BRITISH JOURNAL OF BIOMEDICAL SCIENCE
2021, VOL. 78, NO. 4, 244–247
https://doi.org/10.1080/09674845.2021.1927308

CASE REPORT

# Management of mixed acute rejection driven by a *de novo* donor-specific complement-binding anti-DQB1*03:01 antibody and intraepithelial CD8 T-cells in a kidney recipient: a case report

F Boix[a], J Feito[b], A Rodríguez-Campón[c], MC Chillón[a], S García-Sánchez[a], G Tabernero[c], P Fraile[c] and R García-Sanz[a]

[a]Department of Haematology, University Hospital of Salamanca (HUS-IBSAL), CIBERONC, and Cancer Research Institute of Salamanca-IBMCC (CSIC-USAL University), Salamanca, Spain; [b]Department of Clinical Pathology, University Hospital of Salamanca (HUS-IBSAL), Salamanca, Spain; [c]Department of Nephrology, University Hospital of Salamanca (HUS-IBSAL), Salamanca, Spain

**ABSTRACT**

Mixed acute rejection is a clinicopathological entity that is difficult to accurately diagnose, and so may be under-reported. Allografts are lost more often than in either humoral or cellular rejection. The diagnosis requires both histological and immunological studies on renal biopsy and blood specimens from the transplant recipient to provide the required rescue therapy to abolish the allogeneic response against the graft. We present a clinical case report of an active mixed acute rejection driven by a *de novo* donor-specific complement-binding anti-DQB1*03:01 antibody and intraepithelial CD8 T-cells in a patient with a kidney transplant. The patient was diagnosed, treated, and followed up as per the local institution's procedure with a full recovery of graft function. Our case emphasises the challenge of a mixed acute rejection and supports the need to improve the post-transplant outcome of recipients and their grafts.

**ARTICLE HISTORY**
Received 11 April 2021
Accepted 4 May 2021

**KEYWORDS**
Antibody-mediated rejection; T-cell mediated rejection; mixed acute rejection; complement binding; desensitization; donor specific antibodies; HLA

## Introduction

Acute rejection can be broadly categorised into T cell-mediated (cellular) rejection and antibody-mediated rejection. Although both may coexist at the same time in the renal allograft, namely mixed acute rejection (MAR) [1], its frequency is lower, leading to patients being undertreated or incorrectly treated [2]. MAR is associated with antibody-mediated acute vascular thrombotic microangiopathy, which is further linked with the lowest long-term post-transplant rate of graft survival [3]. Given the importance of the need for the correct treatment of patients with acute vascular thrombotic microangiopathy, MAR diagnosis is essential in improving the outcome of these patients.

In kidney biopsies, MAR is characterised by the presence of prominent tubulitis and interstitial inflammatory infiltrate with microcirculation inflammation alone, or both microcirculation inflammation and deterioration [2]. However, any humoral component of the MAR needs to be demonstrated using both histopathology and laboratory findings [4], leading characteristics include the presence of donor-specific antibodies (DSA), assessing their avidity, subclass, complement-binding capacity, specificity, and response to treatment [5–8]. Furthermore, histopathology study should determine for complement factor C4d deposition on kidney biopsies, as it serves as a footprint of antibody–antigen interaction on the surface of endothelial cells, whose positivity is used for the diagnosis of antibody-mediated rejection [9]. Combined therapeutic regimens have been reported to ineffectively reverse MAR in different observational studies and yet the desired approach remains elusive. To this end, some ongoing trials are trying to address the optimal therapy for MAR [10], while current evidence-based protocols are applied.

We report a case report of the diagnosis and treatment of a MAR episode in a kidney transplant recipient during post-transplant follow-up.

## Clinical case presentation

The case is a 45-year-old man with a medical history of hypertension, hypercholesterolemia, hyperuricaemia, gouty arthritis, and end-stage chronic kidney disease, secondary to nephroangiosclerosis, as well as nephrotoxicity due to anti-inflammatory drugs. In 2017, the patient had negative complement-dependent cytotoxicity (CDC)-based cross-match against a 9/10 HLA mismatch 42-years-old deceased donor. The patient was transplanted with no detectable anti-HLA antibodies.

Before transplantation, induction therapy was based on two cycles of anti-CD25 mAb basiliximab, 20 mg. Following implantation, the graft had delayed

CONTACT F Boix fjboix@saludcastillayleon.es Department of Haematology, Molecular Biology and HLA Unit, University Hospital of Salamanca (HUS-IBSAL), CIBERONC, and Cancer Research Institute of Salamanca-IBMCC (CSIC-USAL University), Salamanca 37007, Spain

© 2021 British Journal of Biomedical Science

function, but subsequently showed stable renal function, allowing discharge 2 weeks later. Post-transplant immunosuppression was tacrolimus (1.5 g/24 h), mycophenolate mofetil (500 mg/24 h), and corticosteroids (methylprednisolone 5 mg/24 h).

During the 32-month post-transplant follow-up visit, the patient reported general discomfort, shivers, asthenia, occasional low-grade fever, dyspnoea on moderate exertion. Anxiety related to a family problem caused the patient to discontinue medication over the previous weeks. Laboratory data are shown in Table 1 with grossly increased serum CRP, urea and creatinine, a modest neutrophil leukocytosis, a lymphopenia and a thrombocytosis. Levels of tacrolimus were significantly decreased, providing a cause for the rejection crisis. Given the acute deterioration of the renal function, the patient was admitted and treatment with boluses of 500 mg of intravenous methylprednisolone was started. An anti-HLA antibody study was requested, and a renal biopsy was scheduled.

The single antigen study (LIFECODES LSA, Immucor) revealed the presence of anti-HLA class I and class II *de novo* donor-specific antibodies namely anti-A*02, anti-DQB1*03:01 and anti-DQA1*02:01, respectively. Furthermore, a complement-binding capacity assay (LIFECODES C3d Detection, Immucor) was subsequently applied to confirm whether the detected anti-HLA antibodies were capable of binding C3d, thus causing damage to the graft. From the antibody panel the anti-DQB1*03:01, -DQB1*03:02 and -DQB1*03:03 were shown to bind C3d. Furthermore, the *de novo* donor-specific antibody anti-DQB1*03:01 was present in the allograft corroborating the humoral component of the MAR. The anti-DQB1*03:01 antibody found in the patient was specific to the epitope 45EV, which is formed by the glutamic acid and valine amino acids at position 45 of this HLA antigen, present in the donor allograft. The anti-A*02 antibody present in the patient's serum was specific to the epitope 66RKH (arginine, lysine, histidine at position 66) shared between different HLA-A*02 antigens, for instance, A*02:01, A*02:02, A*02:03, and A*02:05. However, this antibody does not bind to the C3b complement factor. The results from the anti-HLA antibody analysis are shown in Table 2.

The kidney biopsy revealed the presence of a mild-to-moderate interstitial inflammatory infiltrate with partial sclerosis of many glomeruli, with mild tubular atrophy. There was a strong inflammatory as well as immune component, based on the existence of widespread endothelial expression of C4d in both peritubular and glomerular capillaries, and the presence of vast numbers of intraepithelial T-cells, predominantly CD8 + T, corroborating the diagnosis of active mixed acute rejection [4] (Figure 1).

After corticosteroid-based treatment initiation, creatinine levels fell, with progressive improvement of the renal function and no haemodialysis needed during the entire admission. Following the immunological and pathological findings, a desensitization treatment was initiated based on serial plasma exchange every 48 hours along with a slow-paced administration of intravenous immunoglobulin (IVIG) (500 mg/kg). Overall, a total of 5 boluses of methylprednisolone, 7 sessions of plasmapheresis, and 5 doses of IVIG were administered during the entire hospital admission, that began 4 days after the initiation of the treatment. The success of the desensitization treatment was confirmed in a further anti-HLA antibody study where, although the calculated panel reactive antibody did not change following the Single Antigen assay analysis, the C3d assay was otherwise negative. When we examined the results of the anti-HLA antibody data, the MFI of the anti-DQB1*03:01 and anti-DQA1*02:01 antibodies decreased considerably in comparison with

Table 1. Laboratory parameters of the patient before and after receiving the desensitization treatment.

| | Reference range | Pre-AMR treatment (July 2020) | Post-AMR treatment (August 2020) | Post-AMR follow-up (February 2021) |
|---|---|---|---|---|
| **Serum and plasma biomarkers** | | | | |
| C-reactive protein (mg/L) | [0–50] | 13,900 | 120 | 160 |
| Urea (µmol/L) | [3–8] | 25 | 17 | 12 |
| Creatinine (µmol/L) | [62–107] | 408 | 143 | 164 |
| Glomerular filtration rate (mL/min/1.73 $m^2$) | [≥90] | 14 | 51 | 43 |
| Tacrolimus (ng/ml) | [6–8] | 1.4 | 6.8 | - |
| **Urine biomarkers** | | | | |
| 24 h diuresis (ml) | [1000–1500] | 3300 | 3000 | 2300 |
| Creatinine urine (µmol/L) | [3500–22,900] | 7337 | 5304 | 8486 |
| 24 h urine Creatinine (µmol/L) | [1040–2350] | 2740 | 1790 | 2030 |
| Urine protein (g/L) | [0–0.15] | 0.49 | 0.19 | 0.16 |
| 24 h urine protein (g/24 h) | [1.04–2.35] | 1.62 | 0.58 | 0.36 |
| **Full blood count** | | | | |
| Leukocyte (x$10^9$/L) | [4.50–10.80] | 13.50 | 6.85 | 8.89 |
| Neutrophil (x$10^9$/L) | [1.40–6.50] | 10.80 | 5.03 | 5.35 |
| Lymphocytes (x$10^9$/L) | [1.20–3.50] | 0.96 | 1.16 | 2.12 |
| Monocytes (x$10^9$/L) | [0.30–0.90] | 1.39 | 0.49 | 0.99 |
| Eosinophil (x$10^9$/L) | [0.00–0.50] | 0.39 | 0.17 | 0.40 |
| Basophil (x$10^9$/L) | [0.00–0.10] | 0.03 | 0.01 | 0.04 |
| Platelets (x$10^9$/L) | [150–450] | 597 | 346 | 307 |

AMR, acute mixed rejection.

**Table 2.** Immunological details of the patient before and after receiving the desensitization treatment.

| **Immunological parameters** | | | | | | | |
|---|---|---|---|---|---|---|---|
| *HLA tissue type*[1] | HLA-A | HLA-B | HLA-C | HLA-DRB1 | HLA-DQB1 | | HLA-DQA1 |
| Patient | *01,*11 | *15,*40 | *08,*15 | *03,*15 | *02,*06 | | *01, *05 |
| Donor | *02,- | *27,*53 | *02,*04 | *07,*11 | *02,*03(7) | | *02, *01 |
| | Pre-AMR treatment | | Post-AMR treatment | | Post-AMR follow-up | | |
| *Anti-HLA antibody study*[2] | Single Antigen | C3d assay[#] | Single Antigen | C3d assay[#] | Single Antigen | C3d assay[#] | Epitope analysis |
| *Class I anti-HLA antibodies* | | | | | | | |
| ***A*02:01*** | 826 | 208 | 416 | 221 | 161 | 52 | ***66RKH**** |
| ***A*02:02*** | 1081 | 272 | 540 | 245 | 222 | 59 | 44rRM,***66RKH**** |
| ***A*02:03*** | 1286 | 258 | 570 | 236 | 214 | 49 | 145KHT,***66RKH**** |
| ***A*02:05*** | 1090 | 254 | 533 | 213 | 240 | 48 | 44rRM,***66RKH**** |
| HLA class I cPRA* | 48 | | 48 | | 48 | | |
| *Class II anti-HLA antibodies* | | | | | | | |
| ***DQB1*03:01/DQA1*03:01*** | 4634 | 8898 | 6937 | 224 | 6236 | 44 | ***45EV****,47QL |
| ***DQB1*03:01/DQA1*03:02*** | 7313 | 5914 | 6744 | 236 | 5244 | 38 | ***45EV****,160DD,47QL |
| ***DQB1*03:01/DQA1*06:01*** | 6134 | 1932 | 5030 | 103 | 3962 | 35 | ***45EV**** |
| ***DQB1*03:01***/DQA1*05:01 | 3971 | 17,305 | 6609 | 507 | 6543 | 90 | ***45EV**** |
| ***DQB1*03:02/DQA1*02:01*** | 9129 | 10,599 | 10,357 | 290 | 10,424 | 60 | ***47EK2****,45GV+55PPP |
| ***DQB1*03:02/DQA1*03:01*** | 4634 | 18,991 | 8276 | 689 | 9066 | 150 | 45GV+55PPP,47QL |
| ***DQB1*03:02/DQA1*03:02*** | 6660 | 12,756 | 7434 | 257 | 7687 | 115 | 45GV+55PPP,160DD,47QL |
| ***DQB1*03:03***/DQA1*04:01 | 6400 | 3755 | 6640 | 125 | 4910 | 35 | 45GV+55PPP |
| ***DQB1*03:03/DQA1*06:01*** | 6787 | 1192 | 6200 | 91 | 4311 | 27 | 45GV+55PPP |
| ***DQB1*03:03/DQA1*03:02*** | 5468 | 13,513 | 7977 | 189 | 7126 | 73 | 45GV+55PPP,160DD,47QL |
| DQB1*04:01/***DQA1*02:01*** | 10,852 | 1095 | 5867 | 90 | 4595 | 29 | ***47EK2**** |
| DQB1*04:02/***DQA1*03:01*** | 964 | 128 | 493 | 102 | 137 | 30 | 47QL |
| DQB1*04:02/***DQA1*06:01*** | 784 | 115 | 345 | 80 | 149 | 34 | - |
| DQB1*02:01/***DQA1*02:01*** | 6391 | 199 | 4442 | 92 | 2402 | 37 | ***47EK2**** |
| DQB1*02:02/***DQA1*02:01*** | 5541 | 166 | 2452 | 87 | 1792 | 41 | ***47EK2**** |
| DQB1*02:02/***DQA1*03:02*** | 1418 | 109 | 348 | 85 | 122 | 40 | 160DD,47QL |
| HLA class II cPRA* | 70 | | 70 | | 70 | | |

cPRA = calculated panel reactive antibody; HLA = human leukocyte antigen; MFI = mean fluorescence intensity. [1]The HLA tissue type of the patient and the donor was performed prior transplantation. [2]The anti-HLA antibody study of the patient was performed post-transplantation. [‡]MFI (Raw value) cut-off [≥750]. [#]MFI (Raw value) cut-off [≥1500]. *cPRA is expressed in percentage (%).**donor-specific epitopes for which patient's antibodies are bound to the donor's HLA antigens in the allograft; 66RKH for HLA-A*02; 45EV for HLA-DQB1*03:01(7) and 47EK2 for HLA-DQA1*02:01. The donor-specific antibodies specificities against the donor's HLA antigens are in bold and italics.

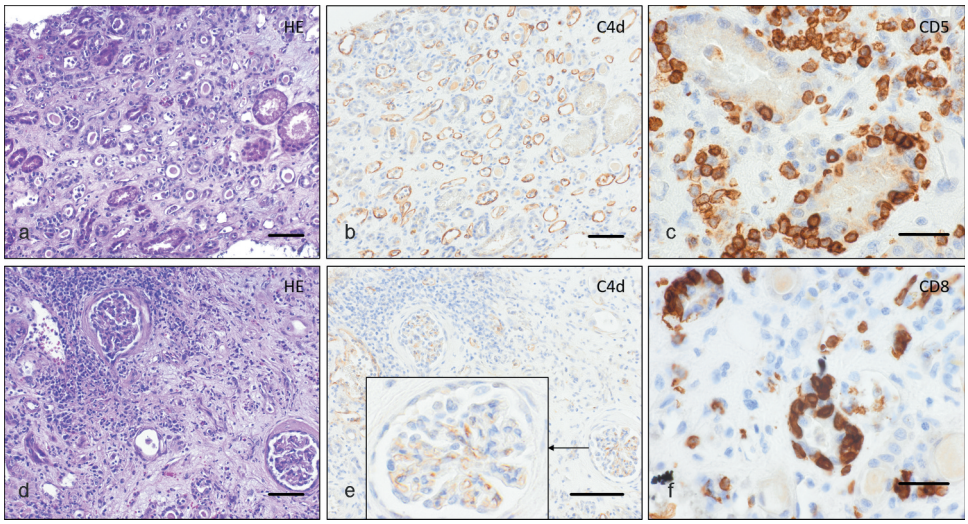


**Figure 1.** Serial section of medullary (a-b) and cortical (d-e) regions of the kidney stained with haematoxylin-eosin (HE) (a, d), and immunohistochemistry targeted against C4d (b, e). Image E has an insert with a magnified glomerulus. There are illustrated, as well, some tubules labelled with CD5 (c) and CD8 (f) antibodies, revealing intraepithelial T lymphocytes. There is a mild (a) to moderate (d) interstitial inflammatory infiltrate, with partial sclerosis of several glomeruli (d). C4d staining reveals widespread endothelial expression over the peritubular capillaries (b). Tubulitis is prominent, with numerous intraepithelial T lymphocytes, which on some tubules outnumbers epithelial cells (c). T lymphocytes are mainly of the CD8 subtype (f).

the same observed in the pre-desensitization treatment serum sample. Furthermore, 6 months following the anti-MAR treatment, the patient remains stable with good renal function and laboratory parameters. Importantly, the MFI of the *de novo* donor-specific antibodies continued to fall (Table 2).

## Discussion

Optimal therapy for MAR, defined as the coexisting cell-mediated and antibody-mediated allo-responses, has not yet been fully defined. We present a case report of a kidney transplant recipient who developed a MAR due to the development of an anti-DQB1*03:01 *de novo* donor-specific antibody capable of binding C3d as well as the activation and infiltration of alloreactive CD8 + T-cells, confirmed in a renal biopsy. The patient was treated with boluses of methylprednisolone with a clear improvement of the renal function, allowing a subsequent desensitization protocol based on plasma exchange and IVIG achieving complete clinical remission.

The current anti-MAR protocol applied in our institution was able to reverse the cellular alloresponse against the graft, as well as reduce the circulating anti-HLA antibodies, so the remaining donor-specific antibody was not able to complement. This approach controlled both the cellular and humoral active rejection already established. Although there was no post-transplant biopsy to demonstrate the reduction of T-cells, the anti-HLA antibody data was negative after the post-MAR treatment.

This case underlines the importance of identifying patients who develop active MAR with alloreactive T-cells and complement-binding *de novo* donor-specific antibodies who are good candidates to benefit from a rescue therapy used in our institution. The participation of nephrologists, immunologists, and pathologists in such cases is considered vital to properly diagnose patients with MAR. We conclude that the study of the renal biopsy in this subphenotype of acute rejection by immunohistochemistry staining of C4d and T-cells is primordial to properly guide the forthcoming treatment of the patient [11]. Moreover, the study of anti-HLA antibodies, by Single Antigen and C3d assays, is a strong confirmatory non-invasive tool for the diagnosis and monitoring of the desensitization protocol in patients with MAR [3–5].

## Acknowledgements

The authors warmly appreciate the generosity of the patient whose case is described in this manuscript. Written informed consent was obtained from the patient for publication of this case report and any accompanying images. This study was approved by the local ethics committee (CEIm PI 2021 01 656).

## Disclosure statement

All the authors who participated in this work certify no competing interests in the subject matter or materials discussed in this manuscript.

## Funding

This work was partially funded by the Gerencia Regional de Salud (GRS) of Castilla y León [GRS 2080/A/19].

## ORCID

F Boix http://orcid.org/0000-0002-3926-7920
J Feito http://orcid.org/0000-0003-2667-5332
MC Chillón http://orcid.org/0000-0003-1624-6059
S García-Sánchez http://orcid.org/0000-0002-8306-8478
R García-Sanz http://orcid.org/0000-0003-4120-2787

## References

[1] Nickeleit V, Andreoni K. The classification and treatment of antibody-mediated renal allograft injury: where do we stand? Kidney Int. 2007;71:7–11.
[2] Halloran PF, Merino Lopez M, Barreto Pereira A. Identifying subphenotypes of antibody-mediated rejection in kidney transplants. Am J Transplant. 2016;16:908–920.
[3] Lefaucheur C, Loupy A, Vernerey D, et al. Antibody-mediated vascular rejection of kidney allografts: a population-based study. Lancet. 2013;381:313–319.
[4] Haas M, Loupy A, Lefaucheur C, et al. The Banff 2017 kidney meeting report: revised diagnostic criteria for chronic active T cell–mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. Am J Transplant. 2018;18:293–307.
[5] Haas M, Mirocha J, Reinsmoen NL, et al. Differences in pathologic features and graft outcomes in antibody-mediated rejection of renal allografts due to persistent/recurrent versus de novo donor-specific antibodies. Kidney Int. 2017;91:729–737.
[6] Lefaucheur C, Viglietti D, Bentlejewski C, et al. IgG donor-specific anti-human HLA antibody subclasses and kidney allograft antibody-mediated injury. J Am Soc Nephrol. 2016;27:293–304.
[7] Loupy A, Lefaucheur C, Vernerey D, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. N Engl J Med. 2013;369:1215–1226.
[8] Wiebe C, Gibson IW, Blydt-Hansen TD, et al. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. Am J Transplant. 2012;12:1157–1167.
[9] Djamali A, Kaufman DB, Ellis TM, et al. Diagnosis and management of antibody-mediated rejection: current status and novel approaches. Am J Transplant. 2014;14:255–271.
[10] Leino A, Lichvar A, Abu-Jawdeh B, et al. Randomized controlled trial of mixed acute rejection therapy in renal allografts: 3 year follow up. Am J Transplant. 2016;16:601.
[11] Sun Q, Liu Z-H, Yin G, et al. Tacrolimus combined with mycophenolate mofetil can effectively reverse C4d-positive steroid-resistant acute rejection in Chinese renal allograft recipients. Nephrol Dial Transplant. 2006;21:510–517.