Original research

Histological findings in protocol biopsy associated with reduction in renal function 12 months post-transplant in renal transplant recipients with low immunological risk

Hallazgos histológicos en biopsia por protocolo asociados con reducción en función renal 12 meses postrasplante, en receptores de trasplante renal con bajo riesgo inmunológico, recibiendo inducción con basiliximab

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Abstract

Introduction: Identifying factors that are associated with allograft function loss might be an important step toward prolonging kidney allograft survival.

Purpose: In this study we found to determine the association between histologic changes on 1-year surveillance biopsies and changes in graft function.

Methods: Recipients of kidneys from deceased donors (95%) or living donors (5%) transplanted between 2007 and 2012. The primary end point was reduction in calculated glomerular filtration rate (Cockroft and Gault) higher than 5ml/min at 12 months post-transplant vs. calculated glomerular filtration rate previous surveillance biopsy.

Results: This analysis included 114 adults, recipients of kidneys from deceased donors (95%) or living donors (5%), with low immunological risk receiving basiliximab induction, transplanted between August 2007 and July 2012. The primary end point was reduction in calculated glomerular filtration rate (Cockroft & Gault) higher than 5ml/min at 12 months post-transplant, 25 of 114 patients showing reduction. The histologic changes associated with renal function reduction were glomerulitis (p=0.024), interstitial inflammation (p=0.001), tubulitis (p=0.001), capillaritis (p=0.001), glomerulitis + capillaritis (p=0.001), polyoma virus nephropathy (p=0.04) and subclinical rejection (p=0.015). By regression analyses, interstitial inflammation (OR = 2.11; 95% CI: 1.13-3.95) and capillaritis (0R=7.12; 95% CI: 1.57-32.27) were associated with renal function reduction 12 month post-transplant.

Conclusion: inflammation and capillaritis in protocol biopsies in the first year post-transplant predict loss of graft function independently of other variables.

Key words: Kidney transplant, surveillance biopsies, renal function, low immunological risk, basiliximab.

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Resumen

Introducción: identificar los factores asociados con pérdida de la función del injerto puede ser un paso importante hacia la prolongación de la sobrevida del injerto renal.

Objetivo: determinar la asociación entre los cambios histológicos presentes en las biopsias por protocolo, en el primer año postrasplante en receptores de bajo riesgo inmunológico, recibiendo inducción con basiliximab y pérdida en la función del injerto 12 meses postrasplante.

Métodos: se incluyeron pacientes receptores de riñones de donante cadavérico (95 %) o donante vivo (5 %) trasplantados entre agosto de 2007 y julio de 2012. El desenlace primario fue pérdida en la tasa de filtración glomerular calculada (Cockroft & Gault) mayor a 5ml/min 12 meses postrasplante, en comparación con la función renal previa a la biopsia por protocolo.

Resultados: la cohorte de estudio estuvo conformada por 114 pacientes, de los cuales 25 presentaron el desenlace principal. Los hallazgos asociados con pérdida de función fueron glomerulitis (p=0,024), inflamación intersticial (p=0,001), tubulitis (p=0,001), capilaritis (p=0,001), glomerulitis + capilaritis (p=0,001), nefropatía por polioma virus (p=0,04) y la presencia de rechazo subclínico (p=0,015). Por análisis de regresión logística la presencia de inflamación intersticial (OR = 2,11; IC 95 %: 1,13-3,95) y capilaritis (0R=7,12; IC 95 %:1,57-32,27) fueron las variables asociadas con pérdida de función del injerto renal 12 meses postrasplante renal.

Conclusión: la inflamación intersticial y capilaritis son variables histológicas asociadas con pérdida de función del injerto renal, 12 meses postrasplante, independiente de otras variables.

Palabras clave: trasplante renal, biopsia por protocolo, bajo riesgo inmunológico, basiliximab, función renal.

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Introduction

Renal transplantation is the best treatment option for patients with end stage chronic renal failure, given its impact on patient's survival, quality of life and reduction in long-term care costs.[1] The advances in immunosuppressive therapy have allowed the increase in patient and renal graft survival in the first year after transplant, from 70 % reported in the early nineties to 90 % today,[1,2] however, the survival of the renal graft beyond the first year post-transplant has not improved in parallel, and between 50 and 80% of renal grafts are lost within the first 10 years.[3]

Some studies have shown that the function of the renal graft in the first 6 and 12 months post-transplant have a strong relationship with the survival of the graft,[4] this study seeks to identify early histological changes in protocol biopsies performed during the first year of transplant, associated with calculated loss of function of the renal graft (Cockroft & Gault), which allows to implement preventive and therapeutic behaviors seeking to improve the survival of the graft and the patient; in this regard there are some reports such as the one published by Cosio et al., mainly in patients recipients of living donor in North America, who found an association between the presence of inflammation and glomerulopathy in the protocol biopsies in the first year and loss of graft function.[5]

However, in the Latin American population it is not clear which are the findings of the protocol biopsy in the first year post-transplant that correlate with loss of function of the renal graft in the first 12 months post-transplant. The hypothesis is that early histological changes in protocol biopsies may be associated with loss of renal graft function 12 months post-transplant.

Materials and methods

Retrospective cohort study in three kidney transplant centers in the city of Bogotá, between August 2007 and July 2012. The inclusion criteria were patients with low immunological risk renal transplant who received induction with basiliximab and immunosuppression scheme based on tacrolimus, mycophenolic acid and steroids with protocol biopsy between days 60 and 240 post-transplant. Patients under 15 years of age, patients with high immunological risk defined by a result of positive panel reactive antibodies or antecedent of renal transplantation, those who received induction with thymoglobulin and those who did not receive induction were excluded.

As independent variables, the histological findings of the biopsies with more than 7 glomeruli, 1 artery with internal elastic lamina (representative biopsies), characterized as glomerulitis, interstitial inflammation, vasculitis, tubulitis, capillaritis, interstitial fibrosis, increased mesangial matrix, glomerulitis + capillaritis, arterial hyalinosis, vascular intima, tubular atrophy, c1q immunofluorescence, polyoma virus, and subclinical rejection were analyzed. The dependent variable was the deterioration of the renal graft function 12 months post-transplant defined as the loss of more than 5 ml/min calculated by the delta of the glomerular filtration rate (GFR) by the Crockoft-Gault formula between the GFR pre-protocol biopsy of the renal graft and the GFR 12 months after the transplant.

Statistical analysis

A descriptive analysis of the information was performed. The categorical variables are shown as absolute frequencies and percentages, and the quantitative variables as measures of central tendency and dispersion according to the normality of the data, which was evaluated using the Shapiro-Wilk test.

A logistic regression model was constructed to determine the association between the histological findings of the protocol biopsy and the outcome; to the initial model were entered all the variables that presented an association ($p < 0.20$) with the outcome in the bivariate analysis, which was performed using the Mann-Whitney test when the independent variable was quantitative and by the Fisher 's exact test or the chi-square test when the variable was categorical. HLA mismatch and the age of the donor were assessed as possible confounding variables.

The final model consisted of the variables that had statistical significance, considering for this a p-value less than a 0.05.

Results

The initial cohort consisted of 209 patients and after verifying the inclusion and exclusion criteria, we obtained 114 patients who set up the study population, (Figure 1) all of them recipients with renal transplant of low immunological risk who received induction with basiliximab, of them, 89 patients did not have loss of function of the renal graft, compared with 25 patients who lost more than 5 ml/min in calculated GFR. No statistically significant differences were found in the demographic characteristics of the population that presented deterioration in renal graft function compared to those who did not. (Table 1).

The individual histological findings associated with statistically significant loss of graft function were: presence of glomerulitis, interstitial inflammation, tubulitis, capillaritis, glomerulitis + capillaritis, polyoma virus nephropathy and the presence of subclinical rejection. The frequency of findings in protocol biopsy is shown in Table 2.

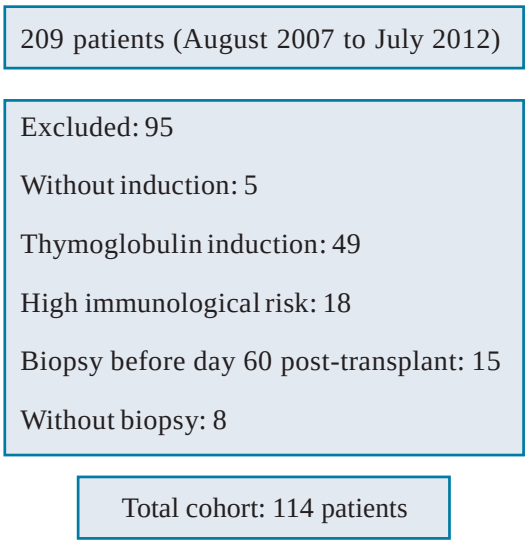

Figure 1. Patients selection.

When performing the logistic regression model, it was found that the presence of interstitial inflammation (OR = 2.11; 95% CI: 1.13-3.95) and capillaritis (0R = 7.12; 95% CI: 1.57-32.27) were the individual variables associated with loss of renal graft function 12 months after renal transplantation. It was not found that the variables HLA mismatch and age of the donor were confounders in the model.

Discussion

Protocol biopsy remains the best available diagnostic strategy to date, becoming the "gold standard" to evaluate the state of the renal graft, identifying histological alterations early before evidencing substantial changes in renal function, which has allowed to progress in the understanding of the pathophysiological keys that explain the injury and the loss of the graft in the long term. However, protocol biopsy is not part of the routine practice in transplant centers, so that our study represents the first report in Colombia, backed up by a standardized program of clinical surveillance to detect histological patterns that are associated with dysfunction of the renal graft in the short and long term. There are few data that support the findings of the protocol biopsy as predictors of loss of function, however, in our study we found an important association between the presence of glomerulitis, interstitial inflammation, tubulitis, capillaritis, glomerulitis + capillaritis, polyoma virus nephropathy and subclinical rejection with the delta of glomerular filtration change, thus becoming findings associated with deterioration of the renal graft function 12 months post-transplant.

We identified that interstitial inflammation and capillaritis are significantly associated with an increased risk of impaired renal function. In this sense, there has been evidence of an association between the presence of tubulitis in protocol biopsies at 3 months with progression to tubular atrophy, interstitial inflammation of mononuclear cells and chronic interstitial fibrosis.[6] In such a way that the presence of interstitial inflammation has a significant impact on kidney function at 12 and 24 months, regardless of whether it meets the Banff criteria for subclinical rejection.[7]

Table 1. Clinical and sociodemographic characteristics of the patients.

Characteristics		Total n=114 (%)	Stability n=89 (%)	Loss of function =25 (%)	p-value
Recipient age	<18 19 a 34 35 a 49 50 a 64 > 65	7(6,1) 28(24,6) 27(23,7) 36(31,6) 16(14)	3(3,4) 23(25,8) 21(23,6) 28(31,5) 14(15,7)	4(16) 5(20) 6(24) 8(32) 2(8)	0,325
Recipient gender	Masculino Femenino	78(68,4) 36(31,6)	63(70,8) 26(29,2)	15(60) 10(40)	0.336
Recipient race	Blanco Negro	108(98,2) 2(1,8)	83(97,7) 2(2,4)	25(100)	1,000
Donor age	<18 19 a 34 35 a 49 50 a 64	11(9,8) 48(42,9) 33(29,5) 20(17,9) 2(1,75)	9(10,1) 37(41,6) 24(26) 17(19,1) 2(2,25)	2(8) 11(44) 9(36) 3(12)	0,556
HLA Mismatch	0 1 2 3 4 5	1(0,9) 3(2,7) 25(22,3) 48(42,9) 33(29,5) 1(1,8)	1(1,15) 3(3,5) 21(24,1) 34(39,1) 26(29,9) 2(2,3)	4(16) 14(56) 7(28)	0,732
HLA DR Mismatch	0 1 2	33(29,5) 69(61,6) 10(8,9)	28(32,18) 53(60,92) 6(6,9)	5(20) 16(64) 4(16)	0,264
Type of donor	Vivo Cadavérico	6(5,3) 108(94,7)	84(94,38) 5(5,62)	24(96) 1(4)	1,000
Ischemia time (hours)*			15 (4 - 28)	13,25(4,5 - 20)	0,765
Expanded Criteria		6(5,36)	6(6,9%)		0,335
Biopsy time*			136 (7 - 206)	130 (88 - 172)	0,407

* Median (IQR). P-value of the Mann Whitney test.

It was found an incidence of subclinical rejection of 18.6 %, results that are in contrast with data reported by other studies in low immunological risk renal transplant recipients, in tacrolimus-based schemes, which range between 0.7 % and 15.2%.[8-11] The presence of subclinical rejection has a significant impact on renal graft function at 12 and 24 months despite receiving steroid treatment.

Table 2. Frequency of individual findings in the protocol biopsy and association with calculated loss of function of the renal graft 12 months post-transplant.

Variable	Stability n = 89 (%)	Loss of function n = 25 (%)	Valor p*
Glomerulitis	7(7,9)	6 (24)	0,024
Interstitial inflammation	41(46,1)	20 (80)	0,001
Vasculitis	1(1,2)	0 (0)	
Tubulitis	17(19,1)	14 (56)	0,001
Capillaritis	3(3,4)	7 (28)	0,001
Glomerulitis + capillaritis	11(12,4)	10 (40)	0,003
Arteriolar hyalinosis	10(11,6)	1 (4,4)	
Double contours	7(7,9)	2 (8)	
Mesangial matrix	25(28,1)	5 (20)	
Interstitial fibrosis	35(39,3)	13(52)	
Tubular atrophy	31(34,8)	10(40)	
Vascular intima	48(53,9)	12(48)	
Polyoma virus	3(3,4)	4(16)	0.040
c1q	11(13,1)	1(4,6)	
Double contours EM	20(26,3)	5(25)	
Immune complexes EM	9(11,8)	0(0)	
CNI toxicity	9(10,6)	4(16)	
Subclinical rejection (Borderline)	12(13,5)	9(37,5)	0.015

* Result of Fisher's exact test significant at 0.05

Nankivell, et al., reported that the presence of subclinical rejection at 3 months post-transplant predicts interstitial fibrosis at 12 months post-transplant.[12] It is probable that the interstitial infiltrate associated with tubulitis is the epiphenomenon of alterations in the tubular basement membrane that generate loss of matrix proteins, compromising the glomerular adaptive process, which in the long term will be reflected in chronic nephropathy and reduction of glomerular filtrate.[8,13,14] Roberts, et al., found a direct relationship between subclinical rejection and the subsequent occurrence of acute rejection with clinical expression and chronic rejection in cases of patients who do not receive treatment;[15] Rush, et al., describe that the treatment of subclinical rejection with corticosteroids can lead to better histological and functional outcomes in renal transplant recipients.[16] The importance of treatment of subclinical rejection was suggested by a randomized study that showed that subclinical rejection treatment in months 1, 2, and 3 was associated with a reduction in interstitial fibrosis and tubular atrophy in 6 months and with the preservation of graft function at 2 years, compared with a control group in which protocol biopsies were not performed.[17]

Glomerulitis is a histological marker associated with multiple entities such as antibody-mediated rejection, T-cell mediated cellular rejection and

glomerular disease, with a noticeable impact in terms of short-term and long-term graft function.[18,19]

Our experience demonstrates that there is a significant association of peritubular capillaritis with renal dysfunction at one year post-transplant, however, it can be a prognostic marker of deterioration in renal function in the long term.[20]

Subclinical peritubular capillaritis can become an early histological precursor of chronic rejection, as an epiphenomenon of histological changes developed from an endothelial lesion, which together with tissue repair mechanisms lead to progressive endohelial cell injury, as well as fragmentation and multilamination of the basement membrane of the peritubular capillaries, findings that have been documented in follow-up biopsies at 12 months post transplantation[21] and in sequential biopsy studies in patients with acute antibody-mediated rejection.[22]

Hence, the impact of peritubular capillaritis in predicting functional changes and chronic rejection supports the need to incorporate a classification system for peritubular capillaritis in Banff's criteria.[23]

The presence of glomerulitis plus peritubular capillaritis (c + ptc) constitutes a true marker of microinflammation and correlates directly with the deterioration of glomerular filtration at one year, as we have evidenced in our experience, but in the same way with the development of chronic graft injury.[24] Likewise, multiple studies have found that along with the presence of donor-specific antibodies they are a determinant of long-term loss of function.[20-23]

The main strength of our study was to find factors associated with renal graft loss in our population; however, it is limited by being a retrospective cohort with short-term follow-up, requiring studies that show whether such findings maintain their long-term predictive capacity.

Conclusion

The protocol biopsy is a valuable tool and it should be part of the post-transplant follow-up standard, since it is the objective way to systematically evidence the early histological changes that allow to implement prevention and management strategies that impact long term outcomes. The association between interstitial inflammation and capillaritis and its impact on graft function can mark clues for the understanding of the pathophysiological mechanisms that determine the prognosis of the renal graft and the patient.

Contribution of the authors

Conflict of interest

The authors of this work have no conflict of interest.

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Ethical responsibilities

Protection of people and animals

The authors declare that no experiments were performed on human beings or animals for this research.

Data confidentiality

The authors declare that they have followed the protocols of their workplace on the publication of patient data.

Right to privacy and informed consent

The authors declare that patient data do not appear in this article.

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