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The Spectrum of Histopathological Changes in the Renal Allograft - a 12 Months Protocol Biopsy Study

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Abstract

INTRODUCTION: Renal transplantation became a routine and successful medical treatment for Chronic Kidney Disease in the last 30 years all over the world. Introduction of Luminex based Single Antigen Beads (SAB) and recent BANFF consensus of histopathological phenotypes of different forms of rejection enables more precise diagnosis and changes the therapeutic approach. The graft biopsies, protocol or cause, indicated, remain a golden diagnostic tool for clinical follow up of kidney transplant recipients (KTR).

AIM: The study aimed to analyse the histopathological changes in renal grafts 12 months after the surgery in KTR with satisfactory kidney function.

MATERIAL AND METHODS: A 12-month protocol biopsy study was performed in a cohort of 50 Kidney transplant recipients (42 from living and 8 from deceased donors). Usual work-up for suitable donors and recipients, standard surgical procedure, basic principles of peri and postoperative care and follow up were done in all KTR. Sequential quadruple immunosuppression including induction with Anti-thymocyte globulin (ATG) or Interleukin-2R antagonist (IL-2R), and triple drug maintenance therapy with Calcineurin Inhibitors (CNI), Mycophenolate Mofetil (MMF) and Steroids were prescribed to all pts. Different forms of Glomerulonephritis (16), Hypertension (10), End Stage Renal Disease (13), Hereditary Nephropathies (6), Diabetes (3) and Vesicoureteral Reflux (2) were the underlying diseases. All biopsies were performed under ultrasound guidance. The 16 gauge needles with automated "gun" were used to take 2 cores of tissue. The samples were stained with HE, PAS, Trichrome Masson and Silver and reviewed by the same pathologist. A revised and uploaded BANFF 2013 classification in 6 categories (Cat) was used.

RESULTS: Out of 48 biopsies, 15 (31%) were considered as normal, 4 (8%), Borderline (BL-Cat 3), 5 (10%) as Interstitial Fibrosis/Tubular Atrophy (IF/TA-Cat 5), 5 (10%) were classified as non-immunological (Cat 6), 2 as a pure antibody-mediated rejection (ABMR-Cat 2) and T-cell Mediated Rejection (TCMR-Cat 4). The remaining 17 samples were classified as a "mixed" rejection: 7 (41%) ABMR + IF/TA, 5 (29%) ABMR + BL + IF/TA, 2 (11%) BL + IF/TA, 1 (5%) ABMR + BL, 1 (5%) ABMR + TCMR and 1 (5%) TCMR + IF/TA. The mean serum creatinine at the time of the biopsy was 126.7 ± 23.4 μmol/L, while GFR-MDRD 63.4 ± 20.7 ml/min, which means that the majority of the findings were subclinical. Among the non-immunological histological findings (Cat 6), 3 cases belonged to CNI toxicity, 1 to BK nephropathy and 1 to recurrence of the primary disease.

CONCLUSION: Our 12-month protocol biopsy study revealed the presence of different forms of mixed subclinical rejection. Use of recent BANFF classification and scoring system enables more precise diagnosis and subsequently different approach to the further treatment of the KTR. More correlative long-term studies including Anti HLA antibodies and Endothelial Cell Activation- Associated Transcripts (ENDAT) are needed.

Introduction

Kidney transplantation is an incredible success of modern medicine. The better understanding of the basic immunological mechanism and the introduction of some new molecules in everyday practice enables improved long-term graft and patient's survival, better quality of life and practically uneventful clinical course for many years [1] [2] [3] [4]. According to the recent data, 20 years graft survival could be expected in 60% of kidney transplant recipients [5] [6]. However, there are still unsolved problems and questions that remain to be investigated from the scientific and practical point of view. One of the major causes of graft loss is still a rejection, either cellular or humoral. It has been accepted today that approximately 60% of the long-term graft loss belongs to the acute or chronic antibody-mediated rejection (ABMR) [7] [8] [9] [10]

The introduction of some new diagnostic tools such as Luminex based Single Antigen Beads (SAB), enables to detect a huge amount of anti HLA antibodies, non HLA antibodies and some fraction of activated complement system. The presence of anti HLA antibodies, especially Donor Specific Antibodies (DSA) in the patient's sera leads to chronic allograft nephropathy and long-term graft loss [11] [12] [13].

Thus, overcoming kidney allograft rejection could have a beneficial effect on long-term graft survival. However, other important pathological features rated as CNI toxicity, BK nephropathy, and recurrence of the primary kidney disease have also a substantial impact on the graft survival rate [14]. Despite modern diagnostic procedures implemented in everyday clinical practice, the kidney allograft biopsy remains a gold standard to determine the cause of graft dysfunction. Biopsy findings change the clinical diagnosis in an average of 36% of patients (range 24-76) and immunosuppressive therapy in 59% [15] [16]. But, the allograft biopsy does not contribute only to clinical diagnosis. It could also be used as a prognostic marker and guide to individual therapeutic approach to different patients [17] [18]. The development of so-called Banff scoring system in the last 25 years enables a much better understanding what is happening inside of the grafts [19] [20]. Introduction of the protocol biopsies gave very useful information about relevant histopathological changes in patients without any clinical evidence of graft dysfunction. They created practically new pathological entity named "subclinical acute or chronic rejection" which was very important regarding possible treatment and further clinical course of transplant recipients [21] [22] [23]. It is true that in the new era of potent immunosuppression therapy, the frequency of acute cellular or antibody-mediated rejection falls between 8-12 % and, therefore, the use protocol biopsies became a little bit controversial, but they are still very useful regarding treatment changes or individual approach to different patient circumstances. In any case, either protocol or clinically indicated, allograft biopsy is a condition sine qua non for modern clinical follow up of any organ transplant patient [24] [25] [26].

The study aimed to analyse the histopathological changes in renal grafts 12 months after the surgery in KTR with satisfactory kidney function.

Material and Methods

Forty-eight successful biopsied patients with haploidentical living (40) and 8 deceased donor transplantation were included in a 12-month prospective study. Renal transplantation was performed at the University Clinical Centre Mother Teresa-Skopje, Republic of Macedonia, by the well-known principles from the surgical and nephrological aspect. Hypertension, Glomerulonephritis, Hereditary nephropathies and End Stage Renal Disease (ESRD) were predominant underlying kidney diseases. Standard pre-transplant workup was done to all potential donors and recipients.

Table 1: Updated 2013 Banff classification categories

1. Normal
2. Antibody-mediated
Acute/active ABMR; all three features must be present for a diagnosis ***1. Histologic evidence of acute tissue injury, including one or more of the following:*** • Microvascular inflammation (g > 0 and/or ptc > 0) Intimal or transmural arteritis (v > 0) • Acute thrombotic microangiopathy, in the absence of any other cause ***2. Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following:*** • Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections) • At least moderate microvascular inflammation ([g +ptc] > 2) • Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury if thoroughly validated ***3. Serologic evidence of donor-specific antibodies (DSAs) (HLA or other antigens*)** **Chronic, active ABMR; all three features must be present for a diagnosis** ***1. Morphologic evidence of chronic tissue injury, including one or more of the following:*** • Transplant glomerulopathy (TG) (e.g.> 0), if no evidence of chronic thrombotic microangiopathy Severe peritubular capillary basement membrane multilayering (requires EM) • Arterial intimal fibrosis of new onset, excluding other causes ***2. Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following:*** • Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections) • At least moderate microvascular inflammation ([g +ptc] > 2) • Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated ***3. Serologic evidence of DSAs (HLA or other antigens)*** **C4d staining without evidence of rejection; all three features must be present for a diagnosis** 1. Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections) 2. g = 0, ptc = 0, eg = 0 (by light microscopy and by EM if available), v = 0; no TMA, no peritubular capillary basement membrane multilayering, no acute tubular injury (in the absence of another apparent cause for this) 3. No acute cell-mediated rejection (Banff 97 type 1A or greater) or borderline changes
3. Borderline changes: 'Suspicious' for acute T-cell mediated rejection (may coincide with categories 2 and 5, and 6)
This category is used when no intimal arteritis is present, but there are foci of tubulitis (t1, t2, or t3) with minor interstitial infiltration (i0, or i1) or interstitial infiltration (i2, i3) with mild (t1) tubulitis
4. T cell-mediated rejection (TCMR, may coincide with categories 2 and 5 and 6) **Acute T-cell mediated rejection (Type/Grade:)** **I A**. Cases with significant interstitial infiltration (>25% of parenchyma affected, i2 or i3) and foci of moderate tubulitis (t2) **I B.** Cases with significant interstitial infiltration (>25% of parenchyma affected, i2 or i3) and foci of severe tubulitis (t3) **II A**. Cases with mild to moderate intimal arteritis (v1) **II B**. Cases with severe intimal arteritis comprising >25% of the luminal area (v2) **III**. Cases with 'transmural' arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells with accompanying lymphocytic inflammation (v3) **Chronic active T-cell mediated rejection** 'chronic allograft arteriopathy' (arterial intimal fibrosis with mononuclear cell infiltration in fibrosis, the formation of neo-intima)
5. Interstitial fibrosis and tubular atrophy, no evidence of any specific aetiology
(may include nonspecific vascular and glomerular sclerosis, but severity graded by tubulointerstitial features) **I**. Mild interstitial fibrosis and tubular atrophy (>25% of cortical area) **II**. Moderate interstitial fibrosis and tubular atrophy (26-50% of cortical area) **III**. Severe interstitial fibrosis and tubular atrophy/loss (>50% of cortical area)
6. Other: Changes not considered to be due to rejection-acute and/or chronic

Cg, Banff chronic glomerulopathy score; EM, electron microscopy; ENDAT, endothelial activation and injury transcript; g, Banff glomerulitis score; GBM, glomerular basement membrane; IF, immunofluorescence; IHC, immunohistochemistry; ptc, peritubular capillary; TCMR. T cell-mediated rejection; v. Banff arteritis score.

According to the centre policy, 50% was a minimum accepted HLA matching in both, living and deceased donor transplantation. A sequential quadruple Immunosuppression including ATG or IL-2R antagonist induction and Prednisolone, MMF and CNI as a triple-drug maintenance therapy was

introduced to all recipients. After the surgery, the patients were followed by the same team according to the KDIGO recommendations. Usual Lab analyses, proteinuria, GFR, trough immunosuppressant levels, graft ultrasound tomography including Doppler were done practically every month on the outpatient basis.

Table 2: Pathological features and Banff score

Feature	Banff term	Banff Score			
		0	1	2	3
Interstitial inflammation (% of nonfibrotic cortex)	i	<10%	10–25%	26–50%	>50%
Total inflammation (% all cortex)	ti	<10%	10–25%	26–50%	>50%
Tubulitis (maximum mononuclear cells/tubule)	t	0	1–4	5–10	>10
Arterial inflammation (% lumen endarteritis)	V	None	<25%	>25%	Transmural or necrosis
Glomerulitis (% glomeruli involved)	g	None	<25%	26–50%	>50%
Capillaritis (cells per cortical PTC, requires >10% of PTC to be affected for scoring)	ptc	<10%	<5/PTC	5–10/PTC	>10/PTC
C4d deposition in PTC (% positive)	C4d	0%	I–9%	10–50%	>50%
Interstitial fibrosis (% of cortex)	ci	<5%	6–25%	26–50%	>50%
Tubular atrophy (% cortex)	ct	0%	<25%	26–50%	>50%
Arterial intimal thickening (% narrowing lumen of most severely affected glomerulus)	cv	0%	<25%	26–50%	>50%
Transplant glomerulopathy (% of capillaries with duplication in most severely affected glomerulus)	cg	0%	<25%	26–50%	>50%
Arteriolar hyalinosis (number with focal or circumferential hyaline)	ah	None	1 focal	>1 focal	1 circumferential >50%
Mesangial matrix increase (% affected glomeruli)	mm	0%	<25%	26–50%	>50%

A total of 48 biopsies (one for each patient included in the study) were done exactly on the 12th month after the transplantation. All the biopsies were performed under ultrasound guidance 16 gauge needle was used with an automated “gun”. Samples routinely comprised 2 cores to get a sufficient amount of glomeruli. The formalin-fixed biopsies were embedded in paraffin, serially sectioned at 3 and 5 μm thickness and stained with hematoxylin-eosin (HE), Periodic Acid-Schiff (PAS), Masson trichrome as well as methenamine silver. Biopsies were considered adequate when they contained ≥ 7 glomeruli and at least one artery.

Renal lesions were reviewed for evidence of chronic and acute changes by the same pathologist using descriptive criteria according to the Banff 2013 scoring schema using a scale from 0-3. At the same time, a frozen section sample was used for Complement (C3) immunofluorescence microscopy. C4d immunohistochemistry was performed on 3 μm thick paraffin sections using “Novolink” Polymer detection system with rabbit anti-human C4d monoclonal antibody [27] [28].

Histological findings were classified into six categories according to BANFF 13 modified and uploaded system: Normal (category 1), Antibody-mediated rejection (ABMR category 2), Borderline (BL -category 3), T – cell-mediated rejection (TCMR – category 4), Interstitial fibrosis and tubular atrophy (IF/TA-category 5) and other non-immunological changes (category 6). The BANFF scoring system (from 0-3) was used for the grading of acute and chronic changes occurring in the interstitium, tubules, glomeruli, arteries and arterioles. For diagnosis of ABMR, a revised BANFF 13 criteria which include “C4d negative ABMR” were used [29] [30], (Table 1 and 2). The research was performed following the tenets of the Declaration of Helsinki. Informed consent was obtained from the patients for the protocol biopsies. The research was approved by the Ethical Committee of the Medical Faculty Skopje.

Descriptive statistic was used, frequencies and percentages for categorical data; average values and standard deviation for continuous data.

Results

The demographic and clinical characteristics are present in Table 3.

Table 3: Clinical and demographic data

Data	Number
Age	34.5 ± 11.7
Gender W/M	14/37
Underlying disease	
Glomerulonephritis	16
Hereditary Nephropathy	6
Hypertension	10
Diabetes	3
VUR	2
ESRD	13
HLA Missmatch	3.1± 0.4
Living/deceased donors	40/8
CIT Living/ deceased donor	3.7 ± 0.3/ 10.4 ± 4.2
Induction therapy-Sim/ATG	19/31
Maintenance immunosuppression	
CNI-Cyclosporin/ Tacrolimus	19/31
MMF / Steroids	50/50
Rejection (clinical)	6 (11%)
Serum creatinine (12 month)	126.7 ± 23.4
GFR-MDRD (12 month)	63.4 ± 20.7

VUR – Vesico-ureteral Reflux, ESRD-End Stage Renal Disease, CIT-Cold ischemia time, CNI-Calcineurin Inhibitors, MMF-Mycophenolat Acid, Sim-Simulect, ATG – Anti -thimocyte Globulin.

A total of 50 biopsies in 50 patients were performed. Forty-eight were successful and available for analysis (> 8 glomeruli). The histopathological findings of the 12th month's protocol biopsies were categorised according to the updated Banff 13 scoring system. (Table 4). We noticed that only 15 (31%) cases belong to the category 1 which means normal biopsy. On the other hand, respecting the Banff 2013 criteria strictly, only 1 as sample could be determined as a pure ABMR and one pure TCMR which represents 4% of the cases. Five samples were classified as IF/TA and other 5 to “others”, which mean non-immunological histological changes including CNI nephrotoxicity, BK nephropathy or recurrence of the primary disease.

Table 4: Categorization of biopsies according to the updated Banff 2013 scoring system (n = 48)

Banff diagnostic category	Number of cases	Percentage
Normal (Category 1)	15	31
Pure ABMR (Category 2)	1	2
Borderline T-cell rejection (Category 3)	4	8
T-cell mediated rejection (Category 4)	1	2
IF/TA (Category 5)	5	10
Other (Category 6)	5	10
Mixed	17	35

The crucial point of every kidney allograft biopsy, protocol or indicated, is the issue of rejection, whether cellular or humoral. For TCMR (Cat 4) and TCMR borderline rejection (Cat 3) we used Banff scoring for interstitial infiltration (i), tubulitis (t) and arterial inflammation (v) whereas for ABMR glomerulitis (g), transplant glomerulopathy (cg), peritubular capillaritis (ptc), arterial inflammation (v), tubulitis (t) and positive C4d [31] [32] [33].

Table 5: Analysis of "mixed" category (n = 17)

Category	Number of cases	Percentage %
ABMR (Cat 2) + IF/TA (Cat 5)	7	41
ABMR(Cat 2) + BL (Cat 3)	1	5
ABMR (Cat 2) + BL (Cat 3) + IF/TA (Cat 5)	5	29
BL (Cat 3) + IF/TA (Cat 5)	2	11
ABMR (Cat 2) + TCMR (Cat 4)	1	5
TCMR (Cat 4) + IF/TA	1	5

The rest of kidney biopsies belonged to the category of so-called "mixed" rejection which is presented in Table 5 and Figure 1.

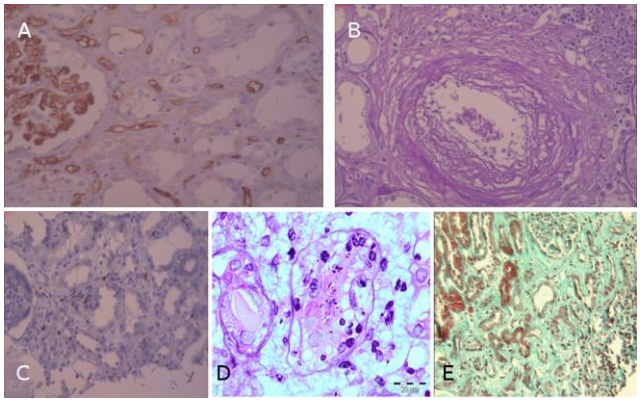

Figure 1: A: C4d positive immunostaining in peritubular capillaries in acute humoral rejection; B: Chronic allograft vasculopathy (arterial blood vessel with fibrointimal thickening); C: CD3 immunostaining for T cell in acute cellular mild rejection. (brown - T lymphocytes); D: Acute cellular rejection – tubulointerstitial – grade 1a: (mild interstitial infiltration and focuses of mild tubulitis > 4 cells cross tubular section/); E: Trichrome Masson histochemical staining: IF/TA (Interstitial fibrosis and tubular atrophy)

Discussion

ABMR

According to literature, the frequencies of ABMR in biopsies 12 months after transplantation differed between patients with/without HLA-DSA (0.2% vs 12%). Our results were closer to the protocol biopsies in patients without HLA-DSA which is 2.2%. Based on the recent BANFF data and a broad spectrum of different opinions about the humoral rejection, we used so-called "expanded criteria" for diagnosis or even "suspicious" for ABMR. It was the reason why we had too much of Category 2 (it means ABMR) in our analysis presented in Table 5. Certainly part of the criteria was also detectable DSA, non-DSA or Non -HLA antibodies. Regarding Cd4 and grading scores, we accept that presence of C4d was not anymore one of the criteria for ABMR. After many controversial findings regarding C4d, a revised Banff 2013 classification included the category of "C4d negative ABMR" [29] [30]. Theoretically, it means that there are alternative non-complement depending reactions of HLA antibodies and graft endothelium. The story of ABMR becomes complicated and this was shown by the fact that some typical histopathological ABMR changes may be seen even if HLA and non HLA antibodies exist in the examined serum samples. In the Banff 2013 scoring system the term borderline or "suspicious" was accepted in the criteria for ABMR. It means that suspicious ABMR may even exist if only C4d staining is present without positive anti HLA antibodies and any other typical changes for definitive ABMR [30]. Additionally, any simple presence of vascular inflammation could be sufficient for the diagnosis of ABMR. Despite the different phenotypes, we unified ABMR as one category including "suspicious", acute/active or chronic/active forms which facilitated further analysis and conclusions [26] [28].

TCMR and Borderline Rejection

The percentage of pure TCMR was less than those in the literature 2% vs 8.2% respectively, but if the number is corrected for the numbers of "borderline" changes and TCMR in a mixed group, the percentage was closer to previous reported. The finding of TCMR which was present in different forms and phenotypes in 13 of the biopsies was a certainly interesting issue: one as a pure TCMR, 4 as a Borderline TCMR, 5 as a Borderline together with ABMR and IF/TA, and 3 with IF/TA. According to the previous Banff session, any classified "borderline" changes" should be understood as a rejection and should be treated appropriately, especially if there is an increase of serum creatinine [29] [30] [42]. Moreover, there is evidence that TCMR or borderline TCMR can provoke real ABMR and shorten the graft survival rate. The presence of some ABMR pattern together with TCMR or 'Borderline" TCMR, also deserves attention. Is it evidence that ABMR is already activated by TCMR, or it is simply two separated parallel findings? We are more inclined to the conclusion that, if there is no anti HLA antibodies and any C4d activity, it could be evident that TCMR had already been initiated ABMR [15] [22] [28].

Mixed Rejection

While analysing the findings and classification presented in Table 5, we can conclude that in 35% of the biopsies there was a broad spectrum of histopathological changes which usually does not correspond with the clinical picture. Wehmeier et al., in their study, reported the percentage of mixed rejection in biopsies from 2.6% in patients without DSA and 14% in patients with DSA, up to 22% in biopsies by indication in DSA patients. Mixed rejection means that both TCMR and ABMR with their different

sub -phenotypes are equally present in the graft biopsy. Potentially it could be quite possible that some interactions among them are happening all the time. Anyway, we don't know who came first in the battle, but it is quite frequent especially in the protocol biopsies. According to the very dynamic issue of BANFF meetings and changes which usually come every other year, we strongly believe that the conclusions are still very far from the real clinical use. Bearing in mind that our study is protocol biopsy based, most of our cases belong to the entity of "subclinical" rejection, or simply, subclinical important histopathological changes [22] [28] [34] [35]. It is obvious that there are many different pathohistological patterns which belong to well-defined forms of rejection as ABMR and TCMR but also, at the same time, to TCMR-borderline or ABMR "suspicious" phenotypes. The recent Banff 2013 recognised so-called "Chronic active TCMR" which is different than the usual acute TCMR with features of chronic allograft arteriopathy [30]. Some of the histological changes are very similar to ABMR. Thus, it is not easy to define "mixed" rejection as an entity not only from diagnosis but much more from the point of further treatment. Hence, the clinical decision "what to do" if there are not sufficient data for definitive diagnosis, could be a very complicated issue. Whether these changes could have a negative impact on the long-term clinical outcome or not, it remains to be seen after several years.

IF/TA

In Table 4 and 5, we noted that interstitial fibrosis and tubular atrophy is more frequent finding compared with others studies. Regarding the severity of the IF/TA pathohistological changes in 4 biopsies, they were present in more than 50% of active cortical tissue, in 13 between 25-50% and only one less than 25%, which is close to normal. As a pure form, IFTA is confirmed in 5 cases, but it was present much more in so-called mixed rejection, usually with ABMR (7 cases), Borderline and ABMR (5 cases), Borderline and TCMR (2 cases). IF/TA, especially without histological signs of rejection is considered as a chronic process [18] [28] [36]. But, the fact that IF/TA is the most frequent finding in our 12 months protocol biopsy study means that probably it is part of a permanent process of rejection, either humoral or cellular. The recent gene expression studies confirmed that even without histological evidence of inflammation IF/TA showed a molecular profile of immune-mediated inflammation [37] [38]. In our study, we noticed that among the cases in group "mixed", 7 (41%) belong to ABMR (Cat 2) and IF/TA (Cat 5). Therefore, the finding of any signs of microvascular inflammation, and/or active glomerular lesions together with IF/TA mean that ABMR was directly involved in the interstitial fibrosis. Equally important was a possible role of TCMR and Borderline changes together with IF/TA [39] [40]. Therefore, interstitial fibrosis may be part of broad immunological events, especially if DSA or Non-DSA are detectable in the patients' sera. However, the presence of inflammation in IF/TA became an unresolved issue even among the experts of the Banff group [15] [18] [41].

Others

In the final 6th group of biopsies, CNI toxicity was a predominant finding, usually as an arterial hyalinosis grade 2 or 3. According to the recent discussion about the nature of chronic CNI toxicity in the era of antibody-mediated rejection, it seems that we should strictly divide what CNI toxicity is and what ABMR is on the other hand [14]. Especially if the anti HLA antibodies are present in the patient's sera. Therefore, the chronic CNI toxicity could be simply replaced by some of the ABMR phenotypes shortly [14]. Until the consensus of that issue is reached, we remain on the actual CNI toxicity pattern among our biopsies despite the fact that we did not observe any potentially toxic CNI level during the 12 months follow up. In the last two biopsies of this group, a BK nephropathy and FSGS recurrence were diagnosed which was also clinically confirmed.

ENDAT

After all that was presented in our analysis, it is evident that additional tests are required to increase the prognostic power of pathohistological assessment in renal transplant patients. In the last two Banff reports, 2013 and 2015, the criteria of "increased expression of gene transcripts indicative of endothelial injury, if thoroughly validated" was included in the whole complicated picture of ABMR. "Thoroughly validated" practically means that it should be confirmed only in a single centre (University of Alberta). Searching for new molecular markers for active endothelial injuries the term "ENDAT" (Endothelial Cell Activation-Associated Transcripts) was broadly introduced [30]. Despite the primary confusion in the definition of rejection as a whole process, the recent data fully justified gene transcripts as a relevant diagnostic tool which should facilitate the diagnosis and clinical use of the biopsy as a golden standard for kidney graft recipients [37] [39].

In conclusion, histologic assessment of kidney transplant recipients by use of 12 months protocol biopsy revealed a huge amount of different histopathological phenotypes and sub-phenotypes of ABMR, TCMR and mixed rejection. Most of them were clinically silent which was very important for further treatment and follow up of kidney transplant recipients. Therefore, 12 months protocol biopsies together with a strict follow up of anti HLA antibodies and clinical picture of kidney transplant recipients is necessary for a successful and long-term graft and patients survival.

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