Classification of Acute Rejection Episodes in Kidney Transplantation

A Proposal Based on Factor Analysis

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Introduction. Kidney transplantation is considered the ideal treatment for end-stage renal disease. Acute rejection can influence graft survival. The aim of this study was to propose a classification system for acute rejection based on factor analysis.
Materials and Methods. Data were collected from kidney transplant recipients with acute rejection diagnosis based on standard histological variables, the presence of peritubular eosinophils, and immunolabeling for lysozyme and myeloperoxidase in kidney tissue. Factor analysis was employed for data reduction and generation of a new case classification, with orthogonal rotation as a strategy to simplify factors, and principal component analysis was used as an extraction method.
Results. Seventy-nine kidney biopsies were obtained from 74 patients. The total population was divided into humoral rejection (39.2%), cellular rejection (34.1%), and mixed acute rejection (26.7%). No significant differences were found between the three groups in clinical and biochemical variables. We extracted 4 factors using factor analysis. The 1st factor was characterized by the presence of capillaritis, plasma cells infiltration, tubulitis, and inflammation. The 2nd factor included positivity for lysozyme and myeloperoxidase, while the 3rd factor included the presence of eosinophils and glomerulitis. The 4th component consisted of the presence of C4d and endarteritis. The cases belonging to the 3rd factor showed the greatest increase in serum creatinine. The cases belonging to the 4th factor exhibited greater urinary excretion of proteins.
Conclusions. This proposal of classification of acute rejection could contribute to evaluate the prognosis of kidney transplant recipients.

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INTRODUCTION

Kidney transplantation is considered the ideal treatment for end-stage renal disease. Nevertheless, one of the factors that has limited graft survival is rejection, which is mediated by different mechanisms and has usually been considered either cellular or humoral. Even though both types of rejection have been defined by serological and histological characteristics,[1] it is common to find an overlap of acute cellular rejection (ACR) and acute humoral rejection (AHR) findings in the same biopsy specimen.[2,3]

This has sparked the search for other markers that allow a better differentiation of both types of rejection. Such markers have included both the presence of peritubular eosinophils and

the immunolabeling of lysozyme (LYS) and myeloperoxidase (MPO) in neutrophils. It has been described that LYS can be taken up by the kidney tubules, where this may lead to the production of inflammatory mediators that could contribute to acute kidney injury during acute allograft rejection. Also, LYS has been found in myelocyte/macrophage cells within capillary loops and arterial walls, when acute necrotizing vasculitis is present. In lung transplantation, MPO was found to be significantly elevated in patients with obliterative bronchiolitis compared with patients without obliterative bronchiolitis. The positivity of these markers has been associated with a shorter graft survival, more severe acute rejection (AR) events, and a poor treatment response.[4-11]

Considering that the different histological variables that define a case of ACR or a case of AHR are frequently present to a greater or lesser extent in both scenarios, the aim of this work was to try to find new axes of classification of cases of AR with the use of factor analysis, also adding to the analysis histological variables that are not commonly used to classify a case of AR, such as the eosinophil count and the labeling for LYS and MPO.

MATERIALS AND METHODS

We included kidney transplant recipients of living related donors, living unrelated donors, and deceased donors from the Nephrology Department of the National Institute of Cardiology. These patients had undergone percutaneous kidney allograft biopsy due to acute kidney function deterioration and had a histological diagnosis of AR based on the Banff 13 criteria, in the period between January 2011 and December 2013. All of the patients received basiliximab-based induction therapy and maintenance therapy with tacrolimus, mycophenolate mofetil, and prednisone.

Samples of kidney tissue, fixed in 10% formaldehyde and embedded in paraffin, were analyzed. Biopsies were stained for microscopy with hematoxylin-eosin, periodic acid-Schiff, Masson trichrome, and silver methenamin, for glomerular, vascular, and tubulointerstitial lesion scoring, and for the peritubular eosinophil count. In addition, 2-µm sections were cut from each of the selected biopsies with tissue available in paraffin blocks to mark C4d with the indirect immunoperoxidase technique, 2 cuts for MPO and 2 more for LYS. In order to give added value to the presence of eosinophils in the kidney biopsy, the possibility of urosepsis was ruled out by means of a urine culture prior to kidney biopsy. To exclude hypersensitivity reactions, cases with a clinical picture suggestive of an allergic process were ruled out.

During the same time period, as a control group for immunolabeling, we included cases of kidney transplantation with time-zero biopsy; that is, a kidney allograft biopsy prior to its implantation in the recipient.

The analyzed histological variables included inflammatory infiltrate, peritubular capillaritis, eosinophil presence, tubulitis, endarteritis, plasma cells presence, and glomerulitis, as well as immunolabeling for C4d, LYS, and MPO, which were coded according to their extension in the biopsy specimen, using a semi-quantitative ordinal scale with values from zero to 3, where zero is the absence of the feature and 3 is its maximum extension. The degree of positivity of the cell infiltrate in the immunolabeling for LYS or MPO was established using the following scale, where degree zero is the absence of infiltrate, degree 1 is the presence of 1 to 4 neutrophils per high-power field, degree 2 corresponds to 5 to 8 neutrophils per high-power field, degree 3 is the presence of 9 to 12 neutrophils per high-power field, and degree 4 is more than 12 neutrophils per high-power field. C4d immunolabeling was evaluated based on the criteria of Banff 13 classification for indirect immunoperoxidase labeling as follows: C4d0, no positivity; C4d1, less than 10% of labeled capillaries; C4d2, 10% to 50%; and C4d3, greater than 50%.

According to the Banff 13 classification, a case of AHR was considered in the presence of one or more of the following findings: glomerulitis, endarteritis, peritubular capillaritis, C4d positivity or plasma cells presence infiltration, and a case of ACR in the presence predominantly of inflammatory infiltrate, tubulitis, or both. Cases of mixed acute rejection (MAR) were considered for those who presented findings of both AHR and ACR. We did not have donor-specific antibody (DSA) determinations; however, we considered that the histological evidence of microvascular inflammation (glomerulitis, endarteritis, and peritubular capillaritis) was enough to suggest

the diagnosis of AHR.

We obtained biochemical and demographic variables corresponding to the study period from each clinical file, mainly including age, etiology of kidney disease, donor type, months of posttransplant evolution, as well as serum creatinine and urinary protein excretion at baseline, at the time of the kidney allograft biopsy, and at the 1st and 2nd months of follow-up after graft biopsy.

Results were expressed as mean ± standard deviation, median with an interquartile range, or proportions, as appropriate. A Spearman correlation analysis was carried out between the histological variables analyzed. Mean comparisons between the groups were made by either a *t* test for independent groups or with a 1-way analysis of variance. The chi-square test was used for proportion comparisons.

For data reduction and a generation of a new case classification, we employed factor analysis with orthogonal rotation as a strategy to simplify factors, and principal component analysis was used as an extraction method. We first confirmed the utility of the data to this end, initially with the Kaiser-Meyer-Olkin test and later with the Bartlett test of sphericity. For the Kaiser-Meyer-Olkin test, values less than 0.5 were considered unacceptable for performing a factor analysis. In the case of Bartlett test, a significant result was expected, which would reject the null hypothesis that our data formed an identity matrix; this, in turn, would indicate a lack of relationship between the variables. Factor analysis is primarily used for data reduction or structure detection. The factor analysis procedure has several extraction methods for constructing a solution. The principal components method of extraction begins by finding a linear combination of variables (a component) that accounts for as much variation in the original variables as possible. It then finds another component that accounts for as much of the remaining variation as possible and is uncorrelated with the previous component, continuing in this way until there are as many components as original variables. Usually, a few components will account for most of the variation, and these components can be used to replace the original variables. The eigenvalue, or amount of variance in the original variables accounted for by each component; the percentage of variance, expressed as a percentage of the variance accounted for by each component to the total variance in all of the variables; and cumulative percentage of variance accounted for by the first n components were calculated.

A value of *P* less than .05 was considered significant. Analyses were done using the SPSS software (Statistical Package for the Social Sciences, version 22.0, IBM Corp, New York, NY, USA).

RESULTS

We obtained 79 kidney biopsies from a total of 74 patients, 34 women (43%) and 45 men (57%). The mean age was 30.9 ± 10.8 years (range, 17 to 61 years). The median of posttransplant evolution was 24.5 months (interquartile range, 11 to 60 months). According to the donor type, 56% of the cases came from a living related donor, 21% from a living unrelated donor, and 23% from a deceased donor.

The total population was divided into 3 groups based on the histopathological diagnosis: group 1 comprised of 31 AHR cases (39.2%), group 2 comprised of 27 ACR cases (34.1%), and group 3 comprised of 21 MAR cases (26.7%). Additionally, 8 time-zero biopsies were included as a control group for C4d, LYS, and MPO immunolabeling. Comparing the 3 groups, no significant differences were found in age, months of posttransplant evolution, serum creatinine, or urinary protein excretion at baseline. There were no significant differences either between groups in sex distribution, chronic kidney disease etiology, or type of donor. We observed a higher proportion of deceased donors in the AHR group and a higher proportion of living related donors in the ACR group (Table 1).

In the correlation analysis, the considered variables for AHR and ACR diagnosis showed a significant positive association. Furthermore, as illustrated in Table 2, variables considered as diagnostic for AHR, exhibited a significant positive correlation with variables considered as diagnostic criteria for ACR. This confirms the overlap of histological findings, which can be found in any AR category.

When we compared serum creatinine values and urinary protein excretion at the time of rejection diagnosis among the groups, as well as at the 1st and 2nd follow-up months, no significant differences were found. However, there was a tendency for a greater increase in both values in the AHR group.

Table 1. Baseline Characteristics of All Kidney Transplant Recipients and by Acute Rejection Group*

Characteristic	Kidney Transplant Recipients With Acute Rejection			
	All (n = 79)	Acute Humoral Rejection (n = 31)	Acute Cellular Rejection (n = 27)	Mixed Acute Rejection (n = 21)
Mean age, y	30.9 ± 10.8	30.2 ± 10.5	33 ± 11.8	29 ± 9.8
Sex, n				
Female	34	11	12	11
Male	45	20	15	10
Donor source, %				
Living related	55.0	47.0	63.6	56.3
Living unrelated	21.0	17.4	22.7	18.8
Deceased	23.0	34.8	13.6	25.0
Median time from transplant, mo	24.5 (11 to 60)	20 (12 to 53)	25 (13 to 64)	24 (4 to 70)
Mean serum creatinine, mg/dL	1.52 ± 0.70	1.58 ± 0.39	1.57 ± 1.00	1.36 ± 0.54

*Variables are expressed as mean ± standard deviation, median (interquartile range), frequency, or percentage as appropriate.

We did not identify significant differences when we compared between groups the intensity of the different histological variables analyzed. Also, considering the small sample size, it was not possible to perform a stratified analysis for each degree of severity of AR. It should be noted that the treatment for rejection was based mainly on pulses of methylprednisolone plus the addition of plasma exchange and rituximab, as appropriate.

Comparing the corresponding proportions for the presence of eosinophil presence, C4d, and neutrophil positivity for LYS and MPO between the groups, no significant differences were found. Regarding C4d, we found diffuse positivity in 70% in group 1 (AHR), 36.4% in group 2 (ACR), and 61% in group 3 (MAR). In the peritubular eosinophil counts, 61.3%, 59.3%, and 62% were identified for the cases of AHR, ACR, and MAR, respectively, with a mild to moderate intensity in all the three groups. For the neutrophil positivity for LYS, we found it in 96% of AHR cases, 83.3% for ACR cases, and 93.8% for MAR cases. A grade 1 to 2 (weak to moderate) staining was present in all the three groups. Finally, for MPO staining, positivity was found in 68% of AHR cases, 85% for ACR cases, and 75% for MAR cases, with a predominant slight to moderate staining.

Taking into account the high correlation between some of the histological variables analyzed, our objective was to identify a new classification structure through the identification of latent variables (also called factors or components) through the use of factor and principal component analyses. A value of 0.776 was obtained with the Kaiser-Meyer-Olkin test, which was considered adequate for the analysis. We also obtained a *P* value less than .001 for the Bartlett test; therefore, we confirmed that our data were suitable for factor analysis.

The variance explained by the initial solution, extracted components, and rotated components are displayed in Table 3. We requested that eigenvalues greater than 1 be extracted, so the first four principal components form the extracted solution. The second section of the table shows the extracted components. They explained more than 72% of the variability in the original 10 variables, so we could considerably reduce the complexity of the data set by using these components. The rotation maintained the cumulative percentage of variation explained by the extracted components, but that variation was spread more evenly over the components. The changes in the individual totals suggested that the rotated component matrix would be easier to interpret than the unrotated matrix. The rotated component matrix helped us to determine what the components represent.

It is noteworthy that the 1st (29.4%) and 2nd (17.9%) components accounted for most of the variance. Four components were extracted, since only 4 had eigenvalues greater than 1 and together explained 72.2% of the variance in cases of rejection. It should be noted that although both the initial solution (not rotated) and the rotated solution explain the same total amount of variance, the amount attributed to each component differs between the two solutions. In the rotated solution, the 1st component explains a smaller amount of variance (24.7%), while components 3 and 4 explain a higher percentage of it (16.4% and 14.2%,

Table 2. Correlations Between Histological Variables

Variable	Correlation Coefficient (*P*)									
	Peritubular Capillaritis	Plasma cells	Inflammatory infiltrate	Tubulitis	Lysozyme	Myeloperoxidase	Endarteritis	C4d	Eosinophil presence	Glomerulitis
Peritubular capilaritis	1	0.681 (< .001)	0.635 (< .001)	0.538 (< .001)	0.239 (.07)	0.254 (.05)	0.244 (.03)	-0.083 (.56)	0.21 (.06)	0.482 (< .001)
Plasma cells	0.681 (< .001)	1	0.542 (< .001)	0.502 (< .001)	0.073 (.58)	0.035 (.79)	0.118 (.30)	-0.036 (.80)	0.207 (.07)	0.418 (< .001)
Inflammatory infiltrate	0.635 (< .001)	0.542 (< .001)	1	0.522 (< .001)	0.236 (.07)	0.213 (.10)	0.205 (.07)	-0.004 (.98)	0.253 (.02)	0.142 (.21)
Tubulitis	0.538 (< .001)	0.502 (< .001)	0.522 (< .001)	1	0.18 (.17)	0.16 (.22)	0.026 (.82)	0.003 (.98)	-0.011 (.93)	0.087 (.45)
Lysozyme	0.239 (.07)	0.073 (.58)	0.236 (.07)	0.18 (.17)	1	0.562 (< .001)	0.275 (.04)	0.033 (.84)	0.152 (.25)	0.08 (.55)
Myeloperoxidase	0.254 (.05)	0.035 (.79)	0.213 (.10)	0.16 (.22)	0.562 (< .001)	1	0.038 (.77)	0.034 (.83)	-0.029 (.83)	0.171 (.19)
Endarteritis	0.244 (.03)	0.118 (.30)	0.205 (.07)	0.026 (.82)	0.275 (.04)	0.038 (.77)	1	0.364 (.008)	0.065 (.57)	0.136 (.23)
C4d	-0.083 (.56)	-0.036 (.80)	-0.004 (.98)	0.003 (.98)	0.033 (.84)	0.034 (.83)	0.364 (.008)	1	-0.276 (.05)	0.038 (.79)
Eosinophil presence	0.21 (.06)	0.207 (.07)	0.253 (.02)	-0.011 (.93)	0.152 (.25)	-0.029 (.83)	0.065 (.57)	-0.276 (.05)	1	0.025 (.83)
Glomerulitis	0.482 (< .001)	0.418 (< .001)	0.142 (.21)	0.087 (.45)	0.08 (.55)	0.171 (.19)	0.136 (.23)	0.038 (.79)	0.025 (.83)	1

respectively).

Table 4 corresponds to the component matrix and shows the weights of each factor for each variable in the initial unrotated solution. The weights corresponded to the correlations between factors and the variables. It was evident that, in the 1st component, the variables with a higher weight were the presence of plasma cells presence, inflammatory infiltrate, peritubular capillaritis, and tubulitis. In the case of the 2nd component, the two variables with the highest weight were LYS and MPO. In the third component, the highest coefficients were for C4d and endarteritis. In the 4th component, the variable with the highest weight was the presence of eosinophil presence.

The matrix of rotated components (Table 5) shows a clearer separation, since the orthogonal rotation (varimax) aimed to simplify the factors and excluded variables with weight coefficients lower than 0.6. It was observed that for the 1st factor, the variables with the higher weight were still the same, albeit with different weight (lower for peritubular capillaritis and plasma cells presence, and higher for tubulitis and inflammatory infiltrate). The 2nd component included the same two variables (LYS and MPO), but their coefficients were greater in comparison with the initial solution. The 3rd component had changed and it included the presence of eosinophil presence and glomerulitis. Finally, the 4th component had also changed, and it consisted of the presence of C4d and endarteritis.

Additionally, based on the coefficients that one variable had for one factor, new variables were generated for every case, corresponding to a score for each factor extracted. Consequently, each case could be assigned membership to a factor according to the highest score obtained. According to this new classification of the total population, which was based on the membership to a factor, 31% of the cases corresponded to the 1st factor, 19% to the 2nd factor, 21.4% to the 3rd factor, and 28.6% to the 4th factor.

When we compared kidney function, age, and serum levels of tacrolimus at the time of the diagnosis of AR, it was possible to observe interesting trends between the four groups formed according to factor membership. We identified that the cases belonging to factor 3 were the ones that showed the greatest increase in serum creatinine, both at the time of AR and at 1 and 2

Table 3. Total Variance Explained*

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	Percentage of Variance	Cumulative Percentage	Total	Percentage of Variance	Cumulative Percentage	Total	Percentage of Variance	Cumulative Percentage
1	2.944	29.442	29.442	2.944	29.442	29.442	2.470	24.700	24.700
2	1.792	17.919	47.361	1.792	17.919	47.361	1.687	16.874	41.574
3	1.271	12.710	60.071	1.271	12.710	60.071	1.643	16.431	58.005
4	1.216	12.164	72.235	1.216	12.164	72.235	1.423	14.230	72.235
5	.902	9.024	81.259	…	…	…	…	…	…
6	.669	6.691	87.949	…	…	…	…	…	…
7	.419	4.187	92.136	…	…	…	…	…	…
8	.319	3.186	95.322	…	…	…	…	…	…
9	.282	2.823	98.145	…	…	…	…	…	…
10	.185	1.855	100.000	…	…	…	…	…	…

*Extraction method was principal component analysis.

Table 4. Component Matrix*

Variable	Component			
	1	2	3	4
C4d	.148	.365	.609	-.398
Plasma cells	.846	-.293	.020	.096
Endarteritis	.252	.505	.631	.182
Eosinophil presence	.415	.016	-.268	.634
Glomerulitis	.382	-.126	.366	.430
Inflammatory infiltrate	.790	.148	-.078	-.266
Lysozyme	-.015	.841	-.263	.052
Myeloperoxidase	-.037	.753	-.347	.122
Peritubular capilaritis	.911	.064	-.067	.078
Tubulitis	.608	-.043	-.310	-.578

*Extraction method was principal component analysis. Four components were extracted.

Table 5. Rotated Component Matrix*

Variable	Component			
	1	2	3	4
Tubulitis	.862			
Inflammatory infiltrate	.814			
Peritubular capilaritis	.755			
Plasma cells	.672			
Lysozyme		.870		
Myeloperoxidase		.839		
Eosinophil presence			.726	
Glomerulitis			.602	
Endarteritis				.785
C4d				.778

*Extraction method was principal component analysis and rotation method was varimax with Kaiser normalization.

months of follow-up. Similarly, these cases had the lowest average age and the lowest serum levels of tacrolimus at the time of AR. On the other hand, the cases belonging to factor 4 showed the highest urinary protein excretion at 1 and 2 months of follow-up.

Finally, when comparing by factor, the proportions of cases with the highest expression intensity were analyzed for each of the histological variables, we found the following significant differences: C4d was higher in cases with factor 4; the eosinophil counts were higher in the cases with factor 3; and LYS and MPO labeling was higher in cases with factor 2. The presence of tubulitis was greater in cases with factor 1 and the presence of endarteritis was higher in the cases with factor 4. Therefore, we could conclude that these 6 variables were the ones that contributed to the greatest capacity of discrimination between factors.

DISCUSSION

The distinction between ACR and AHR is important from the etiopathogenic, diagnostic, and therapeutic points of view, since usually AHR implies a worse prognosis and a greater percentage of management resistance. The problem with establishing an AHR diagnosis opposed to an ACR diagnoses resides in the absence of specific histopathological alterations for the humoral event. However, the alterations may not be representative; they may be present with a minimum intensity or they may coexist with ACR.[12]

While the diagnosis of AHR has typically been based on the presence of C4d+, it may be negative in up to 40 to 50% of the cases.[2] Consequently, the search for other diagnostic criteria is important. Another limitation for AHR diagnosis according to the Banff criteria is the detection of DSA, which may be negative even in the presence of AHR,

or they may not be available in some kidney transplant centers. In addition, accumulating evidence supports the concept that not all DSAs are equivalent and that DSA properties (ability to bind complement or immunoglobulin G subclass), beyond simple positivity and mean fluorescence intensity, are associated with distinct outcomes and injury phenotypes in preexisting or recurrent as well as de novo DSAs. It was also noted that time course, kinetics, and properties of DSA fluctuate. Despite the usefulness of multiplex bead array assays, inherent limitations, technical issues, and lack of available DSA data at the time of biopsy make diagnoses complex. Therefore, the diagnosis of AHR could be based on pathology only, namely, microcirculation inflammation.[13-16]

The mechanisms of tissue damage mediated by cells and antibodies frequently appear simultaneously in cases of AR, to a greater or lesser extent. Consequently, the classification of an AR as purely cellular or humoral seems unhelpful and probably leads to decisions of limited treatment.[17,18] Alternatively, the presence of cases classified as MAR, which, in this series, comprised 26.5% of the cases, shows once again that there are different pathophysiological pathways that can coexist. Furthermore, many cases of AHR in kidney allografts, particularly late AHR associated with de novo DSAs, can present as MAR and ACR. Kidney allograft biopsies with microvascular inflammation plus intimal arteritis also frequently show tubulointerstitial ACR changes. These cases likely represent MAR and, not surprisingly, are often not responsive to treatment for either AHR or ACR alone.[18]

The proposed classification based on factor analysis could represent a useful tool for diagnosis and treatment purposes. As proof of this, we can consider that the cases belonging to the 1st component showed a clearly mixed pattern, with findings of both AHR and ACR; this has, in turn, been associated with a worse long-term prognosis, according to other reported series.[18,19] However, these cases did not show the greatest drop of kidney function in our series with a very short observation period. Another example would be the cases belonging to the 3rd component; these cases showed a clear tendency towards greater deterioration of kidney function at the time of AR and at least within a short follow-up period, suggesting that these patients would require more aggressive initial antirejection therapy at an earlier onset.

Hypereosinophilia preceding the rejection event has been reported in some cases, and activated eosinophils have been shown to be a mechanism of rejection in renal, hepatic, cardiac, and skin grafts.[5] Eosinophils are recruited and activated within the graft through the combined action of interleukins (4, 5, and 13), through local cytotoxic activity and through the synthesis of various cytokines. Eosinophils may form part of the effector pathways of tissue damage during AR episodes.[1,20] In agreement with previously published paper,[21] the patients in our series with peritubular eosinophils were the cases with a more severe clinical course of AR, judging by the tendency to a greater increase of serum creatinine, compared to cases belonging to the other extracted factors. With regard to glomerulitis, in the cases belonging to the third component in this series, the presence of glomerulitis has been described as an independent factor of poor prognosis of the allograft,[22] which could be supported by the higher elevation of serum creatinine at the time of AR, compared to the cases belonging to the other three factors.

The cases pertaining to the 4th component did not exhibit the greatest deterioration of kidney function. However, they did show a clear tendency to increase the urinary excretion of proteins. This warrants a longer-term follow-up to assess the impact that this data might have on graft survival. It has been recognized in other series that the presence of arterial lesions in cases of cell rejection has been associated with a lack of response to steroids and a greater frequency of graft loss 1 year after transplantation.[3,23,24] Furthermore, the presence of C4d and endarteritis could identify cases with increased microvascular endothelial damage,[25] which could translate into an increase in urinary protein excretion.

Finally, the cases associated with the 2nd component can be considered cases with intense inflammation and even a finding related to AHR, owing to the fact that inflammatory cells rich in MPO and LYS have been reported to promote inflammation and tissue destruction through the release of pro-inflammatory proteases and cytokines, such as tumor necrosis factor-α.[7-11]

However, their presence may be somewhat unspecific.[26] Consequently, its translation and role as a potential therapeutic target, in the cases of AR, requires further study.

The value of this proposal as a method for classifying AR cases and its efficiency for the identification of different outcomes, such as the development of chronic nephropathy and graft failure, needs to be further investigated in a study with a larger sample size and long-term follow-up.

CONCLUSIONS

This classification proposal based on factor analysis is a piece of evidence that highlights the multiple pathways involved in the AR process. These pathways, represented by the four extracted factors, are not mutually exclusive or exhaustive events. Nevertheless, they may represent different stages of activation of the immunological processes that explain an AR event, which may be susceptible to various forms of treatment, and may be more aggressive, either simultaneously or sequentially, with an order and intensity to be defined by future studies, whose objective will be to improve allograft prognosis.

CONFLCIT OF INTEREST

None declared.

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