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MONITORIZACIÓN SÉRICA E HISTOLÓGICA DEL RECHAZO MEDIADO POR ANTICUERPOS EN TRASPLANTE RENAL

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Original Article

Impact of the Banff 2013 classification on the diagnosis of suspicious versus conclusive late antibody-mediated rejection in allografts without acute dysfunction

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ABSTRACT

Background. The Banff classification is used worldwide to characterize pathological findings in renal allograft biopsies. During the 11th Banff meeting, relevant changes were introduced in the diagnostic criteria for Category 2 antibody-mediated rejection (ABMR). Here, we assess the effect of these changes on the diagnosis of late chronic ABMR.

Methods. Seventy-three indication renal graft biopsies (chronic dysfunction, proteinuria and/or the presence of *de novo* donor-specific antibodies) from 68 kidney transplant recipients initially classified following the Banff 2009 criteria were reviewed and reclassified as per the new Banff 2013 criteria.

Results. The diagnostic category changed in 18% of the study biopsies with Banff 2013. The reclassification mainly involved Category 2 cases, from which 23.5% of the biopsies from older patients with worse graft function were overlooked by Banff 2009. ABMR was ruled out in 13% of cases under the Banff 2009 criteria. A significant number of the study samples were conclusively diagnosed as ABMR (40% as per Banff 2009 and 74% as per Banff 2013; P = 0.006), because of the inclusion of microvascular inflammation and the acceptance of some ultrastructural diagnostic criteria. However, when following the criteria of the new classification, samples with histological signs of chronic ABMR, in which human leucocyte antigen donor-specific antibodies are not detected or ultrastructural studies are not performed, may be inadequately characterized.

Conclusions. The Banff 2013 classification helps in making a diagnosis of late ABMR, identifying cases, decreasing the percentage of suspected ABMR and making more conclusive diagnoses.

Keywords: antibody-mediated rejection, Banff classification, glomerulitis, microinflammation

INTRODUCTION

The Banff working classification of renal allograft pathology is widely used in international clinical trials of new anti-rejection agents [1]. The first classification focused on T-cell-mediated rejection and so-called chronic rejection [2], while antibody-mediated rejection (ABMR) was not adequately defined until later on [3]. The presence of C4d in peritubular capillaries (PTCs) [4] has been associated with poor graft outcome [5], circulating donor-specific anti-human leucocyte antigen (HLA) antibodies (DSAs) [6] and resistance to drug treatment [7] and, for several years, C4d staining became a requisite to diagnose ABMR [8].

Despite the apparent pivotal role of C4d staining in ABMR, in some patients with DSAs but negative C4d staining in PTCs, graft survival was poor. Biopsies from these cases showed chronic glomerular and interstitial changes evocative of ABMR, thus C4d-independent histological markers capable of revealing antibody/graft endothelium interaction were necessary.

During the 2009 Banff meeting, the 2007 Banff classification was basically reproduced, with only minimal changes [9]. In the 2009 Banff meeting, the diagnostic criteria for ABMR [10] were refined, emphasizing the existence of C4d-negative ABMR. Working groups to evaluate potential changes in the classification were created and the results were presented at the 12th Banff meeting, with apparently significant changes.

The main innovation was the acceptance of moderate to severe microvascular inflammation as evidence of ABMR, which is equivalent to significant C4d staining in PTCs. This, along with other updates, such as recognizing electron microscopic evidence of transplant glomerulopathy, focal C4d in PTCs or at any given time point after transplant DSAs in the presence of chronic lesions related to ABMR, were included in the new classification [11, 12].

Despite the successes of the working classifications, limitations have been related to the potential for sampling error or issues of reproducibility when implemented globally [13]. Since its inception, the classification has evolved in a continuum of active and passive connections, preserving its initial collective thought style, based on morphology [14]. However, the Banff community has performed little evaluation on the usefulness of the changes produced over decades.

The aim of our study was to compare the recently modified Renal Allograft Pathology Classification (Banff 2013) with the 2009 Banff classification. For this, selected renal graft biopsies performed due to a 'non-acute' indication >3 months after transplantation were used. We wanted to assess whether the updates in the 2013 Banff classification lead to a more consistent classification into Category 2 of samples that show histological signs of late ABMR.

MATERIALS AND METHODS

Study population and design

We performed a retrospective analysis of percutaneous biopsies due to a 'non-acute' indication done >3 months after transplantation between February 2011 and February 2014 at our institution. The local internal review board approved the study. All the biopsies were evaluated according to the 2009 Banff classification [9] and reassessed using Banff 2013 by two pathologists (J.G. and D.N.) [11].

In ABMR cases with acute dysfunction, our clinical protocol indicates treatment with plasma exchange and intravenous immunoglobulin, and rituximab in severe or refractory cases. In ABMR cases with no clinical dysfunction, the treatment is optimized (reintroduction of steroids if absent, change from cyclosporine to tacrolimus or initiation of tacrolimus for mycophenolic acid in patients that are being treated with mechanistic target of rapamycin), because, to date, the existing ones cannot be considered clearly effective. Thus, the patients whose samples are included in this study did not receive specific antirejection treatment.

Identification and analysis of anti-HLA antibodies

Serum samples were tested for anti-HLA antibodies several times before the biopsy (3.85 ± 0.99 tests per patient). Determinations were performed in a Luminex platform as described elsewhere [15]. For the anti-HLA screening tests, Lifecodes Life Screen Deluxe kits (Gen-Probe, Stanford, CT, USA) were used. Positive screening samples were further evaluated with Class I and II Lifecodes LSA kits for specificity. The threshold for positivity was set at a mean fluorescence index >1000. Donor HLA antibody specificity was ascribed considering donor HLA-A, -B, -DRB and some -C or -DQB typing. Antibodies against C or DQ antigens were assigned considering linkage disequilibrium in long-term donors.

Light microscopy

For each biopsy, two core biopsies were obtained and sent for analysis in saline solution. The sample for light microscopy was fixed in 4% formaldehyde. Paraffin sections 2–3 μm thick were obtained with a microtome. Conventional haematoxylin and eosin staining was performed using an automated staining platform. Periodic acid-Schiff, methenamine silver and Masson's trichrome staining were done using an autostainer. The sample for fluorescence microscopy examination and C4d immunohistochemical staining was frozen in a histobath and stored at −80°C.

Electron microscopy

From each biopsy, two 1 mm × 1 mm fragments were fixed in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate and post-fixed in 2% osmium tetraoxide buffered with 0.1 M sodium cacodylate for 1 h and then dehydrated in ethanol. Tissue blocks were infiltrated in epon/araldite and transferred to BEEM capsules, where the resin was left to polymerize at 60° C for 24 h. Tissue sections were cut at 90 nm with an ultramicrotome and analysed using a transmission electron microscope (JEOL 1220, Jeol, Tokyo, Japan).

Comparison between the 2009 and 2013 Banff classifications

We focused on the main changes introduced in the Banff classification (Table 1) affecting ABMR. Diagnostic changes are detailed in the figures, particularly for Category 2, which corresponds to the diagnosis of ABMR.

Statistical analysis

Statistical analyses between groups were performed using χ^2 and Fisher's exact tests (SPSS 22.0, IBM, Armonk, NY, USA). Statistical significance was set at $P < 0.05$.

RESULTS

Study population

Seventy-three percutaneous kidney graft biopsies taken from 68 patients between February 2011 and February 2014 for 'non-acute' indications were used in this study, including slow graft function deterioration [$n = 9$ (12.3%)], proteinuria [$n = 13$ (17.8%)], both [$n = 14$ (19.7%)], HLA DSA detection [$n = 32$ (43.8%)] and other [$n = 5$ (6.8%)]. Demographic data, pretransplant immunological data, renal function at biopsy, immunosuppression and DSAs are detailed in Table 2.

Table 1. Banff 2009 versus Banff 2013 classification of study samples, considering relevant changes in the Banff 2013 Working Classification of Renal Allograft Pathology regarding Category 2 (antibody-mediated rejection)

Relevant change in the Banff 2013 classification	Impact
1. More precise definition of glomerulitis	
BANFF 2009: mononuclear cell infiltrate in the glomeruli with endothelial enlargement	Glomerulitis in 34/73 (g1, $n = 24$; g2, $n = 6$; g3, $n = 4$) of the study samples
BANFF 2013: presence of partial or total occlusion of one or more glomerular capillary lumina due to endothelial hypertrophy and leucocyte infiltration	Glomerulitis in 25 samples (g1, $n = 15$; g2, $n = 6$; g3, $n = 4$), 9 g1 disappear. No category change occurs with glomerulitis alone, as it was not considered in Banff 2009
2. Microvascular inflammation	
BANFF 2009: not considered	Microvascular inflammation present in 26 of the study samples: 18 were previously in category 2 and 8 fall into category 2 from other categories
BANFF 2013: glomerulitis + peritubular capillaritis ≥2	
3. Acceptance of focal C4d on frozen sections	
BANFF 2009: C4d diffuse staining (C4d3) as evidence of ABMR	Three cases are moved to Category 2, all from Category 6
BANFF 2013: C4d focal (C4d2) or diffuse staining (C4d3) as evidence of ABMR	
4. Chronic transplant glomerulopathy 1a (CTG only on EM)	
BANFF 2009: the presence of double contours in the glomerular basement membrane in one or more peripheral glomerular capillary walls detected in PAS staining or methenamine silver staining in absence of glomerular immune deposits	Nine new CTG cases (previously had been considered ctg0); three of these nine cases with ctg1a fall into Category 2
BANFF 2013: separates mild CTG (ctg1) in two different subgroups: ctg1a: subtle double contours in at least three peripheral glomerular capillary walls associated with expansion of the subendothelial space due to an electron-lucent material and with endothelial oedema detected using EM in the absence of detection of splitting of this membrane by light microscopy. ctg1b: considers cases that meet the old Banff 2009 criteria for mild CTG	
5. Acceptance of DSA at any moment post-transplantation in cases with histological signs of chronic ABMR	
BANFF 2009: DSA considered exclusively when present at the time of the biopsy	Six cases are now considered HLA DSA positive
BANFF 2013: DSA considered when identified at any time post-transplant, even if these antibodies are not present at the time of the biopsy in samples with histological signs of chronic ABMR (such as CTG) but in the absence of evidence of current/recent antibody interaction with the endothelium	Previously four in Category 6 and two in Category 5 Two of the six fall into Category 2
6. Removal of IFTA as possible morphological criteria for ABMR	
BANFF 2009: IF/TA accepted as one of the histological criteria for chronic ABMR in the presence of DSAs and diffuse C4d staining of peritubular capillaries	Five samples lose Category 2 diagnosis
BANFF 2013: Removal of this histological change from those attributable to ABMR	
7. C4d staining without evidence of rejection	
BANFF 2009: not considered	No samples met the criteria for this subcategory
BANFF 2013: this category includes cases with C4d2 or C4d3 without glomerulitis, peritubular capillaritis, microvascular inflammation or acute tubular injury not explained by other causes	
8. Endarteritis [22]	
BANFF 2009: considered evidence of ATCR	No endarteritic lesions observed[a]
BANFF 2013: in the presence of DSA and histological evidence of antibody interaction with renal graft endothelium (significant C4d staining in peritubular capillaries and/or the presence of significant microvascular inflammation) a possible role of ABMR is suggested	

ATCR, acute T-cell rejection; CTG, chronic transplant glomerulopathy.
[a]This result is partly justifiable because these lesions tend to be associated with an acute deterioration of renal graft function, an exclusion criterion for the present study.

Classification of renal biopsies

Thirty biopsies (41.1%) were identified as chronic active or suspected ABMR (Category 2) as per the 2009 Banff classification (Figure 1A). The reclassification using the Banff 2013 criteria (Figure 1B) affected all categories (Figure 1C). Four cases lost their previous Category 2 status and eight were diagnosed as ABMR. The major difference in the classification of the study biopsies was an increase in the number of cases fulfilling a consistent diagnosis of ABMR (Category 2). From the 30 biopsies diagnosed as Category 2 with Banff 2009, 12 (40%) were conclusive ABMR cases and the other 18 (60%) were suspected of having the disease. From the 34 biopsies diagnosed as Category 2 with Banff 2013, 25 (74%) were conclusive ABMR cases and the other 9 (26%) were suspected cases (P = 0.006, Banff 2009 versus Banff 2013) (Figure 2).

Changes in the new Banff 2013 classification

Impact of the new evaluation of glomerulitis. Assessment using Banff 2009 indicated that 34 study biopsies (46.6%) showed the presence of glomerulitis (g1, $n = 24$; g2, $n = 6$; g3, $n = 4$), while the new Banff 2013 classification reduced this number to 25 (34.2%) (g1, $n = 15$; g2, $n = 6$; g3, $n = 4$). All the biopsies that had previously been identified as moderate (g2) to severe (g3) glomerulitis showed partial or complete occlusion of glomerular capillary lumens, thus conserving the initial classification (Figure 3). However, nine of the samples initially

considered g1 were classified as g0 (no glomerulitis) with re-assessment as per Banff 2013 (Tables 1 and 3A).

At the time of the biopsy and based on Banff 2009, HLA DSAs were detected in 22 samples with glomerulitis (64.7%) and in 11 without it (28.2%) (P = 0.002). With Banff 2013, DSAs were detected in 68 and 33.3%, respectively (P = 0.05).

Two biopsies without glomerulitis (5.1%) and 11 with it (32.3%) (P = 0.002) showed diffuse positive C4d staining with Banff 2009; the percentages were 12.5 and 28% (P = 0.118), respectively, with Banff 2013.

Focusing on the nine samples reclassified from g1 to g0, C4d diffuse positive staining was present in three and DSAs in five at the time of the biopsy. Five of these now g0 cases fall in Category 2 with Banff 2009 (three with full ABMR diagnosis and two suspected ABMR); in the absence of glomerulitis with the 2013 definition, four samples remain in Category 2 (three full and one suspected ABMR) and the other falls in Category 5 [interstitial fibrosis/tabular atrophy (IF/TA) with DSAs]. Four prior g1 cases, reclassified as g0, previously classified in other diagnostic categories different from antibody-mediated changes (Category 2) according to Banff 2009, remain in these categories according to Banff 2013.

Table 2. Baseline data of the 68 patients from which study samples were obtained

Age at transplantation (years), mean ± SD	49.7 ± 14.0
Male gender (%)	41.2
Deceased donor (%)	89.7
Pregnancies (%)	26.5
Re-transplant recipients (%)	29.4
Peak PRA or peak PRA > 5%	25%
Pre-transplant PRA or pre-transplant PRA > 5%	13.5%
HLA mismatches [*n* (%)]	4.1 (1.3)
HLA DSAs at transplantation: no DSAs/DSAs/unknown (%)	45.6/30.9/23.5
Induction treatment: none/anti-IL-2R/ thymoglobulin (%)	5.9/67.6/26.5
Initial immunosuppressive drugs prednisone-MPA-CNI (%)	100
Immunosuppression at biopsy with prednisone/CNI/MPA/mTORi (%)	75.3/75.3/90.5/20.0
DSAs at the time of the biopsy [*n* (%)]	33 (45.2)
DSAs at any time of the clinical follow-up [*n* (%)]	39 (53.4)
DSA type: Class I/Class II/Classes I and II (%)	7.7/87.2/5.1
Serum creatinine (mg/dL), mean ± SD	1.78 ± 0.89
Estimated glomerular filtration rate (mL/min/1.73 m^2), mean ± SD	44.9 ± 22.7
Protein:creatinine ratio (mg/g), median (IQR)	398 (157–1171)
Post-transplantation time to biopsy (months), median (IQR)	36 (14.5–98)

SD, standard deviation; PRA, panel reactive antibodies; DSAs, Luminex-detected donor-specific antibodies; IQR, interquartile range; CNI, calcineurin inhibitors; mTORi, mechanistic target of rapamycin inhibitors; MPA, mycophenolic acid. eGFR was calculated using the Modification of Diet in Renal Disease 4-variable equation.

Interpretation of results when microvascular inflammation is included as histological evidence of humoral response. Microvascular inflammation (g + ptc > 2) was found in 26 (35.6%) study samples whereas no significant microvascular inflammation was found in 47 (64.4%). In the biopsies with microvascular inflammation, C4d staining was diffuse positive (*n* = 9), focally positive (*n* = 3), minimally positive (*n* = 3) or negative (*n* = 11) (Table 3B). C4d diffuse or focally positive staining was significantly associated with microvascular inflammation (P < 0.001). DSAs were detected at the time of the biopsy in 18/26 biopsies with significant microvascular inflammation and in 15/47 biopsies without it (69.2 versus 31.9%; P = 0.002). Chronic transplant glomerulopathy (CTG) was detected in 17/26

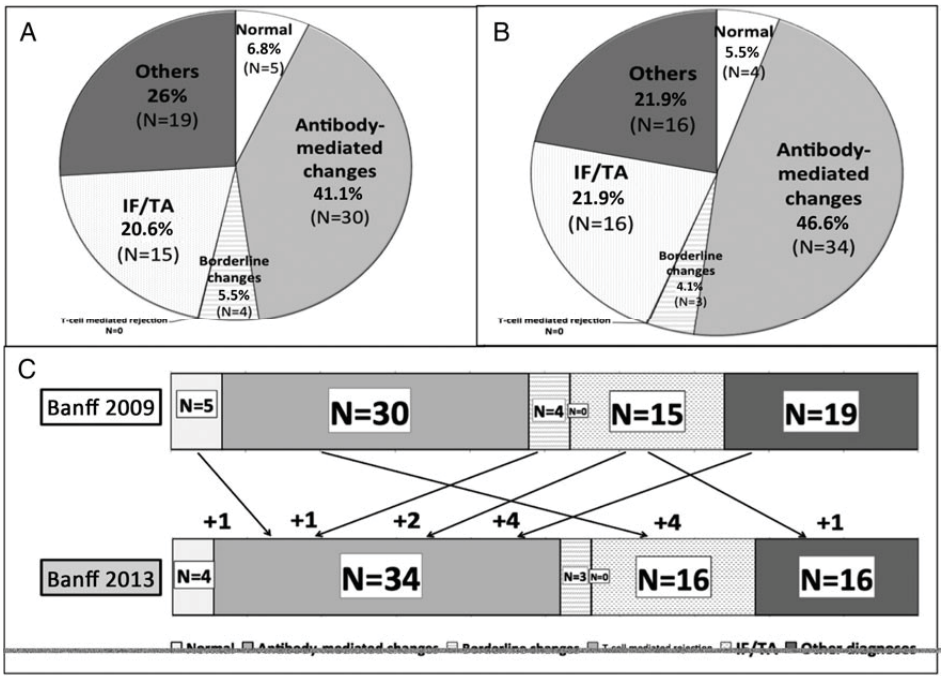

FIGURE 1: Number and percentage of samples (*n* = 73) in each diagnostic category following (**A**) Banff 2009 and (**B**) Banff 2013 criteria. (**C**) Detailed differences between Banff 2009 and Banff 2013.

(65.4%) of the study samples and in 9/47 samples (19.1%) without microvascular inflammation (P < 0.001). Thus, significant microvascular inflammation was associated with the presence of DSAs, significant C4d staining and CTG, considered as traditional markers for chronic ABMR (Table 3B).

Following the 2009 Banff criteria, only 18 of the 26 samples (69.2%) with significant microvascular inflammation were allocated in Category 2 (ABMR). The new Banff 2013 classification placed the 26 biopsies in Category 2, either as suspected acute/active ABMR (without DSAs at the time of the biopsy) ($n = 7$), consistent with acute/active ABMR ($n = 4$) or consistent with chronic active ABMR ($n = 15$; Table 3B).

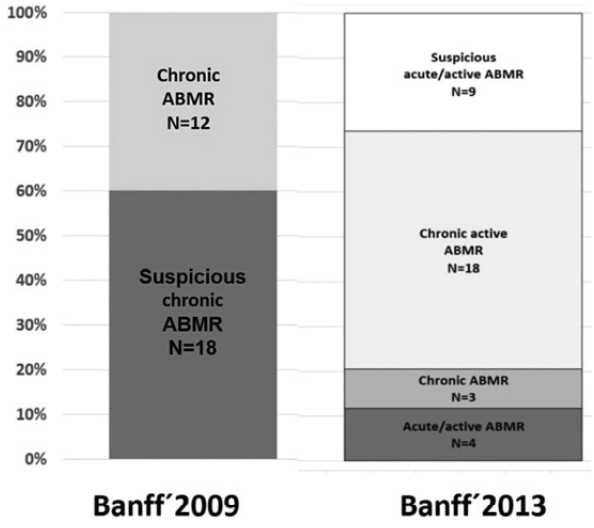

FIGURE 2: Detail of study samples classified as Category 2 as per Banff 2009 and Banff 2013.

Interpretation of results when focally positive C4d PTC staining using frozen material is included as evidence of antibody/endothelium interaction. C4d was diffusely positive (C4d3) in 11 cases, all classified as Category 2 under Banff 2009. Nine coexisted with DSAs and the remaining two with non-DSA HLA.

C4d was focally positive (C4d2) in four samples, of which one simultaneously had DSAs. Three had been classified in Category 6 following the Banff 2009 criteria due to the detection of isolated CTG (one ctg1, one ctg2) with no DSAs; at re-evaluation they fulfilled the Banff 2013 criteria for Category 2. The other sample had already been placed in Category 2, as suspected active ABMR and a conclusive diagnosis of acute/active ABMR as per Banff 2013. Three of these biopsies would have been labelled as active ABMR regardless of C4d staining, since significant microvascular inflammation (g + ptc ≥ 2) was also found.

The combination of focal and diffuse C4d staining in PTCs as evidence of antibody interaction with the endothelium revealed that 15 biopsies (20.5%) were C4d positive and 10 presented with post-transplant DSAs (P = 0.111). The association between C4d staining in PTCs and DSAs, although not statistically significant, seems weaker when following the Banff 2013 criteria compared with the Banff 2009 criteria.

Interpretation of C4d staining in PTCs without evidence of rejection as a subcategory within diagnostic category 2. Only one biopsy classified in Banff 2009 Category 2 as suspected for acute ABMR by light microscopy due to the presence of DSAs and diffuse C4d met the criteria for this subcategory when reassessed following the Banff 2013 criteria. However, an ultrastructural study revealed a focal multilayered

Table 3. Correlation of the new histological evidence on humoral response suggestive of ABMR and classical criteria employed to diagnose ABMR

Glomerulitis	2013, glomerulitis ($n = 25$)	2013, no glomerulitis ($n = 48$)	P-value
Positive C4d (diffuse or focal)	10 (40%)	5 (10.4%)	**0.03***
Donor-specific antibodies	17 (68%)	16 (33.3%)	0.05
Chronic transplant glomerulopathy	14 (56%)	9 (18.7%)	**0.023***
Banff 2013 (Category 2)	22 (88%)	10 (20.8%)	**<0.001***
Banff 2013 (other categories)	3 (12%)	38 (79.2%)	
Microvascular inflammation	**Microvascular inflammation (g + ptc ≥ 2) ($n = 26$)**	**No microvascular inflammation (g + ptc < 2) ($n = 47$)**	**P-value**
Positive C4d (diffuse or focal)	12 (46.2%)	3 (6.4%)	**<0.001***
Donor-specific antibodies	18 (69.2%)	15 (31.9%)	**0.002***
Chronic transplant glomerulopathy	17 (65.4%)	9 (19.1%)	**<0.001***
Banff 2009 (Category 2)	18 (69.2%)	12 (25.5%)	**<0.001***
Banff 2009 (other categories)	8 (30.8%)	35 (74.5%)	
Banff 2013 (Category 2)	26 (100%)	9 (19.1%)	**<0.001***
Banff 2013 (other categories)	0 (0%)	38 (80.9%)	
CTG1a versus absence of CTG (CTG < 1a)	**ctg1a ($n = 9$)**	**ctg < 1a ($n = 45$)**	**P-value**
Protein:creatinine ratio (mg/g), median (IQR)	1163.26 (154.6–1832.1)	244.2 (137.5–698.1)	**0.036***
Microvascular inflammation	6 (66.7%)	8 (17.8%)	**0.006***
Positive C4d (diffuse or focal)	2 (22.2%)	5 (11.1%)	0.395
Donor-specific antibodies	5 (55.6%)	17 (37.8%)	0.461
Banff 2009 (Category 2)	5 (55.6%)	14 (31.1%)	0.251
Banff 2009 (other categories)	4 (44.4%)	31 (68.9%)	
Banff 2013 (Category 2)	7 (77.8%)	13 (28.9%)	**0.009***
Banff 2013 (other categories)	2 (22.2%)	32 (71.1%)	

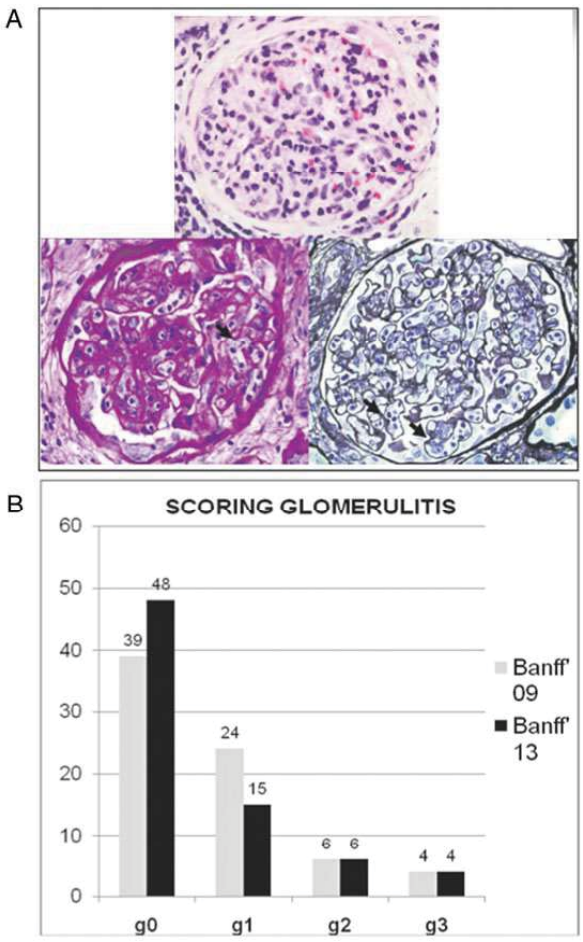

FIGURE 3: (**A**) (haematoxylin and eosin, periodic acid-Schiff and silver stains, ×60) sample with severe glomerulitis (g3) showing associated endothelial enlargement (arrows). (**B**) Distribution of glomerulitis as per Banff 2009 and Banff 2013.

peritubular capillary basement membrane, which defines the sample as chronic active ABMR (Category 2), as per Banff 2013.

Impact of including ultrastructural-only CTG in the Banff schema. Following the Banff 2009 criteria, CTG was detected under light microscopy in 17 study samples (23.6%): 9 mild CTG (ctg1), 5 moderate (ctg2) and 3 severe (ctg3). Nine additional samples also met the ultrastructural criteria for CTG when analysed under electron microscopy (EM) (Figure 4); six of them showed significant microvascular inflammation (g + ptc ≥ 2) and in two, diffusely positive C4d staining was detected in PTCs. DSAs were present in five of these nine samples. Eight had significant IF/TA (ct or ci ≥1). Proteinuria was found in six cases at the time of the biopsy.

Applying the new Banff 2013 criteria, 36.7% of the biopsies showed CTG: nine with EM as previously described (ctg1a), nine with mild CTG (ctg1b), five moderate (ctg2) and three severe (ctg3). The inclusion of ctg1a in Banff 2013 affected the diagnostic category of only 3 of the 73 biopsies (4.1%); for the remaining 6 cases, other ABMR features were reported.

Interestingly, two cases with CTG, but no detectable DSAs at any given time point after transplant, could only be classified as acute active ABMR under Banff 2013 due to the presence of microvascular inflammation.

Impact of introducing chronic, inactive ABMR in Banff 2013 diagnostic category 2. Only three cases (4.1%) fulfilled the criteria for chronic 'inactive' ABMR, all of which had positive DSAs at the time of the biopsy and suspected acute T-cell rejection (Category 3) ('borderline' changes) in one of the three samples. Chronic active ABMR was suspected for the other two biopsies as per Banff 2009 (Category 2) based on the presence of DSAs with IF/TA and CTG.

Impact of considering the presence of DSAs not only at the time of the biopsy in chronic ABMR. Positive post-transplant DSAs were detected in five cases (6.8%) before the biopsy but not at the time of the biopsy. With Banff 2009, three of the cases were placed in Category 5 (IF/TA), as they were negative for diffuse C4d staining, and two in Category 6, one with mild CTG (ctg1) negative for C4d staining and the other with recurrent nodular diabetic nephropathy. Only the sample with CTG was replaced in Banff 2013 Category 2 as chronic active ABMR due to microvascular inflammation with prior DSA detection.

Evaluation of several histological lesions [16] as markers of ABMR with a diagnosis based on the detection of HLA DSAs, showed that microvascular inflammation and peritubular capillaritis were the best histological predictors considering both the sensitivity and specificity of DSAs (Table 4).

Impact of removing IF/TA as histological criteria of chronic ABMR. Eleven of 30 biopsies classified as chronic active ABMR as per Banff 2009 had IF/TA as the only chronic change attributable to ABMR. Following the Banff 2013 criteria, six samples remained in Category 2, three as acute/active ABMR for DSAs with microvascular inflammation, two suspected acute/active ABMR due to DSA presence with mild glomerulitis/capillaritis without microvascular inflammation or positive C4d and one as chronic ABMR due to the detection of ctg1a. Furthermore, 5 of these 11 samples were reclassified as IF/TA (all previously suspected for ABMR) due to the presence of DSAs without microvascular inflammation, C4d staining, CTG or multilayering of the basement membrane.

Clinical impact of the new Banff 2013 classification

Twenty-six study biopsies maintained the diagnosis of ABMR in both classifications, eight cases acquired Category 2 with Banff 2013 classification and four lost it (Table 5). It is worth noting that biopsies labelled as ABMR as per the Banff 2013 classification were taken from older patients and were thus less likely to present with HLA DSAs at biopsy, showing worse graft function. No differences in graft loss at 32 months follow-up were found. Banff 2009 was ineffective for providing a diagnosis of ABMR in 23.5% of biopsies with humoral rejection.

Considering the group of biopsies that maintained the ABMR diagnosis, no major differences were noted between patients whose biopsies were suspected of ABMR as per the Banff 2009 criteria or had a clear diagnosis with Banff 2013 ($n = 11$) and those with a clear diagnosis with both classifications ($n = 12$; data not shown).

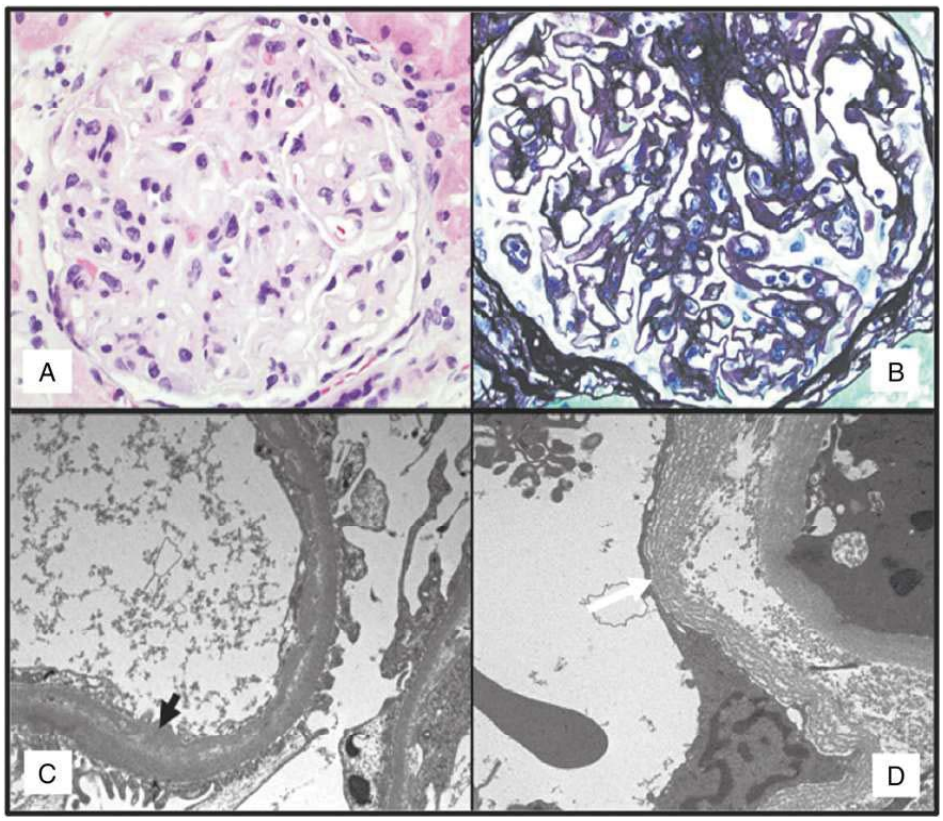

FIGURE 4: Diagnostic changes as per Banff 2013 **(A** and **B)** (haematoxylin and eosin and silver staining, ×60). Glomerulus showing segmental glomerulitis but with no glomerular basement membrane double contours. **(C** and **D)** Ultrastructural study of the same sample revealing double contours (consistent with ctg1a, black arrow) and multilayering of the basement membrane in the peritubular capillaries (white arrow).

Table 4. Sensitivity and specificity of some histological ABMR-related lesions as per Banff 2013 associated with HLA DSAs

Serum HLA DSAs as a criterion for ABMR	Sensitivity (%)	Specificity (%)
Banff 2009		
Diffuse C4d	21.0	90.0
Chronic transplant glomerulopathy, excluding CTG1a	30.0	83.0
Interstitial fibrosis/tubular atrophy	88.0	8.0
Banff 2013		
Focal or diffuse C4d	27.3	87.5
Glomerulitis	51.5	80.0
Peritubular capillaritis	66.7	62.5
Microvascular inflammation	54.5	80.0
Focal or diffuse C4d and/or microvascular inflammation	57.6	75.0
Chronic transplant glomerulopathy	46.9	71.8
Multilayering of peritubular capillaries basement membrane	43.8	79.5

DISCUSSION

Over the years, the Banff classification for allograft pathology has been updated with new knowledge about rejection, hypothetically for more accurate categorization of biopsies. We previously assessed the impact of some of the updates in the Banff 2013 classification [11]. Considering that the aetiology of ABMR lies with DSAs, the diagnosis aims to identify signs of antibody-mediated damage in the graft tissue [17]. Mostly indirect findings, such as C4d or significant microvascular inflammation (recently included), are employed as markers for ABMR. Early studies found a strong correlation between C4d and HLA DSAs for the diagnosis of acute ABMR [16]. Our study confirms that regardless of the criteria used for assessing C4d staining, Banff 2009 or Banff 2013, no significant association exists between this histological marker alone and HLA DSA detection. Despite this, the relationship between HLA DSAs and the presence of the recently approved histological evidence of antibody interaction with the endothelium is statistically significant in the new Banff 2013 classification.

Considering that these histological findings can be used as markers of the presence of DSAs, and therefore an ABMR diagnosis, we believe the new Banff 2013 classification is more conclusive when compared with Banff 2009. Eight of the study biopsies with microvascular inflammation were initially classified as suspected ABMR; with reassessment, either acute/active or chronic active ABMR was determined for these study samples. Moreover, seven other biopsies, previously placed in other categories, were reclassified as Category 2 as per Banff 2013 due to microvascular inflammation. Interestingly, isolated CTG was observed in some of these biopsies under light microscopy, without other evidence of ABMR (negative C4d staining and the absence of DSAs) when Banff 2009 was used. This finding

Table 5. Comparison of biopsies diagnosed with ABMR as per the 2009 and 2013 Banff classifications and those with ABMR only with Banff 2013

	ABMR 2009 and ABMR 2013[a] ($n = 26$)	No ABMR 2009 → ABMR 2013[b] ($n = 8$)	P-value
Age at transplantation (years), mean ± SD	44.2 (15.1)	54.2 (13.1)	**0.009**
Recipient female gender	15 (57.7%)	5 (62.5%)	0.81
Retransplantation	9 (34.6%)	2 (25%)	0.66
DSAs at transplantation	13/22 (59%)	3/5 (60%)	0.43
Immunosuppression at biopsy with prednisone/CNI/MPA/mTORi (%)	21/17/9	5/6/2	0.79
HLA DSAs at biopsy	24 (92.3%)	4 (50%)	**0.018**
MFI, median (IQR)	12 320 (6973–17 123)	2370 (2214–4987)	0.07
Type of DSAs, Class I/II/I + II (%)	3/20/1	0/4/0	0.35
SCr (mg/dL), mean ± SD	1.4 (0.7)	2.1 (0.74)	0.25
eGFR (mL/min/1.73 m^2), mean (SD)	57.3 (25.2)	35.3 (16.1)	**0.035**
Pro/Cr (mg/g), median (IQR)	389 (159.5–1014.1)	231.5 (164.8–789.9)	0.89
SCr at the end of follow-up (mg/dL), mean (SD)	1.7 (1.3)	2.1 (1.8)	0.14
eGFR at the end of follow-up (mL/min/1.73 m^2), mean (SD)	57.1 (28.6)	37.7 (13.6)	**0.04**
Pro/Cr at the end of follow-up (mg/g), median (IQR)	161.7 (125–1454)	456.6 (253.7–1381)	0.41
Graft loss at follow-up (death-censored)	4 (15.3%)	2 (25%)	0.60
Post-transplantation time to biopsy (months), median (IQR)	33.5 (18–112)	99 (35–175)	0.28
Time from biopsy to last follow-up (months), median (IQR)	32 (25–35)	32.5 (29.5–33.5)	0.98

Values given as *n* (%) unless otherwise noted. DSAs, Luminex-detected donor-specific antibodies; CNI, calcineurin inhibitors; MPA, mycophenolic acid; mTORi, mechanistic target of rapamycin inhibitors; MFI, mean fluorescence index; IQR, interquartile range. eGFR was calculated using the Modification of Diet in Renal Disease 4-variable equation.

[a]The group of 26 cases 'ABMR 2009 and ABMR 2013' include cases with lesions suspected of having ABMR as per both Banff classifications ($n = 3$), suspected of having ABMR with Banff 2009 and full ABMR with Banff 2013 ($n = 11$), and with full ABMR as per both Banff classifications ($n = 12$).

[b]Eight cases without an ABMR diagnosis following the Banff 2009 criteria, but full ABMR as per Banff 2013. In addition, four cases had ABMR conclusive criteria as per Banff 2009 but not with Banff 2013.

could suggest that the CTG is not secondary to ABMR, although non-HLA DSAs should be ruled out in these cases.

One of the potential problems with the new classification is that the sole presence of microvascular inflammation allows labelling a biopsy as suspected active ABMR. In the present study, seven biopsies showed significant microvascular inflammation without HLA DSAs or other signs of chronic ABMR, four with proteinuria and a significant increase in serum creatinine at the time of biopsy, one presenting proteinuria and two without proteinuria or an increase in serum creatinine. The last two study samples suggest that the presence of significant microvascular inflammation alone may not necessarily imply ABMR, particularly considering that other causes such as severe acute tubular injury, chronic pyelonephritis and interstitial nephritis may justify the finding, in particular peritubular capillaritis [18]. As per the Banff 2013 classification, it is also possible to find peritubular capillaritis in acute T-cell rejection. In these cases, follow-up clinical data and biopsies can be of help.

Compared with the old criteria used for glomerulitis, the new standards provide ways to evaluate glomerulitis by showing a marginal association with C4d staining (P = 0.05) and a weaker association with DSAs. This discrepancy should be further studied with a larger number of biopsies with previous g1 and current g0. This restrictive way of assessing glomerular inflammation is of great interest given the recent evidence indicating that glomerulitis alone may be an independent histological factor for poor graft survival [19]. On the other hand, occasional inflammatory cells are seen in capillary lumens, most possibly being circulating cells. The new criteria for classifying glomerulitis affect, nine of the study samples with g1, which were recategorized as g0. Overall, only one diagnostic change occurred among the nine cases when the new criteria were applied.

Our study illustrates another potential problem of the Banff 2013 classification; it only accepts certain histological changes as attributable purely to chronic humoral rejection, such as CTG and multilayering of the basement membrane in PTCs, omitting the percentage of glomeruli with global sclerosis or the percentage of IF/TA, which can be the consequence of ABMR in later stages. In fact, five of the study biopsies lost diagnostic Category 2 for this reason.

The new classification includes CTG within the histological diagnostic criteria of chronic ABMR when detected only by EM. In our study, EM allowed detection of CTG in nine study samples and multilayering of the PTC basement membrane in 20 samples from the 73 evaluated. Early detection of ctg1a, within 3 months post-transplantation, has been found to correlate with later development of CTG established by light microscopy [20]. We identified ctg1a lesions in nine patients, but in all cases >1 year post-transplant. A prospective study would confirm the usefulness of ctg1a as a diagnostic tool; our data already show that these patients have higher proteinuria despite very mild CTG. A recent study analysed the clinical impact of both Banff classifications in sequential cohorts of patients with chronic humoral changes in their biopsies and found that the presence of DSAs or C4d and CTG or multilayering, but not the new criterion of micro-inflammation, correlates with the clinical outcome [21].

Our short-term follow-up precludes any meaningful analysis on graft function and outcomes between patients with ABMR as per Banff 2009 and those with ABMR as per Banff 2013. Long-term clinical monitoring could provide functional evidence on which criteria (Banff 2009 or Banff 2013) can better classify renal graft samples with chronic ABMR. Here, we found that biopsies diagnosed with ABMR only following Banff 2013 compared with the biopsies diagnosed with both

classifications occurred more frequently in patients without HLA DSAs at biopsy, and corresponded to grafts with worse GFRs. Thus, following the 2009 Banff classification, 23.5% of the biopsies were not labelled as ABMR, precluding a potential adequate treatment.

One of the limitations of our study is the small number of biopsies. Furthermore, it is a cross-sectional study, with insufficient patient follow-up or subsequent biopsies to evaluate the impact of our findings.

In conclusion, with the new Banff 2013 classification the diagnostic category changed in 18% of the study samples. Considering our results, we believe that the new classification allows a more precise diagnosis of AMBR. Although the percentage of samples included under Category 2 increases only slightly, a consistent diagnosis of ABMR is made for a large number of cases, halving the samples previously considered as suspected ABMR. This more precise classification is justified by (i) considering microvascular inflammation as evidence of antibody-endothelium interaction, (ii) considering DSAs at any time after transplantation when chronic features of ABMR are present in the biopsy and (iii) adding ultrastructural alterations to the classic histological picture of ABMR-related changes.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts 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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DISCUSIÓN

4. DISCUSIÓN

Los artículos incluidos en esta tesis se enmarcan dentro de un proyecto de caracterización del rechazo mediado por anticuerpos en trasplante renal, desde un abordaje serológico e histológico.

Los dos primeros artículos que componen la presente tesis tratan de analizar el impacto de los anticuerpos HLA dirigidos frente al donante en el desarrollo del RMA y en la supervivencia del injerto. En el primero de ellos, analizamos específicamente si el comportamiento postrasplante de estos anticuerpos preformados, su persistencia o no, tiene implicaciones pronósticas en términos de aparición de rechazo humoral y en la supervivencia del injerto. El segundo, se centra en el análisis de anticuerpos anti-HLA poco estudiados hasta el momento en el campo del trasplante renal, los anticuerpos anti-HLA DP y su potencial impacto en la supervivencia del injerto. Los artículos tercero y cuarto artículo se centran en el estudio histológico del RMA: frecuencia de este rechazo en biopsias por indicación en nuestro ámbito clínico e impacto en la supervivencia del injerto, comparado con otros diagnósticos histológicos y la aportación de la clasificación de Banff 2013 en el diagnóstico del RMA.

4.1. Impacto de la evolución postraplante de los DSA preformados en el rechazo mediado por anticuerpos y supervivencia del injerto

En una era donde el estudio de anticuerpos HLA con metodología de fase sólida no se realizaba de forma sistemática antes del trasplante, el análisis retrospectivo del estudio de anticuerpos HLA mediante tecnología Luminex® nos ha permitido describir la historia natural y comportamiento de estos anticuerpos, así como su evolución posterior. En nuestra cohorte, aunque el equipo clínico no lo sabía en el momento del trasplante, el 10.5% de los pacientes se trasplantaron con al menos un DSA. Esta incidencia es similar a la reportada en otras series (49, 51, 58). Este grupo de pacientes, presentaba 10 veces más riesgo de RMA que los pacientes que se trasplantaron sin DSA.

En nuestra serie, los pacientes con DSA frente a antígenos HLA de clase I (con o sin DSA clase II) presentaron mayor riesgo de rechazo humoral agudo precoz severo, que supuso la pérdida del injerto. En los pacientes con DSA preformados clase I que no desarrollaron RMA precoz, el DSA no se detectó mayoritariamente en un seguimiento postrasplante hasta 5 años. Por el contrario, los pacientes con DSA de clase II presentaron un comportamiento diferente, de forma que ningún paciente sufrió RMA precoz con pérdida de injerto y en la mayoría de ellos, el DSA se mantuvo al año del trasplante. En este sentido, WIllicome et al (58) y Caro-Oleas et al (51) también describieron que los pacientes con DSA clase I o clase I y II presentaban una peor supervivencia libre de RMA y del injerto, respectivamente, que los pacientes que tenían solo DSA II o que no tenían anticuerpos. Otten et al (67) relataron peor supervivencia del injerto en el grupo de pacientes con DSA clase I y II. En la experiencia de Lefaucheur et al. (54) los

pacientes con DSA preformados clase I o II exhibieron similar supervivencia del injerto. En nuestra experiencia, los pacientes con DSA clase I pretrasplante sin tratamiento desensibilizador previo tienen alto riesgo de RMA precoz; sin embargo, los pacientes con DSA preformados clase II tienen mayor riesgo de RMA tardío.

Cuando analizamos el comportamiento de los DSA preformados al año del trasplante, observamos que en el 41.2% de los pacientes no se detectaron DSA, sin que ninguno de estos pacientes hubiera recibido tratamiento específico desensibilizador.

Solo cuatro estudios publicados tratan de evaluar la persistencia de los DSA preformados después del trasplante con monitorización sistemática basada en SAB (59, 60, 70, 171). La prevalencia de DSA preformados que no se detectan al año del trasplante en estos estudios es del 62-65%. En dos de estos estudios, los DSA preformados no impactan en la supervivencia del injerto (60, 171); los otros dos trabajos reportan peor supervivencia del injerto solo si el DSA preformado persiste después del trasplante (59, 70). En nuestra cohorte, en un análisis ajustado por otros cofactores, no encontramos diferencias en supervivencia del injerto entre los pacientes con DSA preformados que persisten y los que los eliminan. Sin embargo, los pacientes con DSA preformados presentan mayor riesgo de RMA tanto si el DSA persiste como si no lo hace al año del trasplante.

Calliard et al (70) no encontraron diferencias en función renal ni en supervivencia del injerto entre los cuatro grupos analizados: pacientes sin anticuerpos, pacientes con DSA *de novo*, pacientes con DSA preformados persistentes vs DSA no persistentes. En nuestro trabajo, tratamos de analizar los factores de riesgo de persistencia de DSA preformados y observamos que el único factor independiente asociado fue la MFI del DSA pretrasplante; los pacientes con DSA con MFI mayor de 5100 presentaron de forma independiente mayor riesgo de persistencia de DSA después del trasplante. Solo Caillard et al (70) encontraron similares resultados; ninguno de los otros estudios antes mencionados encuentran características de los DSA asociadas o predictoras de persistencia de DSA preformado postrasplante.

Al año postrasplante, solo el 1.9% de nuestra cohorte había desarrollado DSA *de novo*. Como se ha comentado previamente, los DSA *de novo* se asocian a mayor incidencia de RMA(71, 83, 95, 120). En nuestra experiencia, en un análisis ajustado, los pacientes con DSA *de novo* tienen casi 8 veces más riesgo de RMA que los pacientes sin DSA pre y postrasplante. Un hallazgo relevante en este sentido es que los pacientes con DSA preformado también presentaron mayor riesgo de RMA, tanto si el DSA persiste [HR 5.3] como si no lo hace al año del trasplante [HR 2.7].

La principal limitación nuestro estudio es que analizamos una población de bajo riesgo inmunológico (menos del 2% de desarrollo DSA de novo al año del TR). Además, solo

disponemos de biopsias por indicación, con lo que probablemente el RMA subclínico está probablemente infraestimado.

En resumen, los pacientes con DSA preformados presentan un alto riesgo de RMA, que es precoz y lleva a la pérdida de injerto en aquellos con DSA clase I o clase I y II; y más tardío en los pacientes que se trasplantan con DSA clase II. Este riesgo aumentado de RMA ocurre tanto en los pacientes con DSA preformado persistentes como en los pacientes con DSA preformado que no se aclaran postrasplante.

4.2. Impacto de anticuerpos anti HLA-DP en la supervivencia del injerto

En este segundo estudio, enfocado a evaluar el papel de los anticuerpos anti-HLA, analizamos la prevalencia e impacto en supervivencia del injerto de los anticuerpos anti-HLA DP detectados tanto antes como después del trasplante.

En nuestra experiencia, la prevalencia de anticuerpos anti-HLA DP detectados en plataforma Luminex® es similar pre y postrasplante. Sin embargo, la prevalencia de anticuerpos anti-HLA DP aislados es muy baja, de alrededor del 1%, tanto pre como postrasplante.

De la era anterior al desarrollo de los estudios basados en SAB, son escasos los estudios publicados sobre la detección de anticuerpos anti-HLA DP, probablemente debido a la complejidad técnica de la detección de estos anticuerpos mediante técnicas de linfocitoxicidad. El estudio más relevante en este sentido fue publicado por Pfeiffer et al.(125), que evaluaron la presencia de anticuerpos anti-HLA DP en el suero de 505 pacientes en lista de espera de trasplante mediante una técnica alternativa de inmovilización de antígenos leucocitarios con anticuerpos monoclonales (*monoclonal antibody immobilization of leukocyte antigens technique MAILA*). Detectaron la presencia de estos anticuerpos en el 7.3% de los pacientes en lista de espera.

A pesar de que en la última década los ensayos en fase sólida han permitido detectar con mayor precisión los anticuerpos anti-HLA, son escasos los trabajos publicados que han evaluado la prevalencia pretrasplante de anticuerpos anti-HLA DP. Uno de los primeros trabajos, evaluó 738 pacientes en lista de espera mediante microesferas FlowPRA®, detectándo anticuerpos anti-HLA clase II en 171 pacientes (23.1%), prevalencia similar a la detectada pretrasplante en nuestra cohorte de pacientes. En su serie, la presencia de anticuerpos anti-HLA DP es del 12% (172). Otros dos grupos que estudiaron la presencia de anticuerpos antiHLA-DP mediante microesferas Luminex® encontraron una prevalencia pretrasplante del 8% (173) (Billen et al) y postrasplante del 18% (Billen et al). En consonancia con nuestros resultados, más de la mitad (56%) de los pacientes con anticuerpos anti-HLA DP tenían anticuerpos frente a HLA-DQ y DR(124).

Si la prevalencia de anticuerpos anti-HLA DP ha sido poco reportada en la literatura, aún menos estudiado ha sido el impacto de la presencia de estos anticuerpos en la evolución postrasplante a medio y largo plazo. Previo al desarrollo de los ensayos en fase sólida, un estudio importante con más de 3600 pacientes que reciben un trasplante renal intentó evaluar el impacto de las incompatibilidades el locus HLA DPB en la supervivencia del injerto al año postrasplante. No encontraron diferencias en la supervivencia global del injerto en los pacientes con 0, 1o 2 incompatibilidades DPB. El número de incompatibilidades DP pareció influir únicamente en el grupo de pacientes retrasplantados, de tal manera que la supervivencia al año de seguimiento de los pacientes sin incompatibilidades en HLA-DPB fue del 83%, significativamente mayor que la supervivencia de los pacientes con una o dos incompatibilidades (76% y 73% respectivamente) (174).

En uno de los primeros trabajos que aplicaron la tecnología Luminex® se observó que la frecuencia de anticuerpos anti HLA-DP era mayor en pacientes con rechazo que en los que no presentaron rechazo (19.5% vs 5.1%, $p<0.001$). Sin embargo, no se analizó si ambos subgrupos diferían en aspectos clínicos, características del rechazo, y si éste, influyó en la supervivencia del injerto.(175)

Es difícil evaluar el valor de los anticuerpos frente a antígenos DP, dado que el tipaje HLA-DP no se realiza de forma rutinaria. De tal modo que, aunque los estudios actuales mediante SA nos aportan información sobre estos anticuerpos, desconocemos si estos van dirigidos frente al donante; y por tanto, su potencial relevancia desde el punto de vista clínico, relación con el rechazo humoral e implicación en la supervivencia del injerto.

En este sentido, dos estudios han intentado evaluar el impacto de los DSA dirigidos frente a anticuerpos HLA-DP. Jolly et al. detectaron que los pacientes con DSA pretrasplante antiHLA-DP (n=6), sin otro DSA, presentaron una tasa de rechazo mediado por anticuerpos del 50% comparado con el grupo con anti HLA-DP no DSA (n=15), cuya tasa de rechazo fue de solo el 6.6%. La principal limitación de este trabajo, además del número escaso de pacientes, es que los autores no profundizan en la caracterización del tipo de rechazo ni si éste produce la pérdida del injerto (176). En un reciente trabajo, Bachelet et al analizaron 24 pacientes con DSA frente a HLA-DP de una cohorte de 199 pacientes sensibilizados. Los pacientes con DSA pretrasplante frente a HLA-DP y/o HLA-C presentaron una peor supervivencia que los pacientes sin DSA y similar a la que presentaban los pacientes con otros DSA (177).

Existen otras publicaciones de casos aislados de rechazo agudo humoral en presencia de Anticuerpos anti-HLADPB1* donante-específicos, en pacientes sin otros ADS (178-180).

En nuestro estudio, el 53% de los pacientes con anticuerpos anti-HLA pretrasplante tenían DSA y entre ellos casi el 50% anticuerpos anti-HLA DP. Cuando analizamos el impacto de la

presencia de estos anticuerpos en la supervivencia del injerto, observamos que los pacientes con DSA tienen peor supervivencia del injerto comparada con aquellos con anticuerpos anti-HLA no-DSA, pero este impacto en la supervivencia no se modifica cuando realizamos el análisis teniendo en cuenta la presencia o no de anticuerpos anti-HLA DP.

En nuestra población, aproximadamente el 10% de los pacientes trasplantados renales presentan anticuerpos antiHLA-DP en el estudio de SA, tanto pre como postrasplante. La presencia de DSA detectados pretrasplante y en el seguimiento postrasplante tiene un impacto negativo en la supervivencia del injerto en nuestra cohorte de pacientes trasplantados; y la presencia aloanticuerpos anti-HLA DP no parece modificar este impacto. Por tanto, los anticuerpos anti HLA-DP se deben identificar en casos de rechazo humoral en los que no se identifican otros tipos de DSA. Serán necesarios estudios con importante número de donantes tipados para HLA DP para confirmar estos resultados. La principal limitación de nuestro estudio es la falta de información sobre el tipaje HLA DP de los donantes, y por tanto la imposibilidad para identificar si los anticuerpos HLA-DP detectados son o no donante-específicos.

4.3. Supervivencia del injerto renal según la categoría diagnóstica de Banff 2013 en biopsias por indicación

En el tercer trabajo, nos planteamos analizar el impacto de las diferentes categorías diagnósticas histopatológicas en biopsias realizadas por indicación en la supervivencia del injerto. Las biopsias fueron reclasificadas según los criterios de la clasificación de Banff 2013 y establecimos el pronóstico en cuanto a supervivencia renal según cada categoría diagnóstica.

El objetivo de la primera reunión de patólogos y nefrólogos celebrada en Banff en 1991 fue definir y clasificar los hallazgos histopatológicos encontrados en las BR de trasplante(127). Hasta ese momento, el único diagnóstico que tenía relevancia y tratamiento era el de rechazo agudo. En las reuniones posteriores, se han actualizado las categorías diagnósticas, adaptándolas al avance en el conocimiento de la fisiopatología de la pérdida del injerto y definiendo mejor el daño mediado por anticuerpos. La correcta clasificación de diagnósticos histopatológicos podría poner de manifiesto entidades clínicas previamente infradiagnosticadas o no bien definidas.

En el presente estudio, la reclasificación según Banff 2013 (142) permitió obtener un diagnóstico histológico específico en el 95% de las biopsias, lo que traduce el alto rendimiento diagnóstico que tienen las biopsias realizadas por indicación. La categoría más frecuente encontrada fue la correspondiente a la categoría 6 (otros diagnósticos) (28%) y dentro de esta, los diagnósticos de NTA y la recidiva de la enfermedad glomerular de base. La siguiente categoría en frecuencia fue FI/AT (24.8%), catalogada como leve en el 50% de los nuestros casos. Estudios previos realizados en biopsias por indicación muestran resultados heterogéneos con respecto a los diagnósticos más frecuentes. Sellares et al (73), en un estudio multicéntrico

donde evaluaron una cohorte de 315 receptores de TR a los que se había realizado biopsia por disfunción del injerto (412 biopsias) describen que la categoría más frecuente encontrada fue la de BR normal o sin grandes anormalidades con un 29%, seguida de los cambios mediados por anticuerpos con un 18%. Sis et al (181) evaluaron 234 biopsias de 173 pacientes realizadas una media de 16 meses postrasplante, utilizando los criterios de la clasificación de Banff´2007, y encontraron como diagnóstico más frecuente el de rechazo mediado por células T (19%), seguido de "otros diagnósticos" (17%). En este estudio, los diagnósticos de FI/AT y rechazo mediado por anticuerpos C4d positivo representaron solo un 5 y 7%, respectivamente.

Por tanto, atendiendo a nuestros datos, el análisis global de las frecuencias de los diferentes diagnósticos no aportaría información de valor pronóstico, ya que, como podemos observar tanto en este estudio como en otros, los diagnósticos más frecuentes varían sustancialmente dependiendo del tiempo postrasplante. En nuestra cohorte, observamos que categorías como biopsia normal, NTA y cambios mediados por células T se detectaron con mayor frecuencia en las BR realizadas durante el primer año postrasplante. Se encontraron cambios mediados por células T en un 15% de las biopsias realizadas durante los primeros 6 meses, mientras que, en biopsias realizadas después de 5 años del trasplante, este diagnóstico solo representó un 2.5%. Por otro lado, los diagnósticos como daño mediado por anticuerpos y daño crónico por diferentes causas (182) (representado por FI/AT), se encontraron con mayor frecuencia en biopsias más tardías. En el caso del daño mediado por anticuerpos, pasaron de un 17.2% en biopsias realizadas antes del 6º mes a un 46% en biopsias realizadas entre el 3er y 5º año postrasplante. En el caso de FI/AT se pasa de un 6.6% en biopsias tempranas a un 36% en biopsias con más de 5 años. Sellares et al. describieron resultados similares, y reportaron un claro aumento en la frecuencia de diagnóstico histológico de rechazo mediado por anticuerpos en relación con el tiempo postrasplante y un descenso en la frecuencia de rechazo mediado por células T(73).

Al analizar el pronóstico del injerto según la categoría diagnóstica asignada de la clasificación de Banff´13, observamos que el riesgo de pérdida del injerto se multiplica por 4 en los trasplantes con biopsias compatibles con rechazo mediado por anticuerpos, tal y como ya se ha descrito previamente (8, 73, 148, 169, 170). El otro patrón histológico que se relacionó con peor supervivencia fue el de FI/AT moderada-severa, que no corresponde a un diagnóstico atribuido a un proceso específico sino a una descripción histológica sin peso de especificidad, por lo que para caracterizar adecuadamente al paciente con FI/AT es necesario obtener mejor información desde el punto de vista diagnóstico y pronóstico. De acuerdo con esto, El-Zoghby et al. (8) encontraron que un 30.7% de las biopsias de riñones con pérdida del injerto tenían un diagnóstico de FI/AT. Un 80.9% de estos diagnósticos genéricos de FI/AT pudieron ser

atribuidos a una causa específica al reevaluar las biopsias, como nefropatía por poliomavirus BK, RMA o episodios múltiples de rechazo celular agudo.

A pesar de que el rechazo agudo ha sido clásicamente relacionado con una peor supervivencia del injerto (183, 184), en nuestra cohorte de trasplantados renales el rechazo mediado por células T y el diagnóstico de cambios *borderline* no tiene impacto significativo en el pronóstico del injerto renal, lo que indica que el peso atribuido clásicamente a esta entidad con respecto a su impacto en la supervivencia del injerto podría recaer en el RMA. Si excluimos el rechazo agudo celular y el RMA agudo del análisis de supervivencia y agrupamos el rechazo crónico mediado por anticuerpos junto con la presencia de FI/AT moderada-severa (histología desfavorable) y lo enfrentamos al grupo con una histología favorable, el riesgo de pérdida del injerto es el doble. Estas dos entidades son las de mayor peso específico a la hora de pronosticar la supervivencia a largo plazo del injerto.

La principal limitación de nuestro estudio es el carácter retrospectivo del análisis de las biopsias con falta de disponibilidad de otros datos clínicos relacionados con el trasplante (como función renal, proteinuria o tratamiento inmunosupresor) para poder establecer una relación causal más detallada.

Sin embargo, las biopsias por indicación siguen siendo la herramienta diagnóstica fundamental para el estudio de la disfunción del injerto. No hemos encontrado estudios publicados que analicen el valor pronóstico según las distintas categorías de Banff 2013. Nuestro estudio aporta información relevante sobre el peso específico en la supervivencia del injerto de estas categorías, en una cohorte amplia de trasplantados renales con un tiempo de seguimiento adecuado.

4.4. Impacto de la nueva clasificación de Banff´13 en el diagnóstico del rechazo mediado por anticuerpos

En este cuarto trabajo, analizamos el impacto de la nueva clasificación de Banff´13, fundamentalmente centrado en el RMA en biopsias por disfunción del injerto no aguda.

En las últimas actualizaciones de la clasificación de Banff, los cambios más relevantes se están produciendo en el RMA. Por tanto, nos planteamos analizar las implicaciones de cambios en el diagnóstico del rechazo humoral crónico. En este sentido, intentamos evaluar cómo afectan la última actualización de los criterios histológicos del RMA comparada con la clasificación previa de Banff´09.

Principalmente hallazgos indirectos, como C4d o la inflamación microvascular (recientemente incluida), se emplean como marcadores de RMA. En estudios iniciales, se reportó la correlación entre el depósito de C4d y la detección de DSA en el diagnóstico de RMA agudo (132). Nuestro estudio confirma que, independientemente de los criterios utilizados para evaluar la tinción de

C4d, Banff´09 o Banff´13, no existe asociación significativa entre este marcador histológico aislado y la detección de DSA. Sin embargo, sí encontramos una asociación entre la presencia de DSA y la evidencia histológica recientemente aprobada como interacción del anticuerpo con el endotelio.

Considerando estos hallazgos histológicos diagnósticos de RMA, creemos que la nueva clasificación de Banff´13 es más concluyente en comparación con Banff´09. En este sentido, 8 biopsias con inflamación microvascular que fueron inicialmente clasificadas como sospechosas de RMA, se reclasificaron con un diagnóstico concluyente de RMA. Además, otras 7 que se clasificaron con un diagnóstico diferente a RMA con Banff´09 se incluyeron en la categoría 2 con la actual clasificación por la presencia de inflamación microvascular.

Uno de los potenciales problemas de la clasificación de Banff´13 es el peso que se da a la presencia de inflamación microvascular, así las biopsias cuyo único hallazgo sea la inflamación microvascular serán clasificadas como sospechosas de RMA activo. En nuestro estudio, 7 biopsias presentan ese tipo de inflamación sin DSA ni otros hallazgos sugestivos de RMA crónico. Por tanto, hay que considerar la posibilidad de que exista inflamación aislada sin que sea signo de RMA, en particular la capilaritis peritubular, que puede estar presente en otras entidades como el daño agudo tubular severo, la pielonefritis crónica o la nefritis intersticial. (185)

En comparación con los antiguos criterios utilizados para la clasificación de la glomerulitis según Banff´09, la nueva definición para clasificar la glomerulitis mostró una asociación marginal con la tinción de C4d en capilar peritubular y una asociación débil con la detección de DSA. Esta discrepancia debería evaluarse con un número mayor de biopsias clasificadas como g1 con los antiguos criterios y como g0 con los actuales. La evaluación de esta forma más restrictiva de evaluar la glomerulitis según Banff´13 es relevante dado que hay recientes estudios que relacionan la presencia de glomerulitis con una peor supervivencia del injerto. (186). Según nuestros hallazgos, 9 biopsias con g1 fueron re-categorizadas a g0, lo que supuso que una muestra cambiara de categoría diagnóstica al aplicar los nuevos criterios.

Un cambio importante que se propone en la nueva clasificación de Banff´13 es considerar como único criterio de cronicidad la presencia de CGT y la multilaminación de la membrana basal en capilares peritubulares, omitiendo el porcentaje de glomeruloesclerosis y la FI/AT. De hecho, 5 biopsias perdieron la categoría diagnóstica 2 por este motivo.

Otra novedad de la actual clasificación de Banff´13 es considerar como criterio la presencia de GCT ultraestructural detectada solo por microscopía electrónica. Realizamos estudio ultraestructural de todas las muestras incluidas en el estudio, de ellas, 9 presentaban CGT y 20 multilaminación. Se ha descrito que la presencia de cgt1a en biopsias de protocolo en los 3

primeros meses postrasplante se correlaciona con el posterior desarrollo de GCT establecida (161). En nuestro estudio, los pacientes con cgt1a presentan más proteinuria, incluso cuando esta era leve, comparada con los pacientes que no presentaban CGT, lo que indirectamente puede hacer predecir un potencial peor pronóstico del injerto renal. Un estudio reciente que analizó el impacto clínico de ambas clasificaciones de Banff (2007 vs 2013) en pacientes con cambios humorales crónicos en sus biopsias, demostró que los pacientes con RMA que tenían DSA, C4d y CTG o multilaminación, pero no el nuevo criterio de inflamación microvascular, presentan peores resultados clínicos (supervivencia del injerto o aumento del 50% de la creatinina) (187)

Debido al corto seguimiento y al limitado número de pacientes incluidos no podemos realizar estudios de supervivencia para analizar el impacto de ambas clasificaciones en la supervivencia del injerto a medio/largo plazo. Por tanto, serán necesarios estudios clínicos para analizar este el impacto de ambas clasificaciones en resultados a largo plazo.

Para tratar de evaluar el valor diagnóstico y pronostico, comparamos los pacientes que se re-clasificaron a RMA según Banff´13´pero sin rechazo con la antigua clasificación de Banff´09 con aquellos que cumplían criterios de rechazo en ambas clasificaciones, estos tenían una peor función renal tanto en el momento de la biopsias como al final del seguimiento. Así, el 23.5% de las biopsias no cumplían criterios de RMA según la antigua clasificación de Banff´09, lo que las excluye de un potencial tratamiento adecuado.

Una de las limitaciones de nuestro estudio fue un número limitado de biopsias y un corto seguimiento de los pacientes, que impide realizar estudios de impacto a largo plazo.

Aplicando la nueva clasificación de Banff´13, el 18% de las biopsias fueron reclasificadas en una categoría diagnóstica diferente. Considerando nuestros resultados, creemos que la actual clasificación propuesta por Banff es más precisa para el diagnóstico de RMA, debido a:

1.- La inclusión de la inflamación microvascular como criterio histológico de interacción del anticuerpo con el endotelio.
2.- La consideración de DSA en algún momento del seguimiento potrasplante si hay datos de RMA crónico.
3.- La incorporación de criterios ultraestructurales como hallazgos histológicos de RMA.

CONCLUSIONES

5. CONCLUSIONES

El avance en el conocimiento del rechazo mediado por anticuerpos a través de una caracterización actualizada serológica e histológica es imprescindible para poder diseñar estrategias terapéuticas en el futuro.

1.- Los DSA preformados detectados exclusivamente en plataforma Luminex® confieren más riesgo de rechazo mediado por anticuerpos y peor supervivencia del injerto renal, especialmente en los pacientes con DSA preformados clase I o I-II por su asociación con el rechazo humoral precoz que conlleva a pérdida del injerto. Este riesgo incrementado persiste aunque los DSA preformados no se detecten durante el seguimiento postrasplante, de manera que los tratamientos que reducen los DSA pueden modificar el pronóstico pero no resuelven el rechazo mediado por anticuerpos.

2.- En el 10% de los pacientes trasplantados se detectan anticuerpos anti-HLA DP mediante ensayos en fase sólida, tanto pre como postrasplante. La presencia de anticuerpos anti-HLA DP no parece modificar el impacto negativo en supervivencia del injerto de los DSA pre y postrasplante.

3.- El 20% de las biopsias por indicación corresponden a la categoría de rechazo mediado por anticuerpos según los criterios de la clasificación de Banff 2013. Los pacientes con cambios de rechazo humoral en la biopsia presentan mayor riesgo de pérdida de injerto que el resto de las categorías diagnósticas de Banff 2013, de forma que es necesario desarrollar estrategias terapéuticas adecuadas en este campo.

4.- El cambio de criterios histológicos entre la clasificación de Banff 2009 y Banff 2013 supone la reclasificación del 18% de las biopsias de trasplante renal en una categoría diagnóstica, afectando de forma preferente a aquellas con diagnóstico de rechazo mediado por anticuerpos. La clasificación de Banff 2013 es más precisa para el diagnóstico de rechazo mediado por anticuerpos que la clasificación previa de Banff 2009.

PERSPECTIVAS DE FUTURO

6. PERSPECTIVAS DE FUTURO

Nuestras líneas de investigación futuras están encaminadas a evaluar la respuesta celular y humoral combinada HLA y no HLA en una amplia cohorte de trasplantados renales para delimitar el papel de anticuerpos y células en el rechazo mediado por anticuerpos y su valor como biomarcadores.

Para ello, y con objeto de evaluar la incidencia y valor pronóstico de la detección de anticuerpos HLA y anticuerpos no HLA, continuaremos con el estudio prospectivo de la población trasplantada en nuestro centro desde 2006 incorporando los nuevos trasplante renal con muestras de suero desde julio-2006 y células en sangre periférica de forma seriada y biopsias de seguimiento. En los casos con anticuerpos donante específicos HLA se evaluará la calidad de esos anticuerpos (tipo, concentración y capacidad de fijar complemento).

Además, estudiaremos algunos potenciales mecanismos de lesión en el rechazo mediado por anticuerpos, que podrían responder a estrategias terapéuticas diferentes:

1) *Estudio del papel del complemento*
2) *Polimorfismos de receptores Fc de monocitos-macrófagos y células NK*
3) *Activación de vías intracelulares en tejido renal trasplantado*

Para realizar estos diferentes estudios compararemos casos de pacientes con rechazo mediado por anticuerpos con controles; además de estratificar según diferentes estrategias de inmunosupresión

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