Title:
Improved diagnosis and management of paediatric renal transplant recipients using the 2013 Banff histopathological classification.

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Abstract

Background. After the changes in 2013 Banff classification, there has been an improvement in diagnosing antibody-mediated rejection (ABMR) in adult studies but no data in the paediatric population.

Methods. We assessed 56 paediatric renal transplant biopsies due to renal dysfunction in patients with donor-specific antibodies (DSA) in a retrospective single-centre study between January 2006 and March 2012. The results were compared with 2003/2007 Banff classification.

Results. Following the 2013 Banff classification, there were 7 cases (12.5%) diagnosed with ABMR that would have been misclassified when applying the 2003/2007 classification. Evaluating the histological features of all the ABMR-related cases we report the importance of v- (intimal arteritis) and t- (tubulitis) lesions: the absence of v- and t-lesions in the biopsy is related to a significantly higher graft survival (OR 7.3, 95%CI 1.1 - 48.8, p = 0.03 and OR 5.3, 95%CI 1.2 - 25.5, p = 0.04 respectively). Moreover, the absence of t- lesions was associated with significantly less rejection episodes the year after the initial biopsy (OR 5.1, 95%CI 1.4 - 19.8, p = 0.01).

Conclusion. Considering the clinical outcomes in our cohort we support the 2013 Banff classification as a more precise tool in identifying ABMR in the pediatric population.

Introduction

Antibody-mediated rejection remains an underestimated problem in renal transplant recipients (RTR) and unfortunately has generally a worse prognosis than T-cell mediated rejection (TCMR) [1] probably due to late and not always easy diagnosis. The international Banff working classification in renal allograft pathology is the gold standard used in order to diagnose graft changes indicative of any kind of rejection with a prognostic value [2].

The first international standardization of nomenclature and criteria for the histologic diagnosis of renal allograft rejection was developed in 1991 in Banff, Canada by a group of renal pathologists, nephrologists, and transplant surgeons [3]. The first meeting focused on T-cell mediated rejection, whereas antibody-mediated rejection (ABMR) was not fully characterized until 2001 by Racusen et al [4]. It has been well established recently that the most important elements for identifying ABMR are peritubular capillaritis (ptc), glomerulitis (g), glomerular double contours (cg), the presence of donor specific antibodies (DSA) and positive C4d staining [5]. However, there is no histological specificity for ABMR, as ptc-lesions can occur in both TCMR and acute kidney injury, with the diagnostic criteria for ABMR continuing to evolve over time [6,7]. We have previously demonstrated the presence of tubulitis lesions (t), vasculitis (v) and microvascular injury (g+ptc) has been independently associated with de novo DSA and poorer renal allograft outcome [8] in paediatric RTR (pRTR).
Moreover, Halloran et al, showed that DSA positivity improved the accuracy of the diagnosis of ABMR, but the up-to-date diagnosis of ABMR still requires serologic evidence of DSA against human leukocyte antigens (HLA), evidence of antibody interacting with endothelium and the presence of graft injury. Recent molecular analyses have shown that the microvascular inflammation may be another independent biomarker of rejection [9].

The revised 2017 Banff classification criteria highlight the importance of the presence of DSA for risk stratification and evaluating the response to treatment. In patients with ABMR, ''acute'' was removed from the acute/active ABMR and the 2017 classification highlights the importance of specific molecular markers especially in DSA negative patients or in patients where antibodies are unavailable at the time of renal transplant biopsy [10].

The aim of this study was to compare the modified Renal Allograft Pathology 2013 Banff Classification with the previous 2003/2007 Banff classification in pRTR DSA positive patients in order to find if there is any significant differences after following their evolution based on their histological findings.

Materials and Methods
Study population and design
We performed a retrospective analysis in 56 pRTR with positive de novo DSA who had percutaneous renal transplant biopsies due to acute renal allograft dysfunction. The study period was between January 2006 and March 2012. All the biopsies were independently re-scored by a histopathologist specialist (T.S.) trained in Banff classification.

Renal transplant biopsies: Light microscopy
The sample for light microscopy was fixed in 10% phosphated buffered formalin. Paraffin sections 2 μm thick were obtained with a microtome. Conventional haemotoxylin and eosin staining was performed using an automated staining platform. Periodic acid-Schiff, Periodic acid-silver methenamine and Masson trichrome were hand stained. CD3, CD8, CD20, SV40, cytomegalovirus and C4d immunohistochemical staining was performed using an automated staining platform.

Renal transplant biopsies: Electron microscopy
From the biopsy a small piece (1-2mm) was fixed in 2.5% glutaraldehyde buffered in 0.1M sodium cacodylate (pH7.2), post-fixed in 1% osmium tetroxide and then dehydrated in ascending grades of ethanol. Tissue blocks were passed through a transitional fluid , propylene oxide, and then infiltrated overnight in Epon resin. The blocks were then transferred to BEEM capsules with fresh resin and polymerised at 60 degree Celsius for 24hrs. Ultrathin sections were cut with a Diatome diamond knife at 90nm on a Leica Ultracut UCT ultramicrotome, placed on copper grids and stained with uranyl acetate and lead citrate. Examination was carried out using a JEOL 1400 transmission electron microscope (JEOL Ltd. Tokyo, Japan).

Comparison between the 2003/2007 and 2013 Banff classification
We focused on the main changes introduced in the 2013 Banff classification compared to the 2003/2007 classification (Table 1) affecting ABMR.

Identification and analysis of anti-HLA antibodies
Patients were tested for the presence of Class I, II and III specific antibodies using the LABScreen mixed kits (One Lambda, Thermo Fisher Scientific, Canoga Park, CA) and analyzed using the manufacturer supplied HLA Fusion software. Positivity was defined against a pre-set ratio compared to the negative control. The ratio used is Lot number dependent and varied over the course of the study based on local verification of each new Lot. HLA antibody specificity as indentified in screening positive samples using the LABScreen Class I, II and III single antigen bead kits. A median fluorescence intensity of >1000 was used to assign a positive result. Samples were analysed for all loci – HLA-A, -B, -C, -DRB, -DQA, -DQB and – DPB – with reference to donor and recipient HLA types, with additional loci typing on stored samples where required.

Statistical analysis
Continuous variables (quantitative data) are expressed with their median and interquartile range (IQR), while categorical variables (qualitative data) are expressed with their frequencies and respective percentages. To explore the differences between groups for quantitative factors we used non-parametric Mann-Whitney U-test for medians and associations among qualitative factors were tested with the Pearson Chi-squared test. The Spearman correlations were reported among biopsy lesions, while the association between Banff classification and biopsy lesions was also tested using the Pearson exact chi-squared test for independence.
The Kaplan-Meier survival curves were estimated and compared with the log-rank test for homogeneity among groups. Logistic regression models were used for the multivariate analysis in order to model the effects of multiple variables on the probability of a failing graft and/or the probability of having rejection. Statistical

analyses were performed using JMP software, version 11.0, SAS Institute Inc., Cary, NC, 2007. The level of statistical significance was set at 5% (p=0.05).

Results

Study population

Fifty-six percutaneous renal transplant biopsies taken from 56 patients between January 2006 and March 2012 for acute graft dysfunction were analysed in our study.
The results of their first 'for-cause' biopsy were compared with previous classification as per 2003/2007 Banff criteria with results presented in Table 2(a,b).
Most patients were male (69.6%), having congenital anomalies of the kidney and the urinary tract (CAKUT; 48.2%), having received their first kidney transplant (85.7%) from a deceased donor (53.6%). Almost half of our cohort received a live related donation (46.4%) and for the huge majority represents their first kidney transplant (85.7%) (Table 3).

The median follow-up visit post transplant was 6 (IQR 2.25-9.75) years with the under investigation biopsy to have been performed in 1.4 (IQR 0.1-5.9) years post-transplant. At the last follow up visit, there was a 100% patient survival. Concerning the graft, 47 patients had still a functioning graft (83.9%). Among the pRTR who presented renal allograft failure, 3 (33.3%) had an ABMR not shown by the previous 2003/2007 classification, 3 had a diagnosis of TCMR shown by both classifications, 2 patients were diagnosed with chronic active ABMR and one patient had a biopsy scored as normal. At last follow-up visit, the 8/9 patients with renal allograft failure were on dialysis and one patient had received his second transplant already.

Graft survival and patients' outcome

The patients were followed for a median period of 6 (IQR 2.25-9.75) years after the biopsy under investigation with 100% patient survival at the last follow-up visit and 83.9% graft survival. Among the 9 grafts that failed during the follow-up period, 1 patient had already received their second kidney transplant at the last follow-up visit and 8 patients were on dialysis.
Almost half of our patients 24/56 (42.8%) did not have a renal allograft biopsy and there was no suspicion of rejection on their graft until last review.

Concerning renal allograft survival, initially we checked if there was any difference in survival (primary endpoint) between the 7 under diagnosed cases with ABMR scored after the new 2013 Banff classification and the rest of the cohort [Figure 1]. The result did not reveal any difference between those 2 groups (p=0.169) due to the fact that the rest 49 cases included a combination of ABMR, TCMR, with normal biopsies and mixed lesion biopsies. Also, most of the under-diagnosed ABMR cases were treated as per ABMR or TCMR/ABMR protocols due to the severity of their clinical presentation despite the biopsy conclusion [Table 4].

Secondly, we examined the graft survival rates of every biopsy category in the different Banff classifications [Figure 2]. The Kaplan-Meyer curve is mainly underlying the statistically different survival in between the different biopsy scorings as per Banff 2007 classification, which indicates how severe the TCMR cases, were comparing to the other categories (logrank p=0.013). Patients with TCMR had the worst outcome followed by patients with ABMR.

Nonetheless, in the updated 2013 classification 7 cases were scored correctly as ABMR (a diagnosis that would have been missed previously) and that led to a better stratification of the overall allograft outcomes as shown by the severe graft failure closer to TCMR cases. Subsequently, there was no more any significant difference in between the renal allograft diagnosis's outcome (logrank p= 0.069). As a conclusion, the correct diagnosis of the missed ABMR cases are of significant clinical importance as it can lead to important mortality and needs to be treated properly.

Classification of renal biopsies
There were 14 (25%) active, chronic or suspected ABMR according to the 2003/2007 Banff classification, whereas with the 2013 Banff classification there were 17 (30.4%) cases.
There were 14 cases (25%) of TCMR (borderline or confirmed) using the 2003/2007 Banff classification, while with the 2013 classification there were only 10 (17.9%) cases of TCMR, as two among those cases (2/4) were scored as definite ABMR and the remaining two cases were scored as mixed cases of ABMR and TCMR.
However, one case, which was scored as a TCMR with 2003/2007 classification, was classified as borderline ABMR by the 2013 Banff classification criteria due to severe tubulitis but also presence of Cd4+ staining. Overall, the 2013 Banff reclassification affected all categories (Table 2a,b).
In total, 7 (12.5%) cases of ABMR would have been missed if the clinician had only relied on the old Banff classification. Details of those 7 patients are given in Table 4.

Impact of the 2013 Banff classification in our paediatric renal transplant recipients

a) **Impact of glomerulitis (g):** Assessment of renal biopsies indicated that only one case showed signs of glomerulitis (g1 scoring). In our cohort, the only patient presented with g1 lesions in his biopsy, presented other elements significant for ABMR as diffuse staining of C4d (C4d 3, ti 1, ci 1, gi 1, cv 3, ah 1 and ptc 1) and the histological diagnosis did not change between the two classifications.

b) **Impact of microvascular inflammation (MVI) as histological evidence of humoral response:** Microvascular inflammation (g+ptc) >2 was found in only one sample (the only patient with g1+) who had a definite diagnosis of ABMR. Nonetheless, in one patient where MVI was scored as one (ptc1,g0), this patient presented diffuse t, ti, ci, ct and C4d elements and was one of the six missed ABMR cases based on the 2003/2007 Banff classification as we would have scored his biopsy as grade Ib TCMR , while with the 2013 Banff classification this patient was classified as mixed ABMR with TCMR.

c) **Impact of C4d staining (diffuse versus focal) with and without PTCs:**
C4d staining was present in 27 (48.2%) biopsies in total.
C4d diffuse staining (C4d+3) was present in 10 biopsies (17.9%), all classified under different categories:
- 30% (3/10) classified as no ABMR with both classifications, and 33% (1/3) classified as borderline TCMR (no ABMR) by both classifications
- 40% (4/10) classified as category 2 (chronic active ABMR) by both classifications

- 10% (1/10) classified as borderline TCMR 2A, while with the 2013 classification the diagnosis was suspicious acute ABMR. This patient had a v1, cv1, ah1 and C4d 3 pattern in his biopsy.
- 20% (2/10) classified as ABMR with both classifications.

C4d diffuse staining with graft dysfunction in a DSA positive patient in our study remains a strong and reliable indicator of ABMR.

C4d focal staining (C4d+2) was present in six biopsies (10.7%), all classified under different categories as well:
- 50% (3/6) classified as category 2 by both classifications
- 33.4% (2/6) classified as no ABMR with the old classification, but one of those had a borderline ABMR due to the presence of diffuse tubulitis lesions and widespread C4d staining (that patient had a t1, ti1, ci2, ct2, cv1, ah2 and C4d 2 pattern).
- 16.6% (1/6) was classified as a suspicious chronic ABMR (+TCMR 1A) pattern by the old category and with the 2013 Banff classification it was scored as chronic inactive ABMR with grade Ia TCMR features.

The focal C4d staining appears an important additional element for the ABMR diagnosis, as it increased sensibility up to 16.7% (1 out of 6 cases).

d) **Impact of chronic active ABMR:** There were seven cases of ABMR that were clearly identified independently of the classification used.

e) **Impact of t and v features in our DSA positive patients':** Fourteen biopsies had evidence of t lesions (25%) and six patients presented with v lesions (10.7%). Figure 3 summarizes the increase in diagnosis of ABMR on patients presenting t and/or v lesions, a statement that previously would have argued towards TCMR but not ABMR diagnosis.
- t lesions: One patient with normal biopsy as per 2003/2007 classification (t1, ti1, ci2, ct2, cv1, ah2, c4d2), was scored as having ABMR with the 2013 Banff classification. Moreover, one patient with suspicion of ABMR as per 2003/2007 classification (t1, i2, ti3, ct2, cv1, ah1, c4d1) when applying the new classification 2013 was diagnosed as ABMR.
Two patients with TCMR based on the 2003/2007 classification, were scored as having a mixed picture ABMR-TCMR with 2013 classification. The details of those patients' biopsies revealed high grade t element scoring associated with c4d staining and MVI score of 1 with either g or ptc element (t3, ti3, ci3, ct3, cv1, ah1, ptc1, c4d3 and t3, i3, ti3, g1, cv1, ah1, c4d1 respectively).
- v lesions: All six patients with lesions of vasculitis were initially diagnosed as having TCMR , whereas two out of six (33.3%), were finally re-scored as having pure ABMR when applying the 2013 Banff classification.

f) **Impact of removing IF/TA as histological criteria of chronic ABMR:** Among eight biopsies classified as chronic active ABMR (similar scoring with both classifications), 2 had tubular atrophy (ct) lesions, 5 had both tubular atrophy and intertitial fibrosis lesions (ci) and one biopsy didn't have any of those lesions.

g) **Distribution of ABMR- related biopsy lesions in-between the different classifications and correlation between them.** The sum of ptc+g features were

not found as much as expected, but the presence of vasculitis and/or tubulitis in pure ABMR-classified biopsies was significant [Table 5]. Furthermore, the C4d positive status independently of the grade was clinically relevant while using the new classification, showing that focal staining might be a high indicator of ABMR with or without other histological elements.

There were significant correlations between i+t (r=0.52, p= <0.0001), g+ptc (r=0.48, p= 0.0002), ti+i (r=0.51, p= <0.0001; Table 6) indicating that mononuclear interstitial inflammation may coexist with tubulitis lesions in the same way that glomerulitis coexists with peritubular capillary infiltrates by mononuclears and neutrophils in the above DSA positive pRTR allograft biopsies. Characteristically, histological evidence of ABMR has been divided into four types, based on light microscopy [11]: (1) MVI with neutrophils and mononuclear cells in capillaries which was not observed so frequently in our cohort, (2) intimal or transmural arteritis, which was significantly present in our cohort (Table 7) but not specifically in association with other lesions, (3) acute thrombotic microangiopathy (TMA) and (4) acute tubular injury in the absence of other cause which seems to be clinically relevant in our cohort.

Finally we examined if there is any association between the different diagnosis when applying the 2013 Banff classification and the different biopsy lesions [Table 7]. The biopsy characteristics who showed a difference when comparing their presence between the different biopsy categories were t, v, i cg, mm, ah and c4d. 11.8%, 80% and 100% of the patients having ABMR, TCMR and mixed ABMR and TCMR phenotype respectively presented with t elements (not shown). Overall, the presence of t lesions in the different biopsies was significantly different distributed, obviously more related to the TCMR results but surprisingly present as an important element to the pure ABMR biopsies as well.

Equally, the v lesions were distributed in 11.8% of the ABMR patients, in 40.0% of the TCMR patients showing an additional v effect in a sole ABMR biopsy. There was a significant different distribution of certain histological lesions between the biopsies; for instance almost half of the C4d staining were on the biopsies presenting ABMR, while the majority of t and v lesions were present in biopsies scored as TCMR (Table 7). ABMR biopsies presented v lesions in 33.3% and cg lesions in 85.7%. The raised cg presence in ABMR biopsies can be an indicator of chronic transplant glomerulopathy and this feature is one of the most characteristic ones in chronic ABMR (defined as the widespread duplication or multilayering of glomerular basement membrane in the absence of specific de novo or recurrent glomerular disease or evidence of TMA [11].

The g and ptc lesions did not reveal any difference in their pattern of presentation in between the different biopsy categories, but the number of patients having those lesions in our cohort was small. C4d lesions were largely found in ABMR biopsies (76.5% of the patients with ABMR had positive c4d lesions) while some of TCMR biopsies presented as well c4d positive results (20.0%). The association between biopsy lesions and biopsy result as per 2013 Banff classification is presented in the Table 7.

Multivariate analysis and clinical outcome
Multivariate analysis with three variables per model: biopsy lesion, confirmed diagnosis of ABMR by the 2013 classification and clinical outcome (functioning versus failing graft

at last follow-up, rejection episodes necessitating biopsy/blind treatment in the following year showed in Figure 4. Biopsies with c4d+ and v+ lesions had the worst prognosis among all with 22.2% and 50% failing grafts respectively (figure 4). The absence of t lesions was significantly associated with a better outcome (p= 0.02). In cases scored as ABMR (pure ABMR and mixed TCMR/ABMR cases) there was a strong association between functioning graft and the absence of t+ and v+ lesions separately (p=0.03, OR 5.3, CI 95% 1.2-25.5 and p= 0.04, OR 7.3, CI 95% 1.1-48.8 respectively). Furthermore, we found a strong association between rejection episodes during the following year and the presence of t+ lesions including all ABMR cases (p=0.01, OR 5.1, CI 95% 1.4-19.8) [Figure 4].

Treatment modality and outcomes
Overall, 19 patients received Rituximab, 9 patients received intravenous immunoglobulins (IVIG) and 18 patients received intravenous steroids (pulses of methylprednisolone). The treatment modality received by each patient and their biopsy results is presented in Figure 5.

At the last follow-up visit, 78.9% (15/19) of the patients who received Rituximab still had a functioning graft and four patients had a failing graft with two out of four having received their second transplant already. Among the four patients who lost their graft two had a misleading biopsy diagnosis using the 2003/2007 Banff classification. One patient was scored as normal biopsy initially whereas it was re-scored as borderline ABMR with tubulitis and widespread c4d lesions and the other patient was classified as TCMR whereas with the 2013 Banff classification it was scored as acute ABMR. The other two patients had the same histological diagnosis independently of the classification used (one had chronic active ABMR and the other one TCMR).
Among the pRTR who received IVIG, five out of nine (55.5%) had a failing graft at the last follow-up visit, two patients were the same ones with the misleading diagnosis in the Rituximab group, two others had a TCMR diagnosis with both classifications and the last out of the five patients was scored as a normal biopsy. This last patient with the no ABMR, no TCMR biopsy had primary renal diagnosis focal segmental glomerulosclerosis and he lost his graft in one year post-transplant with the suspicion of recurrence.
All the patients who received Rituximab received high doses of steroids or pulses of methylprednisolone. One patient with kidney allograft biopsy revealed mild TCMR –IA grade had increasing baseline immunosuppression with high dose of steroids and a functioning graft at his last follow-up visit 7 years after the biopsy in question.

The re-scoring of the 56 DSA positive biopsies judging retrospectively based on their follow-up evolution revealed that less normal biopsies would receive unnecessary treatment, treatment against ABMR will be much better matched in the ABMR cases (purely ABMR or mixed lesions) and patients with TCMR will less incorrectly be treated as ABMR. The above results underscore the importance of 2013 Banff classification which makes ABMR diagnosis more accurate in children [Figure 5].

Discussion

In this study, 56 'for-cause' biopsies in 56 DSA positive patients were analysed. Biopsies were done due to allograft dysfunction to assess for ABMR, and were relatively early post-transplant. In the paediatric literature, there is conflicting evidence on the

role of DSA alone in causing graft dysfunction in the absence of any histological findings [12-14]. Nonetheless diagnosis of ABMR has evolved over time and the Banff 2013 classification requires the following criteria to be met:

- *Histologic evidence of acute tissue injury*, including 1 or more of the following: 1) MVI (G>0 and/or ptc>0) in the absence of glomerulitis although in the presence of acute TCMR, borderline infiltrate, or infection, whereas ptc≥1 alone is not sufficient and g must be ≥1, 2) intimal or transmural arteritis (v>0), 3) acute thrombotic microangiopathy or acute tubular injury (in the absence of any other cause)
- *Evidence of current/recent antibody interaction with vascular endothelium*, including 1 or more of the following: 1)linear c4d staining in peritubular capillaries (c4d2 or c4d3 by IF on frozen sections, or c4d>0 by IHC on parrafin sections), 2) at least moderate microvascular inflammation [(g+ptc)≥2] in the absence of other lesiosn mentionned above and 3) increased expression of gene transcripts/classifiers in the biopsy tissue strongly associated with ABMR, if thoroughly validated.
- Serologic evidence of donor-specific antibodies

The main difference concerning biopsy elements identification in order to conclude in ABMR between the 2013 Banff classification and the 2017 Banff classification is that the more recent one, does accept as MVI when we have a score of ≥1, if that is a g element. In our cohort, we only had one patient with ABMR and MVI score ≥ 2 (g1, ptc1) and one patient with ptc1 (but g0) being one of the missed 7 ABMR cases though. We found clinically relevant the co-existence of MVI score of 1 and t or c4d elements in our ABMR biopsies as 2 out of 6 patients were correctly diagnosed with ABMR instead of TCMR. Analyzing separately the c4d staining, including the focal c4d staining in the 2013 Banff classification was one of the major important hallmarks in the diagnosis of ABMR. Gimeno et al, found that the focal or diffuse c4d staining in the 2013 Banff classification in the ABMR DSA positive biopsies had a high specificity of 87.5%, higher than the glomerulitis alone (80%), peritubular capillaritis alone (62.5%) and microvascular inflammation (80%).Their study revealed that c4d focal/diffuse staining is significantly associated with microvascular inflammation as well [15].

In this study, we found a high proportion of t lesions in ABMR. ABMR patients with t lesions had significantly worse outcome [8]. DSA do not mediate acute graft rejection as a solitary event, but activation of other components of innate and adaptive immunity contributes to graft injury: T-cell infiltration and tubulitis have been observed in for-cause biopsies with diagnosis of ABMR and such mixed pathology may occur in 10-90% of graft biopsies [16]. The above finding is in line with recent observations showing that the occurrence of intimal or transmural arteritis was more often observed in cases with ABMR (21%) than TCMR (9%), and the grade of intimal arteritis in acute ABMR was 52% in v1, followed by 30% in v2, and 19% in v3 [17].

Furthermore, recently a distinct phenotype of rejection, named antibody-mediated vascular rejection has shown surprisingly elevated risk of graft loss [9·07 times (95 CI 3·62–19·7)] than T cell-mediated rejection without vasculitis [17]. Thus the presence of tubulitis elements in association with the presence of ptc and/or C4d staining in DSA positive patients is another important finding in ABMR cases; suggesting that the presence of g elements is less compulsory. Obviously, is difficult to conclude with certainty due to the small amount of patients and as such larger paediatric studies are required to confirm this hypothesis.

Traditionally, the combination of C4d staining (focal/diffuse) and ptc is evidence of antibody interaction with the endothelium with or without the presence of DSAs. One patient was incorrectly diagnosed as having TCMR and was reclassified as mixed rejection (ABMR and TCMR) after the 2013 Banff classification. Adult studies have shown the same effect of C4d in allograft biopsies in DSA positive patients [17].

ABMR diagnosis remains delicate and requires prompt suspicion as the clinical outcome can be detrimental for both the graft and the patient [18]. Comparison of the predictive value of 2013 Banff classification versus 2003/2007 criteria for chronic-active ABMR in a single center adult study followed retrospectively for 8 years, showed that the endpoint of graft loss or doubling creatinine was better associated with the 2013 criteria (PPV 46% versus 48% and NPV 65% versus 70% for the 2003/2007 and 2013 classification criteria respectively). The same study suggested that 2013 criteria led to an overall improvement of diagnosis and a better association with clinical outcomes, particularly with the lower threshold for c4d positivity; whereas the g+ptc component was not a significant predictor [19].

In our centre, pRTR with ABMR presenting acute dysfunction, are treated with high dose intravenous corticosteroids with consideration of plasma exchange (PEX), rituximab and intravenous immunoglobulins (IVIG). Baseline immunosuppresion is also optimized by ensuring tacrolimus levels within target range and considering conversion of azathioprine to MMF. However, pRTR with TCMR are treated with high dose IV corticosteroids, anti-thymoglobulin (ATG) with or without IVIG [20].

On the other hand, for TCMR our protocol indicates anti-thymoglobulin (ATG) and high dose intravenous steroids with intravenous immunoglobulins (IVIG), so the correct diagnosis influences the treatment modality chosen. In our study we showed that treatment would be much more adaptive to the correct diagnosis after applying the 2013 Banff classification criteria.

In a high technology sphere in the near future where other biomarkers will be used for diagnosis, an improved biopsy scoring will help clinician not only to better target their treatment but also to reduce side effects from incorrect hypothesis and treatments. Hereby to mention that, even if DSA remains strongly recommended in all cases with biopsy specimens meeting the phenotypic criteria for ABMR, the DSA testing should be used as well for risk stratification, evaluating the response to treatment and further patient monitoring [10]. Our study was not designed to look for evolution in patients' DSA but only their clinical evolution in terms of patients and graft survival.

In summary, the 2003/2007 Banff classifications recognised the significance of the focal (not only diffuse) C4d staining in biopsies as a poorer outcome, the significance of the microvascular inflammation (MVI) and injury and the active arterial lesions other than fibroid necrosis, namely intimal arteritis (endarteritis) as it could be a manifestation of acute ABMR (rather than/in addition to, acute TCMR) [21].

Additionally to the above, in the 2003/2007 classification it was discussed the role of DSA and significantly the reduced 1- and 4- year survival in kidney recipients with the presence of de novo HLA DSA. Despite the significant role of DSA (HLA and non-HLA) to the renal allograft dysfunction, their underlying mechanism is not entirely understood. Multiple studies in animals have shown that ABMR can be induced by either complement [22 23] or non-complement activating DSA [24 25]. Moreover, we can have a mixed picture of T-cell-antibody mediated pathology when T cell infiltration and

tubulitis is observed in for-cause biopsies with diagnosis of ABMR and this particular mixed histological picture may occur in 10-90% of graft biopsies [16].

The importance of 2013 Banff classification is that the evidence of current/recent antibody interaction with vascular endothelium was recognised, including but not limited to C4d deposition, requiring a higher threshold for MVI (glomerulitis and/or peritubular capillaritis) for diagnosis of ABMR in the absence of C4d (to limit false-positive diagnoses). Moreover, the 2013 Banff classification included focal (10-50%) and diffuse (>50%) C4d staining in peritubular capillaries and any peritubular capillary staining; and it recognised intimal arteritis in addition to arterial fibrinoid necrosis as histologic evidence of acute tissue injury. In our cohort, both local C4d staining and arteritis lesions have shown to be important elements in the diagnosis and prognosis of ABMR.

Conclusion

Considering the results of our paediatric cohort, we support the updated 2013 Banff classification for a more precise diagnosis of ABMR. This more precise classification of ABMR was justified by the presence of focal C4d and the fact that mononuclear interstitial inflammation may coexist with tubulitis lesions in the same way that glomerulitis coexists with peritubular capillary infiltrates. Our results indicate that special attention should be paid in the intimal or transmural arteritis which was more often observed in cases with ABMR rather than TCMR on those DSA positive pRTR and the fact that there was a strong association between functioning graft and the absence of t+ and v+ lesions, as well as less rejection episodes over the next year when t lesions were absent in the biopsy under investigation.

Conflict of interest

The authors declare no conflict of interest. The results presented in this article have not been published previously in whole, or part, except in abstract form.

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