46 (96)	17 (94)	29 (97)	1.00
Grade 1/2/3, *N* (%)	21/21/4 (46/46/8)	8/8/1 (47/47/6)	13/13/3 (45/45/10)	1.00
Glomerular inflammation (g), *N* (%)	40 (83)	18 (100)	22 (83)	0.02
Grade 1/2/3, *N* (%)	19/15/6 (47/38/15)	6/9/3 (33/50/17)	13/6/3 (59/27/14)	0.0007
g+ptc, median (IQR)	3 (2–3)	3 (3–4)	2 (2–3)	0.02
Intimal arteritis, *N* (%)	5 (10)	1 (6)	4 (14)	0.64
Transplant glomerulopathy (cg), *N* (%)	16 (34)	9 (53)	7 (23)	0.04
Grade 1/2/3, *N* (%)	6/7/3 (37/44/19)	3/5/1 (33/56/11)	3/2/2 (42/29/29)	0.0001
IFTA, *N* (%)	26 (58)	8 (50)	18 (62)	0.43
Grade 1/2/3, *N* (%)	13/9/4 (50/35/15)	4/3/1 (50/38/12)	9/6/3 (50/33/17)	0.54
Fibrous intimal thickening (cv), *N* (%)	22 (46)	8 (44)	14 (47)	1.00
Grade 1/2/3, *N* (%)	9/13/0 (41/59/0)	4/4/0 (50/50/0)	5/9/0 (36/64/0)	0.04
ACR, *N* (%)	21 (44)	6 (33)	15 (50)	0.26
Treatment				
Standard treatment, *N* (%)	42 (88)	15 (83)	27 (90)	0.66
Rituximab, *N* (%)	27 (56)	12 (67)	15 (50)	0.26
Antithymocyte globulin, *N* (%)	16 (3)	6 (33)	10 (33)	1.00
Outcome				
After 1 year				
eGFR, ml/min/1.73 m^2, median (IQR)	35 (25–48)	38 (25–48)	35 (26–61)	0.86
Graft loss, *N* (%)	16 (33)	7 (39)	9 (30)	0.53
At the end of follow-up				
Duration, months, median (IQR)	23 (10–40)	18 (2–30)	27 (13–45)	0.21

Table 2. Continued.

Variables	Whole cohort N = *48* (100%)	C4d positive *N* = 18 (38%)	C4d negative *N* = 30 (62%)	*P*-value*
eGFR, ml/min/1.73 m^2, median (IQR)	26 (18–39)	26 (24–34)	25 (17–40)	0.94
Graft loss, *N* (%)	23 (48)	12 (67)	11 (37)	0.04
Graft survival, (months), median (IQR)	23 (10–39)	17 (2–29)	27 (13–44)	0.21
Recipient death, *N* (%)	5 (10)	2 (11)	3 (10)	1.00

**P*-value between the two groups C4d+ and C4d− by Student's t-test or Mann–Whitney test for continuous variables and by chi-square test or Fisher's test for categorical variables, as appropriate.

time of AMR ($P = 0.08$). The number and the MFI sum of class I DSA were significantly higher in the C4d+ group ($P = 0.02$ and $P = 0.01$, respectively). However, the immunodominant DSA were significantly more class II in the C4d− group ($P = 0.03$). Class I C1q characteristics (incidence, number of C1q DSA, MFI sum C1q and highest MFI C1q) were significantly higher in C4d+ patients ($P = 0.04$; $P = 0.02$; $P = 0.01$ and $P = 0.01$, respectively), while class II C1q DSA were similar in both groups. The presence of at least one C1q-positive DSA was significantly higher in the C4d+ ($N = 11$ [65%]) compared to the C4d− group ($N = 9$ [30%]) ($P = 0.03$). Median DSA max MFI was significantly higher in patients with C1q-binding DSA [11 898 (7787–15 486) vs. 2797 (1333–4905); $P < 0.001$].

Considering kidney allograft histopathology, glomerulitis was significantly more frequent ($P = 0.02$) and more severe ($P = 0.0007$) in the C4d+ group, while ptc frequency and severity were similar in both groups. Microvascular score (g + ptc) was significantly higher in the C4d+ group [3 (3,4) vs. 2 (2,3) in the C4d− group; $P = 0.02$]. Transplant glomerulopathy (cg) was significantly more frequent and more severe in the C4d+ group ($P = 0.04$ and $P = 0.0001$, respectively). Interstitial fibrosis – tubular atrophy IFTA and chronic vasculopathy were similar in both groups. AMR was concurrent with an ACR episode in 6 (33%) biopsies in the C4d+ group and in 15 (50%) biopsies in the C4d− group ($P = 0.26$).

The AMR therapy was not different between the groups. Within 12 months after the AMR episode, eGFR was 38 (5–48) ml/min/1 73 m^2 in the C4d+ group and 35 (5–61) ml/min/1 73 m^2 in the C4d− group ($P = 0.86$). However, at the end of the 23 (0–40) months' follow-up, the number of graft losses was significantly higher in the C4d+ group [$N = 12$ (67%) vs. $N = 11$ (37%) in the C4d− group; $P = 0.04$]. Median graft survival time after AMR was comparable between both groups [27 (13–44) vs. 18 (2–29) months; $P = 0.21$].

C1q+ and C1q− AMR episodes

C1q data were available in 45 (94%) patients. AMR with C1q-binding DSA at the time of the rejection episode included 20 (44%) biopsies, while AMR with C1q-nonbinding included 25 (66%) biopsies (Table 3). C1q+ AMR presented with significantly more class I and class II DSA [$N = 1$ (1–3) and $N = 2$ (1–3), $P = 0.005$ and 0.04, respectively] than C1q− AMR. Class I and class II MFI sums were significantly higher in the C1q+ group ($P = 0.01$ and $P = 0.0004$, respectively). Pathology was similar in both groups outside of C4d+ staining, which was significantly more frequent in C1q+ AMR ($P = 0.01$). A moderate statistical correlation between C4d+ staining and C1q binding has been found (Cramer's $V = 0.41$). Allograft loss, eGFR (12 months after and at the end of follow-up), and patient survival were comparable between the groups.

Survival analysis

Univariate analysis did not individualize any clinical, immunological, and histological variables associated with allograft survival (Table 4). Allograft survival was similar whatever the C4d staining or the C1q-binding status were ($P = 0.09$ and $P = 0.67$, respectively) (Fig. 1).

C4d status, IFTA, and standard and antithymocyte globulin therapy for AMR were considered in the multivariable model (Table 5). Twenty-three events were considered with these three explicative variables. No significant interaction was found between the three variables. C4d-positive staining and IFTA were independently associated with allograft loss [HR = 2.65, 95% CI (1.11–6.34), $P = 0.028$ and HR=3.07, 95% CI (1.16–8.1), $P = 0.024$, respectively]. However, antithymocyte globulin therapy is not associated with allograft loss [HR = 0.56, 95% CI (0.21–1.47), $P = 0.238$].

Table 3. Acute antibody-mediated rejection episode characteristics with C1q-positive and C1q-negative donor-specific anti-HLA antibodies ($N = 45$).

Variables	C1q positive $N = 20$ (38%)	C1q negative $N = 25$ (62%)	*P*-value*
Cause of biopsy			
Delayed graft function, *N* (%)	0 (0)	4 (16)	0.15
Acute kidney injury, *N* (%)	19 (95)	19 (76)	
Proteinuria, *N* (%)	1 (5)	2 (8)	
Clinical presentation			
eGFR, ml/min/1.73 m^2, median (IQR)	23 (12–31)	29 (15–32)	0.58
Proteinuria >1 g/l *N* (%)	4 (20)	7 (28)	0.73
Time to rejection, months, median (IQR)	31 (6–61)	7 (1–41)	0.17
>12 months *N* (%)	13 (65)	12 (48)	0.36
Anti-HLA DSA			
Both DSA class I and class II, *N* (%)	20 (100)	25 (100)	1.00
DSA class I, *N* (%)	16 (80)	13 (52)	0.07
Number, median (IQR)	1 (1–3)	1 (0–1)	0.005
Sum of MFI, median (IQR)	2097 (505–12 455)	411 (0–1761)	0.01
Class I iDSA, *N* (%)	4 (20)	6 (24)	1.00
DSA class II, *N* (%)	19 (95)	22 (88)	0.62
Number, median (IQR)	2 (1–3)	1 (1–2)	0.04
Sum of MFI, median (IQR)	13 555 (4544–20 393)	2885 (655–5668)	0.0004
Class II iDSA, *N* (%)	16 (80)	19 (76)	1.00
Both C1q class I and class II DSA, *N* (%)	20 (100)	–	–
C1q class I DSA, *N* (%)	9 (20)	–	–
Number, median (IQR)	0 (0–0)	–	–
MFI max, median (IQR)	0 (0–0)	–	–
Sum of MFI, median (IQR)	0 (0–0)	–	–
C1q Class II DSA, *N* (%)	14 (31)	–	–
Number, median (IQR)	0 (0–1)	–	–
MFI max, median (IQR)	0 (0–2799)	–	–
Sum of MFI, median (IQR)	0 (0–2799)	–	–
Pathology			
Acute tubular injury, *N* (%)	8 (40)	14 (80)	0.37
Peritubular capillary inflammation (ptc), *N* (%)	19 (95)	24 (96)	1.00
Grade 1/2/3, *N* (%)	9/8/2 (47/42/11)	10/12/2 (42/50/8)	0.87
Glomerular inflammation (g), *N* (%)	18 (90)	19 (76)	0.26
Grade 1/2/3, *N* (%)	8/8/2 (44/44/12)	11/4/4 (58/21/21)	0.29
g + ptc, median (IQR)	3 (2–3)	2 (2–3)	0.18
Intimal arteritis, *N* (%)	2 (10)	3 (12)	1.00
Transplant glomerulopathy (cg), *N* (%)	9 (45)	6 (24)	0.20
Grade 1/2/3, *N* (%)	2/6/1 (22/67/11)	4/1/1 (68/16/16)	0.15
IFTA, *N* (%)	11 (55)	13 (52)	0.76
Grade 1/2/3, *N* (%)	7/2/2 (64/18/18)	6/5/2 (46/38/16)	0.55
Fibrous intimal thickening (cv), *N* (%)	10 (50)	11 (44)	0.77
Grade 1/2/3, *N* (%)	6/4/0 (60/40/0)	3/8/0 (27/73/0)	0.13
ACR, *N* (%)	6 (30)	7 (28)	1.00
C4d+, *N* (%)	11 (55)	4 (16)	0.01
Treatment			
Standard treatment, *N* (%)	19 (95)	22 (88)	0.66
Rituximab, *N* (%)	11 (55)	14 (56)	0.26
Antithymocyte globulin, *N* (%)	8 (40)	7 (28)	1.00
Outcome			
After 1 year			
eGFR, ml/min/1.73 m^2, median (IQR)	30 (24–39)	38 (27–61)	0.30
Graft loss, *N* (%)	7 (35)	9 (28)	1.00
At the end of follow-up			

Table 3. Continued.

Variables	C1q positive *N* = 20 (38%)	C1q negative *N* = 25 (62%)	*P*-value*
Duration, months, median (IQR)	17 (3–46)	27 (8–39)	0.58
eGFR, ml/min/1.73 m^2, median (IQR)	26 (21–39)	26 (16–68)	1.00
Graft loss, *N* (%)	9 (45)	11 (44)	0.95
Graft survival, (months), median (IQR)	17 (3–46)	27 (8–39)	0.58
Recipient death, *N* (%)	2 (10)	0 (0)	–

**P*-value between the two groups C1q positive and C1q negative by Student's t-test or Mann–Whitney test for continuous variables and by chi-square test or Fisher's test for categorical variables, as appropriate.

Calibration of the Cox regression model was good reflecting fit statistical analysis to the observed data (test of the slope $P = 0.97$), and discrimination was correct with Harrell's C 0.69.

Discussion

In this study, we aimed to analyse the capability of the C4d staining and C1q binding to predict allograft loss after clinical AMR episode. We found that C4d+ staining but not C1q is associated with allograft loss after an AMR episode.

Currently, C4d-positive staining is not required for a diagnosis of an AMR episode [7]. In our cohort from two transplant centres, including only clinical AMR, the incidence of C4d− AMR reaches 62%, confirming former data talking about an incidence up to 70% depending on technique used to detect C4d staining and delay from transplant considered [9,12,15,17,25]. Our data confirmed that C4d− AMR might occur anytime after transplant [9,18]. Before transplant, C4d− clinical AMRs have significantly fewer class I DSA than C4d+ AMR and the immunodominant DSA was more frequently class II. Clinical presentation was similar, irrespective of C4d status. At the time of acute rejection, the number of class I and MFI max DSA was significantly lower in C4d− AMR as were class I C1q DSA numbers, MFI max and sum of MFI. The immunodominant DSA was more frequently class II. Our cohort did not confirm exactly the largest described probably because we selected only clinical AMR and the number of patients is smaller [18]. Histological analysis was not included in this recent report [18]. We found that microvascular inflammation and transplant glomerulopathy were significantly more frequent in C4d+ AMR, but IFTA and vascular lesions were not. These results are highly consistent with those suggesting that C4d− AMR is a less severe form of AMR than C4d+ AMR [10,17]. In another hand, clinical C4d− AMR could be the result a complement independent mechanism of microcirculation injury involving NK cells or antibody dependent cellular cytotoxicity [26–29].

At our knowledge, we described for the first time the difference between C1q+ DSA and C1q− DSA clinical AMR. Clinical presentation was similar at the time of the acute rejection episode. The MFI max of class I and class II DSAs was significantly higher in C1q positive group. Higher MFI associated with C1q-binding property has already been described [20]. Kidney pathology was similar in both groups besides C4d+ staining significantly more frequent in DSA binding C1q cases. Association between C1q DSA and C4d+ staining has already been found in the paediatric population [30]. Allograft survival was significantly associated with C4d+ staining but not with C1q+ binding. AMR treatment is not associated with allograft survival and was similar in C4d groups. Conflicting data are published about AMR C4d prognostic value with two recent studies showing opposite results [15,18]. The first one found a negative impact of C4d staining whatever the histological analysis was, AMR or not [15]. The second one analysed a large cohort of clinical and subclinical AMR and did not isolate a difference between C4d− and C4d+ 1, 2 or 3 years post-AMR death censored allograft survival [18]. However, no multivariable analysis was performed [18]. C1q+ binding and C4d staining prognosis value have also been tested with C3d-binding DSA in two cohorts of patients with AMR [16]. C3d-binding DSA was an independent predictor of allograft loss while C4d staining and C1q-binding DSA were not [16]. Unfortunately, we could not test our sera for C3d-binding DSA. C1q-binding DSA may strongly relate to IgG MFI [31]. In the context of clinical AMR, DSA MFI has

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Table 4. Kidney allograft survival univariable analysis after acute antibody-mediated rejection episode.

Variables	Hazard ratio	95% CI	*P*-value*
Recipients			
Age	1.00	0.95–1.04	0.84
Gender, female	1	–	–
Gender, male	0.90	0.34–2.42	0.84
DSA before transplant			
Number	0.91	0.65–1.26	0.56
MFI max	1.00	1.00–1.00	0.89
Sum of MFI	1.00	1.00–1.00	0.62
Transplant characteristics			
Donor age	1.02	0.98–1.05	0.34
Deceased donor	1	–	–
Living donor	1.37	0.31–6.08	0.67
No delayed graft function	1	–	–
Delayed graft function	0.65	0.22–1.88	0.42
No induction immunosuppression	1	–	–
Induction immunosuppression,	0.77	0.22–2.74	0.69
Without Interleukine receptor-2 blockers	1	–	–
Interleukine receptor-2 blockers	0.71	0.25–2.06	0.53
Without antithymocyte globulin	1	–	–
Antithymocyte globulin	1.22	0.42–3.56	0.71
At the time of AMR			
eGFR, ml/min/1.73 m^2	0.98	0.94–1.02	0.34
Proteinuria<1 g/l	1	–	–
Proteinuria >1 g/l	0.64	0.14–2.91	0.56
Time to rejection	1.00	0.99–1.02	0.77
<12 months	1	–	–
>12 months	1.62	0.58–4.50	0.35
Anti-HLA DSA			
Number	0.85	0.55–1.29	0.43
MFI max	1.00	1.00–1.00	0.83
Sum of MFI	1.00	1.00–1.00	0.90
C1q class I DSA	0.91	0.20–4.14	0.90
Number	1.09	0.38–3.13	0.87
MFI max	1.00	1.00–1.00	0.91
Sum of MFI	1.00	1.00–1.00	0.96
C1q Class II DSA	1.09	0.33–3.60	0.89
Number, median	1.07	0.55–2.06	0.84
MFI max, median	1.00	1.00–1.00	0.58
Sum of MFI, median	1.00	1.00–1.00	0.73
Renal allograft biopsy			
C4d–	1	–	–
C4d+	2.23	0.84–5.97	0.099
g + ptc	1.11	0.72–1.72	0.50
Transplant glomerulopathy = 0	1	–	–
Transplant glomerulopathy	1.04	0.36–3.01	0.94
IFTA = 0	1	–	–
IFTA	2.40	0.83–6.95	0.10
Without ACR	1	–	–
With ACR	1.25	0.46–3.35	0.66
AMR therapy			
No standard treatment	1	–	–
Standard treatment	0.37	0.12–1.16	0.08
Without Rituximab	1	–	–
With Rituximab	1.15	0.41–3.19	0.79
Without antithymocyte globulin	1	–	–
With antithymocyte globulin	0.36	0.10–1.29	0.10

**P*-value by log rank test.

already been reported a bad prognosis marker [32]. So more, recently, detecting complement fixation did not show any independent diagnostic advantage to predict late silent AMR compared to DSA IgG MFI [33]. In the context of de novo DSA, C1q+ binding DSA is associated with acute and chronic AMR however impact of allograft survival has been reported negative [22,23].

The major limitation of our study is the relative small number of patients. However, inclusion of patients was consecutive during the study period precluding the selection bias. So more, the number of events is relatively high with 23 (48%) allograft losses at the end of the follow-up. Our Cox model is quite robust for three reasons: (i) we included three explicative variables which is very close to the ten events per variable required ideally, (ii) calibration of the model was good, and (iii) discrimination was correct.

In conclusion, in our cohort of clinical AMR, we found that C4d + staining is independently associated with allograft loss. C4d− AMR presented with fewer histological lesions and fewer C1q+ DSA. Besides the number of DSA and C4d staining, the C1q+ group presented with AMR similar to that of patients in the group without C1q binding. Our results suggest that, in clinical AMR, C4d could be useful in prognosis prediction analysis and that C1q well known to predict AMR is not a reliable prognosis factor.

Authorship

AM, PG and MM: designed the research, wrote the manuscript and performed the research. JPa, FCP, DD

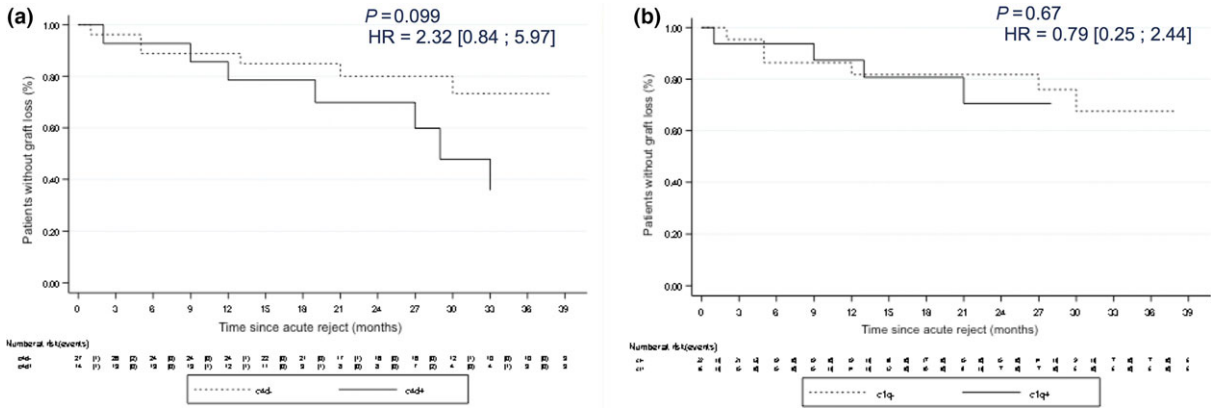

Figure 1 Graft survival univariate analysis using Kaplan–Meier survival curves. (a) Allograft survival was similar in C4d+ staining AMR and in C4d− staining AMR (P = 0.09). (b) Allograft survival after the AMR episode was similar in the presence of or in the absence of complement fixing ability DSA (C1q DSA) at the time of rejection (P = 0.67).

Table 5. Multivariable analysis of risk factors for graft loss by Cox proportional hazards regression model.

Variables	Hazard ratio	95% CI	*P*
C4d+	2.65	1.11–6.34	0.028
AMR therapy: antithymocyte globulin	0.56	0.21–1.47	0.238
Presence of IFTA	3.07	1.16–8.1	0.024

and IB: designed the research and performed the research. JPe, ER, PL, CS, TK and VA: performed the research.

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Conflict of interest

The authors declare no conflict of interests.

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