Clinicopathological Profile Of Antibody-Mediated Rejection In Renal Allograft And Role Of C4d Immunohistochemistry And Anti-HLA Donor-Specific Antibodies In Its Diagnosis.

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<td>Peer Reviewed on 26th July, 2021</td>
<td>Antibody-mediated rejection (ABMR) is a major cause of poor renal allograft outcome. Despite the increased recognition of histopathological changes and molecular characteristics of ABMR, its clinicopathological features, and diagnostic end points are not clear. This study was aimed at evaluating the clinicopathologic features of ABMR and determining the role of C4d immunohistochemistry and anti-HLA Donor-Specific Antibodies (DSA) in its diagnosis.</td>
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<td>This was a single center prospective study conducted on “clinically indicated” renal allograft biopsies, over a 3 years duration at M.S Ramaiah Medical College, Bengaluru. The biopsies were evaluated for light microscopic and immunofluorescence features and C4d immunohistochemistry and anti-HLA DSA testing were performed. Revised Banff 2017 criteria was used for diagnosis. The comparison of C4d immunoexpression and DSA with clinicopathological features was carried using Fisher Exact test, Mann-Whitney U test and Chi square test or Fisher Exact test, as appropriate.</td>
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Keywords: Allograft biopsy, C4d immunostaining, Donor specific antibodies, Microvascular inflammation, Renal transplantation.

Results
A total of 34 cases of ABMR, with mean patient age of 34.8±8.4 years were evaluated. Active ABMR was observed in 61.8% and commonly presented as abrupt increase in serum creatine levels. Chronic active ABMR was observed in 38.2% and commonly presented as proteinuria. The frequency of C4d positive ABMR and DSA-positive ABMR was 70.6% and 76.5% respectively.

Conclusions
C4d-positive ABMR is frequently associated with earlier graft dysfunction and acute clinical presentation. There is significant association between C4d positivity and microvascular inflammation (MVI). However; there is no significant association between DSA-positive ABMR and DSA-negative ABMR, with respect to clinical features, C4d positivity or MVI.
INTRODUCTION:
Antibody-mediated rejection (ABMR) is major cause of renal allograft dysfunction and loss.\(^1\)\(^-\)\(^2\) ABMR occurs as a consequence of alloantibody mediated injury to donor cells exhibiting alloantigen, predominantly HLA class I and II. The diagnostic criteria for ABMR, formulated in Banff 2001 and 2007 conferences, has already undergone significant revisions in the subsequent Banff 2013 and 2017 conferences and is still evolving.\(^3\)\(^-\)\(^4\) The central tenets which determine ABMR diagnosis are: active tissue injury, evidence of recent antibody interaction with vascular endothelium and serological evidence of donor-specific antibody (DSA).\(^3\)\(^-\)\(^4\) Studies have shown that peritubular capillary C4d immunostaining, which was considered as the molecular footprint of an antibody response, has limited sensitivity in diagnosing ABMR.\(^5\)\(^-\)\(^6\) As a consequence, Banff 2013 conference incorporated a new subtype- C4d-negative ABMR. Further it has been recognised that DSA testing has potential shortcomings to be considered as the end point in the diagnosis of ABMR resulting in emergence of the entity “DSA-negative ABMR”, the clinicopathological characteristics and graft outcome of which is unclear.\(^4\)

Objectives
The study was aimed at evaluating the clinicopathologic features of ABMR in renal allograft biopsies, determining the sensitivity of peritubular capillary C4d immunostaining in diagnosing ABMR and determining the frequency of anti-HLA DSA positivity in ABMR. Further we investigated the association between C4d positivity and DSA with microvascular inflammation (MVI).

MATERIAL AND METHODS
This was a unicenter prospective study conducted on “clinically indicated” renal allograft biopsies from renal transplant recipients, received in the Pathology department, M.S Ramaiah Medical College, Bengaluru over a duration of 3 years (between September 2017 to September 2020). The allograft biopsies were processed for light microscopy, direct immunofluorescence study (DIF) and C4d immunohistochemistry (IHC) as per standard protocol. For light microscopy the 3\(\mu\)m sections were stained with Hematoxylin and Eosin, Periodic Acid Schiff, Gomori’s trichrome and Jones silver methenamine. DIF was performed on cryo-sections using Fluorescein isothiocyanate conjugated polyclonal rabbit anti-human immunoglobulin (Ig) IgG, IgM, IgA, Kappa, Lambda, C1q and C3 antibodies. C4d IHC was done on formalin fixed paraffin embedded tissue sections using “Dako REAL™ EnVision™ Detection System” with rabbit anti-human C4d polyclonal antibody (Master Diagnostica, MAD-000672QD-R) and dextran coated polymer coupled with peroxidase molecules and goat secondary antibody against rabbit immunoglobulin’s [Dako REAL™ EnVision™ /HRP, Rabbit/Mouse (ENV)]. DSA testing, was performed by the bead-based Immunoassay (Luminex) method [which detects Anti HLA IgG antibodies against HLA-A & HLA-B (class I) and HLA-DRB1 (Class II)], at the time of biopsy or graft dysfunction. DSA titer was expressed as mean fluorescence intensity (MFI) and MFI value >500 was considered as positive result for DSA. Non-HLA DSA was not tested. The patient demographics including allograft age, immunosuppression, serum creatinine, proteinuria, urine analysis and serum Tacrolimus levels (performed at time of biopsy) were obtained from laboratory database.

Revised Banff 2017 criteria for classification of ABMR was used for ABMR diagnosis.\(^4\) A diagnosis of acute/active ABMR (AcABMR) was made when all the three following criteria were met: 1. Histological evidence of active tissue injury { MVI [glomerulitis(g) and/or peritubular capillaritis (ptc)]; g > 0 and/or ptc > 0}, intimal/ transmural arteritis, acute thrombotic microangiopathy, or acute tubular injury}, 2. Evidence of recent antibody interaction with microvascular endothelium [ Linear C4d immunostaining in peritubular capillaries (PTC’s) or presence of at least moderate MVI (g + ptc ≥2)] and 3. Serologic evidence of DSA or C4d immunostaining in peritubular capillaries.\(^4\)

A diagnosis of chronic active ABMR (ChABMR) was made when all the three following criteria were met: 1. Histological evidence of chronic tissue injury [ Transplant glomerulopathy, peritubular capillary basement membrane multilayering or arterial intimal fibrosis], 2. Similar to criterion 2 for AcABMR and 3. Similar to criterion 3 for AcABMR.\(^4\) The Banff scoring system, with scores ranging from 0 to 3 was used for grading interstitial inflammation (i), tubulitis (t), vascular inflammation (v), transplant glomerulitis (g), peritubular capillaritis (ptc), interstitial inflammation (ci), tubular atrophy (ct), arterial fibrointimal thickening
(cv), transplant glomerulopathy (cg), arteriolar hyalinosis (ah) and mesangial expansion (mm). C4d immunostaining was graded as C4d0- negative (0%); C4d1- minimal positivity (1%<10% of PTC); C4d2- focal positivity (10%-50% of PCT) and C4d3- diffuse positivity (>50% of PTC).

All the renal transplant recipients were under the standard maintenance immunosuppression protocol comprising of corticosteroids (prednisolone), calcineurin inhibitor (tacrolimus) and/or antiproliferative agents (mycofenolate mofetil).

Renal transplants, of all the cases included in the study, were done with negative microlymphocytotoxicity HLA cross-matches and none of the cases were positive for pre-existing donor reactive HLA or blood group antibodies. None of the patients had received a second transplantation.

**Statistical analysis**

All continuous variables were expressed as mean and standard deviation for normally distributed data and as median with range when the data was skewed. Qualitative variables were expressed as frequencies and percentages. The comparison of C4d immunoexpression and MVI was carried using Fisher Exact test. For comparing DSA with age, serum creatinine and serum tacrolimus levels Mann-Whitney U test was used. Chi square test or Fisher Exact test, as appropriate, were used to compare DSA with time from transplantation to diagnosis, PTC C4d immunoexpression and MVI. SPSS version 18.0 was used for statistical analysis. P < 0.05 were considered as significant. The sensitivity of C4d in detecting ABMR was calculated as a/a+b {a= true positive [No. of C4d positive ABMR, b= false negative [No. of C4d negative ABMR]}

**RESULTS**

A total of 122 renal allograft biopsies (performed for evaluation of graft dysfunction) were obtained from 103 patients, over a duration of three years and categorized as per Banff’ 17 revised diagnostic categories. The final diagnosis was made for each case after analyzing the clinical data and results of light microscopy, DIF and C4d IHC. Of these, ABMR was diagnosed only in 34 biopsies (27%), which were obtained from 34 patients. The mean patient age was 34.8±8.4 years (range: 21- 48 years) with male: female ratio of 4.7:1. Induction immunosuppression with Basiliximab was administered to 25 (73.5%) patients.

AcABMR was observed in 21 biopsies (61.8%) with mean patient age of 33.1±8.2 years (range: 21- 46 years), male: female ratio of 4.3:1, mean serum creatinine levels of 2.9±2.24 mg/dl (range: 1.2- 8.5 mg/dl) and mean serum tacrolimus levels of 5.97±1.85 ng/ml (range: 1.8-10.5 ng/ml) (Fig. 1A & 1B). The most common clinical presentation was abrupt and sustained increase in creatinine levels (47.6%) followed by oliguria (28.6%), active urinary sediment (19.1%) and proteinuria (4.8%). Of the 21 cases, 19 were pure AcABMR and 2 were mixed AcABMR and T cell mediated rejection.

ChABMR was observed in 13 biopsies (38.2%) with mean patient age of 37±8.2 years (age range: 25- 48 years), male: female ratio of 5.5:1, mean serum creatinine levels of 3.1±0.86 (range: 1.04-4.42 mg/dl) and mean serum tacrolimus levels of 4.08±1.18 (range: 1.7- 6.3 ng/ml) (Fig. 1C). The most common clinical presentation was proteinuria (53.8%) followed by creeping/insidious increase in creatinine levels (38.5%) and active urinary sediment (7.7%). Of the 13 cases, 12 were pure ChABMR and 1 was mixed ChABMR and T cell mediated rejection.

![Fig 1](A) Active antibody mediated rejection with glomerulus showing infiltrates of neutrophils and mononuclear inflammatory cells (glomerulitis). (B) Active antibody mediated rejection with moderate to severe peritubular capillaritis (arrows). (C) Chronic active antibody mediated rejection with glomerulus showing capillary lumina lined by thickened basement membranes with focal double contoured appearance (arrow) and glomerulitis. (Haematoxylin and Eosin).
The time of allograft biopsy ranged from 8 days to 5 years post transplantation. The median time from transplantation to diagnosis for AcABMR was 2.5 months (0.27-48 months) and for ChABMR was 24 months (20-60 months). 55.9% (19/34) of the biopsies were performed in the first six months and 44.1% (15/34) were performed after 6 months post transplantation.

**PTC C4d immunostaining and ABMR**

C4d-positive ABMR was present in 24 cases (70.6%; 24/34), comprised of 16 AcABMR (76.2%; 16/21) and eight ChABMR (61.5%; 8/13). All these cases showed histological evidence of active tissue injury in the form of MVI (g>0, ptc>0) and recent antibody interaction with microvascular endothelium in the form of PTC C4d immunostaining (C4d>0).

Of the C4d-positive ABMR cases, nine (37.5%) exhibited minimal positivity (C4d1), 10 (41.7%) exhibited focal positivity (C4d2) and five (20.8%) exhibited diffuse positivity (C4d3) (Fig. 2).

![Fig 2 Peritubular capillary C4d immunostaining: (A) Diffuse positivity (C4d3) - staining in >50% of the peritubular capillaries (arrows). (B) Focal positivity (C4d2) - staining in up to 35% of the peritubular capillaries (arrows). (C) Minimal positivity (C4d1) - staining in up to 8% of the peritubular capillaries (arrows).](image)

C4d-negative ABMR was present in 10 cases (29.4%; 10/34), comprising of 5 AcABMR (23.8%; 5/21) and 5 ChABMR (38.5%; 5/13). All these cases showed histological evidence of active tissue injury in the form of MVI (g>0, ptc>0) along with DSA positivity. Thus, the sensitivity of PTC C4d IHC in detecting ABMR was 70.6% (76.2% in AcABMR and 61.5% in ChABMR).

**Table 1 Comparison of PTC C4d immunostaining with microvascular inflammation**

<table>
<thead>
<tr>
<th>C4d Score</th>
<th>Peritubular capillaritis score (total no. 34)</th>
<th>p-value</th>
<th>Glomerulitis score (total no. 34)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ptc 1 Mild</td>
<td>ptc 2 Moderate</td>
<td>ptc 3 Severe</td>
<td>g 1 Mild</td>
</tr>
<tr>
<td>C4d positive</td>
<td>11 (45.8%)</td>
<td>7 (29.2%)</td>
<td>6 (25%)</td>
<td>0.024</td>
</tr>
<tr>
<td>(C4d&gt;0) (n=24)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4d negative</td>
<td>9 (90%)</td>
<td>1 (10%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>(C4d=0) (n=10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>8</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: g, glomerulitis; ptc, peritubular capillaritis.

Comparison of PTC C4d immunostaining with MVI is shown in Table 1. A statistically significant correlation was present between PTC C4d deposition and MVI (p = 0.024 for “ptc” and p = 0.002 for “g”). 54.2% and 58.3% of C4d-positive ABMR exhibited moderate to severe “ptc” and “g” respectively, unlike 10% and 0% in C4d-negative ABMR.
Further, in the C4d-positive group a significant association was obtained between the intensity of PTC C4d immunostaining and severity of MVI (p= 0.002 for “ptc” and p=0.01 for “g”) (Table 2).

Table 2 Comparison of intensity of PTC C4d positivity with severity of microvascular inflammation.

<table>
<thead>
<tr>
<th>C4d Score</th>
<th>Peritubular capillaritis score (total no. 24)</th>
<th>Glomerulitis score (total no. 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ptc 1</td>
<td>ptc 2</td>
</tr>
<tr>
<td>C4d1 (n=9)</td>
<td>8 (88.9%)</td>
<td>1(11.1%)</td>
</tr>
<tr>
<td>C4d2 (n=10)</td>
<td>3 (30%)</td>
<td>5(50%)</td>
</tr>
<tr>
<td>C4d3 (n=5)</td>
<td>0 (0%)</td>
<td>1(20%)</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>7</td>
</tr>
</tbody>
</table>

Abbreviation: g, glomerulitis; ptc, peritubular capillaritis.

**DSA and ABMR**

Of the 34 cases of ABMR serological evidence of significant DSA levels (DSA+ABMR) was detected only in 26 cases (76.5%). The remaining eight cases (23.5%) were negative for DSA, however these cases showed PTC C4d positivity and MVI. HLA class I DSA was present in 53.8% of cases (14/26) and HLA class 2 DSA was present in 76.9% (20/26) of cases.

Comparison of clinical features, PTC C4d staining and MVI between DSA+ABMR and DSA-ABMR (DSA negative ABMR) is shown in Table 3. DSA+ABMR cases showed higher serum creatinine levels (at the time of biopsy) when compared with DSA-ABMR cases (p=0.009).

Table 3. Comparison of characteristics of DSA+ABMR and DSA-ABMR

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>DSA+ABMR n= 26</th>
<th>DSA-ABMR n= 8</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (range)</td>
<td>34.65±8.25 (21-48)</td>
<td>35±8.16 (25-46)</td>
<td>0.95</td>
</tr>
<tr>
<td>Male: female ratio</td>
<td>4.2:1</td>
<td>7:1</td>
<td>-</td>
</tr>
<tr>
<td>Induction immunosuppression</td>
<td>20 (76.9%)</td>
<td>5 (62.5%)</td>
<td>0.65</td>
</tr>
<tr>
<td>Type of transplantation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LRRT</td>
<td>20 (76.9%)</td>
<td>5 (62.5%)</td>
<td>0.63</td>
</tr>
<tr>
<td>DDRT</td>
<td>3 (11.5%)</td>
<td>2 (25%)</td>
<td></td>
</tr>
<tr>
<td>LNRT</td>
<td>3 (11.5%)</td>
<td>1(12.5%)</td>
<td></td>
</tr>
<tr>
<td>Mean serum creatinine levels, mg/dl (range)</td>
<td>2.59±1.55 (1.04-8.24)</td>
<td>3.59±2.12 (1.83-8.5)</td>
<td>0.009</td>
</tr>
<tr>
<td>Mean serum tacrolimus levels, ng/ml (range)</td>
<td>5.43±1.8 (2.5-10.5)</td>
<td>4.67±1.97 (1.7-7.4)</td>
<td>0.49</td>
</tr>
<tr>
<td>Median time from transplantation to diagnosis, months (range)</td>
<td>5 (0.27-60)</td>
<td>11.5 (1-59)</td>
<td>0.94</td>
</tr>
<tr>
<td>PTC C4d immunostaining</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4d0</td>
<td>10 (38.5%)</td>
<td>0 (0%)</td>
<td>0.47</td>
</tr>
<tr>
<td>C4d1</td>
<td>5 (19.2%)</td>
<td>4 (50%)</td>
<td></td>
</tr>
<tr>
<td>C4d2</td>
<td>8 (30.8%)</td>
<td>2 (25%)</td>
<td></td>
</tr>
<tr>
<td>C4d3</td>
<td>3 (11.5%)</td>
<td>2 (25%)</td>
<td></td>
</tr>
<tr>
<td>Microvascular inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ptc1</td>
<td>17 (65.4%)</td>
<td>3 (37.5%)</td>
<td>0.23</td>
</tr>
<tr>
<td>ptc2</td>
<td>5 (19.2%)</td>
<td>3 (37.5%)</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

The use of multimodal immunosuppressive agents has reduced the frequency of cellular rejection (T cell mediated rejection) episodes resulting in significant improvement in the 1-year renal allograft survival. However, the long-term renal allograft survival rates have not significantly improved due to ABMR.\[8\] Being the major reason for graft failure, the pathogenesis and therapeutic management of ABMR has been extensively studied.

AcABMR occurs when DSA reacts with HLA class I or HLA class II or non-HLA antigens or blood group antigens present on the donor endothelial cells. As a result, complement is activated predominantly via classical pathway culminating in recruitment of leukocytes, natural killer cells and macrophages. The latter inflammatory cells activate complex molecular pathways that cause endothelial activation and injury, derangement of vascular integrity and increased coagulation leading on to the morphological manifestations of MVI, intimal or transmural arteritis, acute thrombotic microangiopathy and acute tubular injury.\[9,10\] Repeated episodes of AcABMR or episodic subclinical antibody mediated endothelial activation and injury results in chronic tissue injury and repair leading on to morphological manifestations of ChABMR i.e. transplant glomerulopathy with remodeling and duplication of glomerular capillary basement membrane, PTC basement membrane multilayering and arterial intimal fibrosis.\[11\]

In the present study, the frequency of ABMR, as a cause of graft dysfunction was 27%. Similarly Chand et al found that 27.8% of the 97 biopsies, reviewed for graft failure, showed evidence of ABMR.\[12\] A higher frequency (53.7%) of ABMR was found by Devadass et al who evaluated 67 allograft biopsies for graft dysfunction.\[12\] AcABMR occurs within days, weeks, or months post transplantation, commonly one to three weeks after transplantation.\[6,17\] In our study 81% of active ABMR occurred in the first three months post transplantation. The term “chronic” in rejection does not refer to time since transplantation but refers to the histological changes of tissue remodeling. However; ChABMR usually presents years after transplantation.\[6,17\] In the current study 76.9% of ChABMR occurred two years post transplantation.

Literature review reveals that AcABMR presents commonly as acute renal failure and oliguria and ChABMR presents as chronic renal failure, proteinuria and hypertension.\[10,11\] Our experience also is similar, with 47.6% and 28.6% of AcABMR presenting as abrupt increase in serum creatinine and oliguria respectively and 53.85% and 38.5% of ChABMR presenting as proteinuria and insidious increase in creatinine levels respectively.

C4d staining and ABMR

The positive correlation between PTC C4d deposition and allograft loss was first reported by Feucht et al in 1993.\[9\] C4d is the split product of activated complement factor C4d and has a thioester moiety that forms a strong covalent bonding with the amino or hydroxyl groups of endothelial cells and basement membrane.\[13\] Thus PTC C4d deposits, which can be detected by DIF or IHC, represent footprint/ indirect sign of a current antibody reaction with tissues.\[11,3,5\]

As PTC C4d deposition predicted allograft loss and linked DSA with histology, it was considered as the corner stone of ABMR diagnosis and incorporated as one of the diagnostic triad by Banff 2007.\[5,14\] However; subsequent studies have questioned the sensitivity of C4d staining and recognised the existence of ABMR with no PTC C4d staining.\[3,5,12,14\] Thus the Banff 2013 meeting, revised ABMR diagnostic criteria with inclusion of “C4d negative ABMR”.\[3\] Even though PTC C4d staining is highly specific for interaction of DSA with renal allograft it suffers from poor inter-laboratory reproducibility.\[9\] Because of differences in methodology/techniques and threshold criteria of C4d positivity, the frequency of PTC C4d positivity varies from centre to centre with frequencies ranging from 31% to 61%.\[8\] The other causes of C4d negative staining include non-complement activating DSA, low C4d
deposits beyond the detection limits of IHC, treatment
effects and fluctuation of C4d status post
transplantation.[1,3,15] The frequency of PTC C4d positivity in our study was
70.6% (76.2% in AcABMR and 61.5% in ChABMR). In the
studies conducted by Takeda et al, Devadas et al
and Orandi et al, C4d positivity was seen in 46.9%
(62.5% in AcABMR and 31.3% in ChABMR), 40%
(55.6% in AcABMR and 23.5% in ChABMR) and
75.4% of ABMR cases respectively.[12,15,16]

PTC C4d positivity is an independent risk factor for
allograft loss. C4d-positive ABMR is associated with
deeper course, exhibits more severe and acute clinical
presentation and is caused by complement dependent
cytotoxicity where as C4d-negative ABMR is usually
subclinical or chori onic with late graft dysfunction and
loss and is caused by complement independent pathways.
[1,9,16] Similarly in our study C4d-positive ABMR was
more frequently associated with AcABMR (66.6%,
16/24) with earlier graft dysfunction and acute clinical
presentation whereas only 33.3% (8/24) presented as
ChABMR with late graft dysfunction. In contrast, 50%
(5/10) of C4d-negative ABMR presented as AcABMR and
50% (5/10) presented as ChABMR with late graft
dysfunction.

C4d and microvascular inflammation
Studies have confirmed the validity of MVI in the
histological diagnosis of ABMR and have shown that
MVI is strongly associated with detrimental graft
outcomes and is associated with progression to transplant
glomerulopathy.[4,17,18] Further there is a significant
correlation between PTC C4d deposition and MVI.[8,19]
Increase in PTC C4d score is associated with increased
severity of MVI.[8,12] Similarly in our study there was a
statistically significant correlation between MVI and
C4d positivity. A significant association was also
obtained between the intensity of PTC C4d
immunostaining and severity of MVI.

DSA and ABMR
Presence of DSA is associated with high incidence of
ABMR and poor graft outcome.[1,17] DSA could be
preformed or de novo.[1,17,20] Those DSA which are
present before transplantation are known as “preformed”
and cause hyperacute/accelerated rejection and early
active ABMR.[1,17,20] De novo DSA develop post
transplantation and drive late AcABMR and
ChABMR.[11] In the present study, none of the patients
had preformed DSA. The Banff 2013 ABMR diagnostic
criteria requires presence of DSA.[10] However, it was
recognised in Banff 2015 meeting that (i) DSA especially
against non-HLA antigens was not tested in many
laboratories and (ii) graft survival outcomes were similar
in cases of ABMR with both C4d and DSA positivity
and highly probable ABMR with C4d positivity and no
demonstrable DSA.[21] Subsequently, Banff 2017
consensus considered C4d positivity as an alternative for
DSA criterion and also allowed the diagnosis of ABMR in
presence of C4d positivity and absence of detectable
DSA.[4] However they advised testing for DSA to non-
HLA antigens, whenever DSA to HLA antigens was
negative.[4]

Redfield et al evaluated 160 kidney transplant recipients
with biopsy evidence of ABMR. Of these, 86.9% were
DSA+ and 13.1% were DSA-. In our study DSA was
detected in 76.5% of ABMR and was not detected in
23.5%. The cause of DSA negativity could be due to
prozone effects, presence of inhibitors, technical
limitations of the assay and non-HLA antibodies.[1]
Except for higher serum creatinine levels in
DSA+ABMR, there was no statistically significant
difference between DSA+ABMR and DSA-ABMR,
with respect to clinical features, C4d positivity or MVI
(Table 3). In the study by Senev et al DSA+ABMR cases
exhibited female predilection and increased C4d
positivity and had received repeat transplant more
frequently.[23]
The predominant de novo DSA are HLA class II.[1] In the
study by Bouatou et al majority of the de novo DSA
belonged to HLA class II (68.1%) followed by HLA class
I+II (27%) and HLA class I (4.5%).[20] Our
experience also is similar.

Our study has several limitations. First, this being a uni-
centre study, the sample size was less. Second, follow up
and determination of graft outcome was not possible.
Third, we did not test for non-HLA DSA.

We tried to capture the phenotypic data of ABMR in a
single south Indian centre; however, addition of
molecular phenotype (validated ABMR classifiers) will
further enhance the utility of such studies.

CONCLUSION
The present study represents a contribution to
understanding the clinicopathological features of ABMR
in south India. The sensitivity of PTC C4d IHC in
detecting ABMR is 70.6%. In contrast to C4d-negative
ABMR, C4d+positive ABMR is frequently associated
with AcABMR, earlier graft dysfunction and acute clinical presentation. There is a statistically significant association between C4d positivity and MVI with C4d-positive ABMR exhibiting more intense MVI when compared with C4d-negative ABMR. Further increase in PTC C4d score is associated with increased severity of MVI. Anti-HLA DSA is detected only in 76.5% of ABMR. Higher serum creatinine levels are associated with DSA+ABMR, however; there is no statistically significant difference between DSA+ABMR and DSA-ABMR, with respect to C4d positivity or MVI. As C4d staining is not sensitive enough and anti-HLA DSA testing has potential limitations, these alone can’t be used as the sole endpoints for diagnosis of ABMR. Further large-scale studies have to be conducted on the use of molecular diagnostics (gene transcripts/ classifiers like endothelial cell activation- associated transcripts, and donor specific antibody transcript) to improve ABMR diagnosis, beyond what is possible with biopsy, C4d staining and DSA detection.

CONFLICT OF INTEREST
None declared

ACKNOWLEDGMENT
We are grateful to the Management of MS Ramaiah Medical College, Bangalore, for logistic support and guidance.

We are grateful to Department of Health Research (Ministry of Health and Family Welfare), New Delhi, for funding of the project (DHR project no. V.25011/541-HRD/2016-HR) for funding the project.

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How To Cite This Article:
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Source of Support: Nil
Conflict of Interest: None declared

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