Vimentin expression is a predictor of renal dysfunction after kidney transplantation

A expressão de vimentina é um preditor de disfunção renal após o transplante renal

Ana Cristina Carvalho de Matos, Marcello Fabiano de Franco, Luiz Antonio Ribeiro Moura, Frederico Rafael Moreira, Alvaro Pacheco e Silva Filho

ABSTRACT

Objective: To identify renal transplant patients with normal and stable renal function, long-term prognostic markers of renal function. Methods: We followed a protocol for renal biopsies in 32 patients at a median time of 180 days (min: 90 – max: 690 days) after the renal transplant. According to Banff’s classification (1997), biopsies were classified according to the presence or absence of chronic allograft nephropathy (CAN), and the sum of each chronic alteration produced the chronic score. All biopsy specimens were stained with picrosirius and observed under polarized light, and fibrotic tissue identified was quantified by histomorphometry. Using immunohistochemistry techniques, markers involved in the epithelium-mesenchymal transdifferentiation phenomenon were evaluated: vimentin (mesenchymal cell marker), alpha-SMA (myofibroblast marker), and cytokeratin (epithelial cell marker). Renal function was evaluated by serum creatinine levels at the time of biopsy, one and two years after the transplant, and current levels (36.5 ± 8.42 months after the biopsy). Statistical tests used were Mann-Whitney, Kruskal-Wallis, Spearman, and Fisher’s exact test. Results: Tubular expression of vimentin correlated with creatinine level at biopsy (r = 0.390 p = 0.033), at one year (r = 0.405 p = 0.026), two years (r = 0.474 p = 0.008), and current (r = 0.415 p = 0.028). The interstitial expression of alpha-SMA correlated with creatinine at biopsy (r = 0.442 p = 0.014), at two years (r = 0.364 p = 0.047), and current (r = 0.376 p = 0.048). The interstitial expression of alpha-SMA was associated with chronic changes (r = 0.412 p = 0.029), with the expression of vimentin (r = 0.502 p = 0.004), with fibrosis estimated by picrosirius (r = 0.402 p = 0.003), and the presence of chronic allograft nephropathy (p = 0.04). Tubular expression of vimentin correlated with chronic allograft nephropathy (p = 0.001) and was the marker with the strongest association to chronic tubulointerstitial changes (r = 0.513 p = 0.003). Conclusions: The increased expression of vimentin in tubules may suggest the presence of acute or chronic tubular injury or a regenerative response of epithelial tubular cells after damage, or an occurrence of the epithelial-mesenchymal phenomenon. The greatest tubular expression of vimentin was observed in areas of greatest tubulointerstitial damage and greatest recruitment of myofibroblasts, the primary matrix-producing cells. Therefore, this study suggests that patients with stable renal function who have undergone kidney transplants and show an increased expression of vimentin in tubules progress with poorer long-term kidney function.

Descriptors: Kidney transplant; Vimentin; Prognosis

RESUMO

Objetivo: Identificar em pacientes transplantados renais, com função renal normal e estável, marcadores prognósticos da função renal a longo prazo. Métodos: Realizamos biópsias renais protocolares em 32 pacientes após um tempo mediano de 180 dias (min: 90 – máx: 690 dias) após o transplante renal. Conforme a classificação de Banff-1997, as biópsias foram classificadas de acordo com a presença ou não de nefropatia crônica do enxerto e foi calculada a soma de cada alteração crônica, obtendo-se a soma dos escores crônicos. Todas as biópsias foram coradas com Picro-sírius, observado sob luz polarizada e o tecido fibrotico encontrado foi quantificado por meio de histomorfometria. Por meio de imunoistoquímica, foram avaliados os marcadores envolvidos no fenômeno de transdiferenciação epiteliomesenquimal: vimentina (marcador de célula de origem mesenquimal), alfa-SMA (marcador de miofibroblastos) e citoceratina (marcador de célula epitelial). A função renal foi avaliada por meio da creatinina sérica, no momento da biópsia, após um e dois anos do transplante e atual (após 36,5 ± 8,42 meses da realização da biópsia). Os testes estatísticos utilizados foram: Mann-Whitney, Kruskal-Wallis, Spearman e o teste exato de Fisher. Resultados: A expressão tubular de vimentina correlacionou-se à creatinina da biópsia (r = 0,390 p = 0,033), de um ano (r = 0,405 p = 0,026), dois anos (r = 0,474 p = 0,008) e atual (r = 0,415 p = 0,028). A expressão intersticial de alfa-SMA, correlacionou-se à creatinina da biópsia (r = 0,442 p = 0,014), de dois anos (r = 0,364 p = 0,047) e atual (r = 0,376 p = 0,048). A expressão intersticial de alfa-SMA esteve associada à creatinina da biópsia (r = 0,412 p = 0,029), dois anos (r = 0,474 p = 0,008) e atual (r = 0,415 p = 0,028). A expressão intersticial de alfa-SMA esteve associada às alterações crônicas (r = 0,412 p = 0,029), à
INTRODUCTION

Chronic allograft nephropathy (CAN) is one of the main causes of renal allograft loss after the first year of transplantation(1). The histological characteristics of CAN most closely associated with the subsequent deterioration of renal function are interstitial fibrosis and tubular atrophy(2). Evidence suggests that interstitial fibrosis and tubular atrophy set in primarily during the first year after the transplant, because of immune and ischemic injury(3). The morphological abnormalities of CAN, such as interstitial fibrosis, can be detected in their initial stage by means of protocol biopsies performed in the first months after transplantation(4). The prevalence of CAN in protocol biopsies carried out between three and 4 months is 25 to 40%, at 6 months it is around 40%, at 12 months it is approximately 50%, and at 24 months the prevalence of CAN in protocol biopsies is between 50 and 90%(5). Several studies have demonstrated that the finding of interstitial fibrosis in protocol biopsies performed between 3 and 6 months after the transplant correlated with worsening renal function and long-term loss of the graft(1,4-5). Specifically in kidney transplants, tubular epithelial cells can suffer several types of aggression related to ischemic injury, abnormalities of the recipient and donor, episodes of acute rejection, prolonged exposure to nephrotoxic drugs, and infections such as cytomegalovirus and poliovirus. Depending on the type, intensity, and duration of the injury, tubular epithelial cells can present several responses. They can recover their characteristics, die by necrosis or apoptosis, or modify their phenotype, adopting characteristics of mesenchymal cells such as myofibroblasts, the primary cells involved in matrix production, by means of a process called epithelial-mesenchymal transition (EMT), thus actively participating in the process of interstitial fibrosis(10-13). The extent of tubular epithelial cell lesion is a marker of renal dysfunction progression in a model of 5/6 ablation(14) and is associated with CAN in patients who have undergone kidney transplants(15).

EMT involves four key events, all of them induced by TGF-beta 1, the main regulator of the EMT process(11,16):
1. loss of epithelial phenotype, i.e., loss of tubular epithelial cell integrity and polarity.
2. expression of a new alpha-SMA and conversion of intermediate cytoplasmatic filaments of the cytoskeleton from an epithelial type of cytokeratin to a mesenchymal type of vimentin. Cells start to produce fibronectin and type I collagen.
3. destruction of the basal tubular membrane.
4. increased migratory and invasive capacity of epithelial cells(11,17-19)

In the renal transplant, few studies have identified the participation of the EMT process in renal fibrogenesis and its influence on renal prognosis(20-22).

In this study, we performed protocol renal biopsies at 3 months or more after the renal transplantation in patients with normal and stable kidney function. We analyzed chronic tubulointerstitial changes, interstitial fibrosis estimated quantitatively by picrosirius staining, and immunohistochemical markers involved in the EMT phenomenon.

OBJECTIVE

To identify morphological characteristics associated with kidney prognosis in renal transplant patients with normal and stable renal function.

METHODS

Thirty-two patients who had undergone renal transplantation, with normal and stable kidney function, were selected for the study. Excluded from the study were patients with serum creatinine levels showing a more than 10% variation, and patients with serum creatinine levels of more than 2.5 mg/dL and a clinical picture of acute allograft dysfunction that caused creatinine level elevation (acute rejection, acute tubular necrosis, nephrotoxicity by calcineurin inhibitors, chronic allograft nephropathy, an infectious condition, relapse of the underlying disease, de novo glomerulonephritis, or any systemic condition that caused repercussions on kidney function).

Thirty-two protocol biopsies were performed in renal transplant patients who satisfied the above mentioned criteria. Renal function was concomitantly assessed by creatinine levels. During the period of 11 April 2001 to
13 January 2004 in patients who had received kidney transplants from 10 April 2001 to 15 April 2003 coming from two transplant centers, the Hospital do Rim e Hipertensão [Kidney and Hypertension Hospital] and the Hospital Israelita Albert Einstein. Between April 2001 and April 2003, 1036 kidney transplants were carried out at the Hospital do Rim e Hipertensão, and 67 kidney transplants at the Hospital Israelita Albert Einstein. The same transplant team operated at both hospitals. All study subjects had been informed of the research objective and had signed written informed consent forms previously approved by the Medical Ethics Committees of both hospitals.

The median time after transplant at which the biopsies were performed was 180 days (min: 90 – max: 690 days). Of the 32 patients, 13 were females and 19 were males. Median age of patients was 38.0 ± 11.1 years. All patients had undergone hemodialysis before the transplant for an average dialysis time of 33.3 ± 25.8 months.

Twenty-two (68.7%) were living donor transplantations and 10 (31.2%) were deceased transplants. As to HLA compatibility between the living donors transplants, 11 (34.3%) were between identical donors and recipients, 8 (25%) between haploidentical donors and recipients, and 3 (9.3%) between distinct donors and recipients. Average cold ischemia time was 20 ± 7.4 hours in the transplants carried out with a deceased donor. Median age of donor was 38.3 ± 11.7 years. Initial immunosuppression used at both institutions comprised a calcineurin inhibitor associated with a corticoid and an antimetabolite. Ten (31.2%) patients used tacrolimus and 22 (68.7%) used cyclosporin; 19 (59.3%) patients used azathioprine (AZA), and 13 (40.6%) used mycophenolate mofetil (MMF). Only two patients received an induction scheme with antilymphocyte antibodies. Five (15.6%) patients experienced acute cellular rejection. Treatment of acute rejections was based on clinical progress or Banff’s Classification (1997). For acute cellular rejections, treatment was given with methylprednisolone for 3 to 5 days. No use of OKT3 was necessary for treatment of rejections.

Four (12.5%) patients experienced delayed graft function (DGF) defined as the need for dialysis in the first week after the transplant.

Average follow-up time of patients after the biopsy was 36 ± 8.42 months.

**Histological analysis**

The renal fragment was submitted to routine stains (hematoxylin-eosin, Jones’s silver stain, periodic acid-Schiff stain (PAS), and Masson’s trichrome stain), picrosirius a collagen I and III stains when observed under polarized light, and immunohistochemistry for evaluation of markers involved in the EMT process.

All biopsies were analyzed by a renal pathologist (the same pathologist who analyzed the biopsies at the two transplant centers). For biopsy analysis, Banff’s Classification (1997) was used\(^{23}\). Biopsies were classified according to the presence or absence of CAN, and each chronic alteration (glomerular, interstitial fibrosis, mesangial proliferation, tubular atrophy, and arteriolar hyalinosis) was semi-quantified from 0 to 3, according Banff’s Classification (1997). The sum of each chronic alteration was calculated for each patient, which could vary from 0 to 18, thus obtaining the sum of chronic scores.

Three immunohistochemical markers involved in the EMT phenomenon were analyzed: cytokeratin (tubular epithelial cell marker), vimentin (mesenchymal cell marker), and alpha -SMA (myofibroblast marker).

**Picrosirius: processing**

For Sirius red staining, 0.1 g of Sirius red and 100 ml of saturated picric acid solution were used. Paraffin blocks were subjected to 3 µm thick histological sections and were deparaffinized with xylol and subsequently hydrated with absolute alcohol (4x) and running water. Subsequently, the sections were immersed in saturated picric acid solution for 15 minutes and then in Sirius red for 20 more minutes. Specimens were then washed with running water and saturated picric acid solution for 5 more minutes. Counter-staining was carried out with fast green solution (0.1%-2%).

**Picrosirius: morphometric analysis**

Image analysis was performed by a clinician blinded to the clinical source of the sample. Sirius red stained sections were analyzed by an Olympus Bx50 microscope. An Olympus camera was attached to the microscope. Manual shots of the cortex magnified 400X and observed under polarized light and whenever possible structures such as: glomeruli, subcapsular cortex, large vessels and medulla were excluded. These pictures were digitalized (HP scanjet 2400) and then the interstitial volume of collagen in the cortex compared to the overall cortex area was quantified by morphometry.

For the morphometric analysis, the Image Processing and Analysis in Java, Image J software was used. The result of each image (or field) is given in percentages, which means a proportion (in pixels) between the interstitial volume of collagen in the cortex (represented by the black dots) and the total cortical interstitial volume (represented by the white dots) and then the arithmetic mean of the analyzed fields is calculated for each slide.

The confidence and reproducibility of interstitial fibrosis volume obtained by the morphometric analysis of the digital image stained with Sirius red was based on previous studies\(^{24}\).
**Immunohistochemistry**

For the immunohistochemistry analysis, paraffin-blocked tissue fixed in 10% formaldehyde was sectioned in 4 to 5 micra sections and kept at 57ºC incubator during 12 hours (overnight). For antigen recovery, slides were heated in citrate buffer in the microwave oven. Endogenous peroxide activity was blocked by 3% hydrogen peroxide. The slides were incubated during 12 hours with the following primary antibodies and their respective dilutions: anti-cytokeratin - epithelial cell marker (clones AE1/AE3; dilution 1:100, DakoCytomation, USA), anti-vimentin - mesenchymal cell marker (clone Vim 3B4, dilution 1:100, DakoCytomation, USA), smooth muscle anti-actin - myofibroblast marker (clone 1A4, DakoCytomation, dilution 1:100, USA). The amplification method was DAKO LSAB (labeled streptavidin biotin) + kit peroxidase, which consists of a solution with a secondary biotinylated antibody solution with a streptavidin-peroxidase conjugate molecule. Diaminobenzidine solution is used to visualize the reaction (3,3-Diaminobenzidine Tetrahydrochloride Sigma Chemical – USA). The primary antibody was omitted from the reaction for negative controls.

**Analysis of immunohistochemical markers**

Anti-cytokeratin: observation with 200x magnification and semi-quantitative analysis of the percentage of cortex tubules marked with this antibody from 0 to 100%.

Anti-vimentin: observation with 200x magnification and semi-quantitative analysis of the percentage of cortex tubules marked with this antibody from 0 to 100%.

Smooth muscle anti-actin: observation with 400x magnification and quantitative analysis by digital image morphometry, followed by the same methodology used for the picrosirius morphometric analysis.

**Evaluation of renal function**

Renal function was assessed by serum creatinine at different time points: on the day of the biopsy, in the first year, in the second year, and current (after an average follow-up time of 36.5 ± 8.42 months post-biopsy).

**Statistical Analysis**

Categorical variables were presented descriptively on tables containing absolute and relative frequencies. Quantitative variables were presented descriptively on tables containing medians, and maximum and minimum values.

Demographic and clinical variables analyzed were age of recipient, age of donor, time under dialysis, type of donor (deceased or living), compatibility of the HLA system (identical, haploidentical, distinct, or deceased), immunosuppression used, and early graft dysfunction.

Morphological findings considered in this study were the sum of the chronic scores, CAN, fibrosis estimated by picrosirius, and immunohistochemical findings.

Because of the sample size, the Mann-Whitney nonparametric test was used in comparing the medians between the two groups; in comparisons between three or more independent groups, Kruskal-Wallis’s test was used. Analysis of linear correlation was carried out by Spearman’s nonparametric correlation coefficient. Comparisons of proportions were performed with Fisher’s exact test. Odds ratios and the respective 95% confidence intervals were calculated.

All significance probabilities (p values) presented are two-tailed and values less than 0.05 are considered statistically significant. Statistical analysis was performed using Minitab 13.31 (State College, PA, USA).

**RESULTS**

**Renal function**

Mean serum creatinine at the time of biopsy was 1.47 ± 0.35 mg/dL, mean serum creatinine 1 year (n = 32) after the transplant was 1.39 ± 0.37 mg/dL, after 2 years (n = 32) it was 1.43 ± 0.64 mg/dL, and after a mean follow-up of 36.5 ± 8.42 months (n = 30) it was 1.29 ± 0.21 mg/dL (table 1). Two patients lost the renal allograft at 25 and 27 months after the transplant, respectively, because of CAN.

<table>
<thead>
<tr>
<th>Creatinine levels</th>
<th>Mean*</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine biopsy (n = 32)</td>
<td>1.47 ± 0.35 mg/dL</td>
<td>0.90 mg/dL</td>
<td>2.40 mg/dL</td>
</tr>
<tr>
<td>Creatinine 1 year (n = 32)</td>
<td>1.39 ± 0.37 mg/dL</td>
<td>0.90 mg/dL</td>
<td>2.80 mg/dL</td>
</tr>
<tr>
<td>Creatinine 2 years (n = 32)</td>
<td>1.43 ± 0.64 mg/dL</td>
<td>0.90 mg/dL</td>
<td>4.7 mg/dL</td>
</tr>
<tr>
<td>Creatinine current (n = 30)</td>
<td>1.29 ± 0.21 mg/dL</td>
<td>1.00 mg/dL</td>
<td>1.90 mg/dL</td>
</tr>
</tbody>
</table>

* Mean and standard deviation

Associations between the demographic and clinical data and renal function (creatinine at the time of biopsy, 1-year creatinine, 2-years creatinine, current creatinine):

Immunosuppression was the only variable correlated with renal function, i.e., the group that used tacrolimus (n = 9) in the initial immunosuppression regime, progressed with a better current level of creatinine (Cr: 1.2 mg/dL vs 1.4 mg/dL, p = 0.004) than the group that used cyclosporin (n = 21).

**Histological and immunohistochemical findings**

Among the 32 biopsies, 6 (18.7%) showed histological modifications consistent with CAN according to Banff’s Classification (1997)\(^{(2)}\) (table 2). The median of the chronic score sum was 1 (min 0 - max 7), revealing a low rate of chronic morphological findings in this population. Mean fibrosis estimated quantitatively by picrosirius was 4.11% ± 2.15% (min: 1.30% - max: 9.75%) (figure 1).

In the normal kidney, cytokeratin is found in all tubules. The average tubular expression of cytokeratin...
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In our study was 71.3% ± 27.4% (min 20% – max 100%). In six biopsies, there was a loss of cytokeratin expression in more than 50% of the tubules (figures 2 e 3).

In the normal kidney, vimentin is present in glomeruli, arterioles, and interstitial fibroblasts (figures 4 and 5). In the normal adult kidney, no vimentin was noted in the tubules. In this study, when vimentin was present outside of habitual areas, it was observed in tubules and the interstitium, and its expression was greater in areas with tubulointerstitial damage (atrophy, fibrosis, and inflammation). Mean percentage of vimentin in tubules was 24.5% ± 24.1% (min 0% - max 100%). In 12 (37.5%) biopsies, there was expression of vimentin in more than 30% of the tubules, a finding that does not occur in normal kidneys.

In the normal kidney, alpha-SMA is present only in smooth muscle cells of vessels. In our study, alpha-SMA was found essentially in the interstitium (figure 6), primarily in areas of tubulointerstitial damage (atrophy, fibrosis, and inflammation), and near the areas where tubular epithelial cells expressed vimentin. No expression of alpha-SMA was found in tubular epithelial cells. Mean alpha-SMA expression was 4.01% ± 2.32% (min 0.8% - max 10.2%).

The presence of CAN at biopsy was associated with a greater expression of vimentin (p = 0.001) and alpha-SMA (p = 0.04), according to table 3.

Fibrosis determined by picrosirius staining was associated with chronic modifications (r = 0.412 p = 0.029) and the expression of alpha-SMA (r = 0.402 p = 0.033), according to table 2.

The expression of vimentin showed a correlation with alpha-SMA expression (r = 0.502; p = 0.004). Even

<table>
<thead>
<tr>
<th>Findings</th>
<th>CGN in biopsy (median)</th>
<th>Absence of CGN in biopsy (median)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokeratin</td>
<td>75%</td>
<td>80%</td>
<td>0.732</td>
</tr>
<tr>
<td>Vimentin</td>
<td>45%</td>
<td>15%</td>
<td>0.014</td>
</tr>
<tr>
<td>Alpha-SMA</td>
<td>5.85</td>
<td>3.27</td>
<td>0.040</td>
</tr>
<tr>
<td>Fibrosis by picrosirius</td>
<td>4.48</td>
<td>3.42</td>
<td>0.186</td>
</tr>
</tbody>
</table>
though the expression of alpha-SMA was associated with chronic modifications \( (r = 0.470; p = 0.008) \), vimentin was the immunohistochemical marker with the strongest association with chronic tubular interstitial changes \( (r = 0.513; p = 0.003) \) (table 2).

**Association between demographic data and clinical and morphological findings (sum of the chronic scores, CAN, fibrosis estimated by picrosirius, and immunohistochemistry).**

The development of DGF correlated with the greater allograft interstitial expression of alpha-SMA, i.e., 7.45% in the group with DGF versus 3.27% in the group without DGF \( (p = 0.003) \).

Patients who used AZA in their immunosuppression scheme showed a lower risk of CAN than patients who used mycophenolate mofetil (MMF) \( (p = 0.029) \). The proportion of CAN observed in the group that used AZA was 5.26%, and in the group that did not use AZA it was 38.46%. A significant 91.11% reduction was noted in the chance of CAN for individuals who used AZA, when compared to those who did not use AZA.

**Association between histological findings (sum of chronic scores, CAN, fibrosis estimated by picrosirius, and immunohistochemistry), and renal function.**

According to data on table 4, the sum of chronic scores, CAN, fibrosis estimated by picrosirius, and chronic modifications related to tubular epithelial cells and the interstitium in patients with normal and stable kidney function after the transplant.

We noted mesenchymal markers in tubules (vimentin) and in the interstitium (vimentin and myofibroblasts), especially in areas with greater tubulointerstitial damage, i.e., atrophy, fibrosis, and inflammation. Depositions of collagen I and III were also observed in these areas of greatest tubulointerstitial injury and where there was a greater concentration of myofibroblasts, the main cells involved in the production of the tubulointerstitial matrix. After suffering injury, proximal tubular epithelial cells can be stimulated to synthesize growth factors involved in renal fibrogenesis, such as endothelin-1, PDGF, and TGF-β1, inducing abnormal peritubular expression of myofibroblasts(30). The unanswered issue in this study is related to the origin of these myofibroblasts, whether they come from resident fibroblasts or originate in EMT, since we did not perform double immunohistochemical marking for epithelial and mesenchymal cell markers.

Fibrosis measures by picrosirius and the sum of chronic scores (tubulointerstitial injury) were associated with current creatinine levels. Prior studies have demonstrated that a more precise quantification of the volume of interstitial fibrosis by means of histomorphometry and the use of stains more specific for collagen, such as picrosirius, in protocol biopsies were related to the prognosis of the kidney graft(24,31). On the other hand, Sund et al., in comparing the semiquantitative analysis of fibrosis by routine staining with Masson’s trichrome and its quantitative analysis by picrosirius staining, observed that semiquantification of fibrosis by Masson’s trichrome showed a better correlation with the renal prognosis(32).

In our study, we noted an association between the expression of myofibroblasts and interstitial fibrosis evaluated both by Masson’s trichrome and by picrosirius. Cortical kidney fibroblasts have the function of mediating the intercellular communication between neighboring

### Table 4. Correlation between renal function and histological and immunohistochemical findings

<table>
<thead>
<tr>
<th>Findings</th>
<th>Sum of chronic scores</th>
<th>Fibrosis by picrosirius</th>
<th>Cytokeratin</th>
<th>Vimentin</th>
<th>Alpha-SMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>( r = 0.241 )</td>
<td>( r = 0.125 )</td>
<td>( r = -0.104 )</td>
<td>( r = 0.390 )</td>
<td>( r = 0.442 )</td>
</tr>
<tr>
<td>Biopsy ( n = 32 )</td>
<td>( p = 0.183 )</td>
<td>( p = 0.525 )</td>
<td>( p = 0.581 )</td>
<td>( p = 0.033 )</td>
<td>( p = 0.014 )</td>
</tr>
<tr>
<td>Creatinine</td>
<td>( r = 0.087 )</td>
<td>( r = 0.297 )</td>
<td>( r = -0.034 )</td>
<td>( r = 0.405 )</td>
<td>( r = 0.310 )</td>
</tr>
<tr>
<td>1 year ( n = 32 )</td>
<td>( p = 0.635 )</td>
<td>( p = 0.124 )</td>
<td>( p = 0.854 )</td>
<td>( p = 0.026 )</td>
<td>( p = 0.094 )</td>
</tr>
<tr>
<td>Creatinine</td>
<td>( r = 0.114 )</td>
<td>( r = 0.356 )</td>
<td>( r = 0.002 )</td>
<td>( r = 0.474 )</td>
<td>( r = 0.364 )</td>
</tr>
<tr>
<td>2 years ( n = 32 )</td>
<td>( p = 0.531 )</td>
<td>( p = 0.062 )</td>
<td>( p = 0.989 )</td>
<td>( p = 0.008 )</td>
<td>( p = 0.047 )</td>
</tr>
<tr>
<td>Creatinine</td>
<td>( r = 0.379 )</td>
<td>( r = 0.392 )</td>
<td>( r = -0.245 )</td>
<td>( r = 0.015 )</td>
<td>( r = 0.376 )</td>
</tr>
<tr>
<td>Current ( n = 30 )</td>
<td>( p = 0.038 )</td>
<td>( p = 0.043 )</td>
<td>( p = 0.208 )</td>
<td>( p = 0.028 )</td>
<td>( p = 0.048 )</td>
</tr>
</tbody>
</table>
cells, infiltrating cells, and the extracellular matrix, and maintaining renal tissue architecture. These cells express a variety of cytokines, chemokines, growth factors, and adhesion molecules, playing an active role in the regulation of fibrogenesis and interstitial inflammatory response. Interstitial fibrosis, characterized by the presence of activated fibroblasts, i.e., that are characterized by a myofibroblast phenotype, can express -SMA, produce CAN, and show a high proliferative capacity.(10) Kidney fibroblasts may derive from resident or circulating fibroblasts, from hematopoietic progenitors, from stromal cells derived from bone marrow, or by EMT.(10,33)

The interstitial fibrosis found in the early period of a transplant when our study biopsies were performed could have come from the donor.(4) Performing biopsies at the time of implantation or soon after renal perfusion and in a series could be strategies used to verify the origin of the interstitial fibrosis and its progression.(4)

The histological markers most closely correlated with kidney function were the expression of vimentin and of myofibroblasts, confirming the finding of a previous study done in human renal biopsies with different kidney diseases.(34)

In the normal adult kidney, vimentin is present in glomeruli, arterioles, and interstitial fibroblasts and no vimentin is observed in the tubules. The expression of vimentin is considered a marker of tubular injury, regeneration, and of EMT in several experimental models such as of ureteral obstruction,(35) proteinuria,(36) kidney aging,(37) nephropathy by adriamycin,(38) and ischemic injury.(39) After acute tubular insult induced by mercury chloride, the regenerated tubular epithelium does not further express vimentin.(40) In rats with chronic nephropathy induced by daunomycin, co-expression was noted of cytokeratin and vimentin in areas of great tubular damage and proliferative activity.(40)

The majority of renal cell carcinomas also co-express vimentin and cytokeratin in tubules.(40) In senile rats with chronic nephropathy, tubules that expressed vimentin also expressed PDGF (platelet-derived growth factor) indicating that these cells are possible sources of factors that promote renal fibrogenesis.(37) In a model of ischemia in rats, it was also observed that progenitor-like renal cells were found in the kidney during the regeneration phase, that is, these cells of uncertain origin, i.e., whether coming from the kidney itself or from bone marrow, may be the source of regeneration cells and may differentiate into epithelial cells. These cells present a characteristically high proliferative activity, and during early regeneration phase, their offspring express vimentin. Consequently, the expression of vimentin may be secondary to the replication of progenitor-like renal cells with a regenerative function.(41) It has also been demonstrated that the extent of the vimentin expression in the tubular epithelial cells of patients submitted to kidney transplants correlates with the severity of tubular damage, glomerular filtration rate, and seriousness of the CAN.(22)

In our study, the increased expression of vimentin may be considered a marker of acute or chronic tubular injury, regeneration of tubular epithelial cells, EMT, and of renal prognosis.

The increased expression of myofibroblasts, the primary matrix-producing cells, in our biopsies was also associated with the worst long-term renal allograft function, confirming what had been found in earlier studies.(10,21,33-34,42-46)

In our study, some clinical and demographic variables were associated with morphological modifications, such as the presence of DGF and the increased expression of myofibroblasts, which probably contributes towards the association between CAN and DGF.(3) The use of AZA correlated with a lower risk of CAN development. Although studies have demonstrated a 50% reduction in the acute rejection rate, improvement of graft and patient survival,(47) and lower association with CAN(3,48) with the used of MMF, an earlier study did not find a better result in graft survival when comparing patients who used MMF or AZA.(49) In our study, MMF was indicated primarily in patients with greater immunological risk, contributing to the result found. As to renal function, the use of tacrolimus was the only clinical variable that correlated with the improved levels of creatinine during follow-up compared with the patients who used cyclosporin, confirming a finding of other studies.(50-51)

**CONCLUSIONS**

We found an increased expression of vimentin and myofibroblasts in renal biopsies carried out during an early phase of the transplant in stable patients with normal kidney function who evolved with worsening renal function 2 years after the transplant. The increased expression of vimentin was associated with chronic histologic lesions, inflammation, and myofibroblasts, and these cells, on the other hand, correlated with the extent of interstitial fibrosis. The increased expression of vimentin can be considered an early marker of long-term renal allograft dysfunction. Evaluation of the expression of vimentin and myofibroblasts could be used as a tool to evaluate the prognosis of the kidney allograft in an early phase after the transplant. We are carrying out studies involving a larger number of patients and considering the influence of other variables (multivariate analysis) in order to confirm these findings.
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