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Cystatin C and inflammatory markers in kidney transplant recipients

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Summary

Objective: Kidney transplantation is the best option for patients with end-stage renal disease. This study evaluated the profile of cystatin C (CysC), interleukin 2 (IL-2), IL-6, and tumor necrosis factor-α (TNF-α) as inflammatory markers in 23 living donor kidney transplant recipients. **Methods:** A descriptive, analytical and prospective study was conducted between January 1st, 2007 and June 30th, 2008 on 23 living donor kidney transplant recipients. The biomarkers were evaluated before and 30 and 180 days after transplantation. **Results:** The mean age of the patients was 34.3 years (± 11.7), females (52%) and non-whites (61%). Significant difference was found in cystatin C and creatinine before and 30 days after transplantation ($p < 0.0001$) and before and 180 days after transplantation ($p < 0.0001$). There was a significant difference in IL-2 between 30 and 180 days post-transplant ($p = 0.0418$) and in TNF-α between pre-transplant and 30 days post-transplant ($p = 0.0001$). A negative correlation was observed between cystatin C and TNF-α at pre-transplant and between cystatin C and IL-6 at 180 days post-transplant. Comparison of biopsied and non-biopsied patients showed a significant difference in creatinine and cystatin C at 30 and 180 days post-transplant in biopsied patients. **Conclusion:** Our results showed no significant correlations between CysC, IL-2, IL-6 and TNF-α levels in kidney transplant recipients at short-term follow-up. Moreover, CysC levels were very similar to creatinine levels in contrast to other inflammatory markers studied in biopsied and non-biopsied patients. Further studies are important to evaluate the long-term profile of these markers.

Keywords: Cystatin C; creatinine; biological markers; graft rejection; kidney transplantation.

Resumo

Cistatina C e marcadores inflamatórios em receptores de transplante renal

Objetivo: O transplante renal é a melhor opção para pacientes renais crônicos em estágio terminal. Este estudo avaliou o perfil da cistatina C (CysC), interleucina 2 (IL-2), IL-6, e fator de necrose tumoral-α (TNF-α) como marcadores inflamatórios em 23 transplantados renais de doador vivo. **Métodos:** Estudo descritivo, analítico e prospectivo conduzido entre 1º de janeiro (2007) e 30 de junho (2008) em 23 transplantados renais de doador vivo. Os biomarcadores foram avaliados no pré, com 30 e 180 dias do pós-transplante. **Resultados:** A média de idade foi de 34,3 anos (± 11,7), 52% do sexo feminino e 61% de negros. Foi encontrada diferença significativa na CysC e creatinina antes do transplante e 30 dias após o procedimento ($p < 0,0001$) e antes do transplante e 180 dias após o procedimento ($p < 0,0001$). Houve uma diferença significativa na IL-2, entre 30 and 180 dias do pós-transplante ($p = 0,0418$) e no TNF-α antes do transplante e 30 dias após o procedimento ($p = 0,0001$). Foi observada uma correlação negativa entre CysC e TNF-α no pré-transplante, e entre CysC e IL-6 com 180 dias do pós-transplante. Em pacientes biopsiados houve uma diferença significante na creatinina e na CysC com 30 e 180 dias do pós-transplante. **Conclusão:** Em seguimento a curto prazo, não houve correlação relevante entre os níveis de CysC, IL-2, IL-6 e TNF-α em transplantados renais. Em pacientes biopsiados e não biopsiados, os níveis de CysC foram muito similares aos da creatinina, ao contrário de outros marcadores inflamatórios. Demais estudos são importantes para avaliar o perfil destes marcadores a longo prazo.

Unitermos: Cistatina C; creatinina; marcadores biológicos; rejeição de enxerto; transplante de rim.

Study conducted at Hospital Universitário Presidente Dutra, Universidade Federal do Maranhão, São Luiz, MA

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Introduction

A kidney transplant is the best therapeutic and rehabilitation option for patients with end-stage renal disease (ESRD) since it provides a better quality of life and life expectancy to the patient than dialysis. However, chronic graft dysfunction is an important cause of renal graft loss and the treatment of this condition is nonspecific in view of its multifactorial etiology. In this setting, for the management of kidney transplant recipients, it is essential to avoid or delay the progression of chronic graft dysfunction by means of its prevention and an early diagnosis[1].

Ischemia-reperfusion injury, which occurs during the removal and implantation of the renal graft, involves immunological and non-immunological mechanisms associated with inflammation, whose mediators include complement, procoagulant factors, cytokines, and fibrinolytic agents[2]. Acute inflammation is the response of tissue to injury and is characterized by an increase in blood flow and vascular permeability as a result of the accumulation of fluids, leukocytes and inflammatory mediators such as cytokines. During the chronic phase, inflammation is characterized by the development of a humoral and cellular immune response[3].

Acute renal graft rejection is a common event during the early and late post-transplant period and a kidney biopsy is the gold standard for its diagnosis. Acute rejection is characterized by endothelitis/arteritis and tubulointerstitial inflammation[4]. Evidence suggests that persistent inflammation and oxidative stress (the formation of reactive oxygen species) start early during the process of renal function decline. Recently there has been interest in the potential value of pretransplant inflammatory markers as predictors of acute rejection and graft loss in adult kidney transplant patients[5,6].

Inflammation directly associated with atherosclerosis may influence graft loss and cardiovascular disease. Several studies have demonstrated that inflammatory markers such as interleukin 2 (IL-2), IL-6 and tumor necrosis factor alpha (TNF-α) are associated with episodes of acute rejection[7]. However, these markers have not always been useful in distinguishing between rejection and infection or tubular damage. In addition, cystatin C, which is known to be a sensitive marker of small reductions in renal function[7], has shown linear associations with inflammatory markers, also suggesting adverse physiopathological consequences[8-10].

More detailed studies investigating markers that could be used for the early and non-invasive evaluation of renal damage are necessary. Therefore, the objective of the present study was to evaluate the short-term profile of cystatin C, IL-2, IL-6 and TNF-α in kidney transplant recipients and the potential role of them as predictors of early renal graft dysfunction.

Methods

A descriptive, analytical and prospective study was conducted between January 1st, 2007 and June 30th, 2008 on 23 kidney transplant patients seen at the transplant unit of *Hospital Universitário, Universidade Federal do Maranhão* (HUUFMA). Patients ranging in age from 18 to 60 years who received a kidney transplant from a living donor and who signed the free informed consent form were included in the study. The hospitalized patients were prepared for transplantation by the medical team, with the evaluation of serology, hepatic, renal and cardiac function, imaging exams, HLA compatibility, and crossmatching. The following triple immunosuppression schemes were used: 1) tacrolimus, mycophenolate mofetil, and prednisone; 2) cyclosporin, azathioprine, and prednisone.

After the selection procedures and admission of the patients for transplantation, data collection was started by signing the free informed consent form and subsequent application of a clinical-laboratory questionnaire. The biomarkers studied were measured before and 30 and 180 days after kidney transplantation. Blood was collected individually at the transplant unit by specialized technicians and the samples were identified, properly stored, and sent for laboratory analysis. The biomarkers were compared between patients submitted to graft biopsy and those not submitted to this procedure.

Cystatin C was measured in serum samples by nephelometry using the BN II ProSpec equipment and reagents (Siemens) according to manufacturer instructions. Serum samples were obtained by coagulation and centrifugation of whole blood at 3,500 rpm for 15 min. Serum levels of IL-2, IL-6 and TNF-α were measured using human TiterZyme enzyme immunoassay (EIA) kits. The reference values provided by the manufacturer were adopted: 2.0 pg/mL for IL-2, up to 6.01 pg/mL for IL-6, and up to 8.43 pg/mL for TNF-α.

The quantitative variables are reported as the mean and standard deviation or median, and qualitative variables are expressed as frequency and percentage. The Shapiro-Wilk test was used to determine whether the quantitative variables showed a normal distribution. Since the variables were not normally distributed, Friedman's test was used to compare the markers between the different time points. Spearman's correlation coefficient was calculated to determine which markers were correlated with cystatin C. A level of significance of 5% was adopted. Statistical analysis was performed using the STATA 9.0 program. The study was approved by the Ethics Committee of *Hospital Universitário, Universidade Federal do Maranhão* (protocol 322/6).

Results

The mean age of the patients was 34.3 years (± 11.7). There was a predominance of females (52%) and non-white (61%). The mean weight and height of the patients, dia-

stolic and systolic blood pressure and hemoglobin, albumin, phosphorus and creatinine levels, as well as underlying diseases, are shown in Table 1.

Table 1 – Demographic and clinical characteristics at admission of the kidney transplant population studied (n = 23)

Variable	
Age (years)	34.3 ± 11.7
Gender	
Female/male (%)	12/11 (52/48)
Race	
Non-white/white (%)	14/9 (61/39)
Weight (kg)	55.2 ± 14
Height (cm)	158 ± 0.1
Diastolic pressure (mmHg)	133 ± 21.6
Systolic pressure (mmHg)	88 ± 15.6
Hemoglobin (g/dL)	11.7 ± 1.9
Albumin (mg/dL)	4.1 ± 0.5
Phosphorus (mg/dL)	6.2 ± 1.7
Creatinine (mg/dL)	8.9 ± 4.1
Underlying disease	
Non-glomerular/glomerular (%)	12/11 (52/48)

Comparison of the biomarkers between the different sampling times showed a significant difference in plasma creatinine and cystatin C between admission and 30 days post-transplant ($p < 0.0001$), and between admission and 180 days post-transplant ($p < 0.0001$). On the other hand, a significant difference in IL-2 was only observed between 30 and 180 days post-transplant ($p = 0.0418$) and in TNF-α between admission and 30 days post-transplant ($p = 0.0001$), whereas there was no significant difference in IL-6 (Table 2).

Analysis of the correlation between cystatin C and the other markers by Spearman's test showed a significant negative correlation between cystatin C and TNF-α at admission ($r = -0.44$) and between cystatin C and IL-6 at 180 days post-transplant ($r = -0.38$).

A graft biopsy was obtained from eight of the 23 patients because of elevated serum levels of creatinine in six and elevated proteinuria in two. A normal biopsy result was obtained in two patients, three presented acute drug nephrotoxicity, recurrence of the underlying disease was observed in two, and one patient had a confirmatory result of cell rejection. The immunosuppression scheme used was tacrolimus, mycophenolate mofetil and prednisone in 19 patients, and cyclosporin, azathioprine and prednisone in four.

The mean plasma concentrations of the biomarkers were compared at admission and 30 and 180 days post-transplant between biopsied (n = 8) and non-biopsied (n = 15) patients. A significant difference was only observed for serum creatinine and cystatin C at 30 days post-transplant ($p = 0.0040$ vs $p = 0.0417$) and 180 days post-transplant ($p = 0.0077$ vs $p = 0.0137$) (Table 3). No significant differences were observed in pretransplant serum levels of cystatin C, IL-2, IL-6 or TNF-α between biopsied (n = 8) and non-biopsied patients (n = 15).

Discussion

The mean age of the patients studied was 34.3 years (± 11.7), in agreement with the literature[9,11-13]. Bohler *et al.*[14] investigated cofactors that potentially influence the expression of immunological biomarkers, exploring the proliferation and activation of T cells (expression of CD25 and CD17) and the production of cytokines by lymphocytes (IL-2 and TNF-α) in healthy volunteers, dialysis patients and stable kidney transplant patients receiving standard immunosuppressive therapy. The authors observed a positive correlation between age and TNF-α expression in all three populations. In contrast, the expression of IL-2 was positively correlated with age only in healthy volunteers and kidney transplant patients, indicating that age exerts diverse effects on the adaptive immune system, which modulates the immunogenicity of donor organs in terms of the recipient's capacity to reject the organ. The same authors also demonstrated a reduction of T cell function in kidney transplant patients when compared

Table 2 – Mean and standard deviation of the biomarkers at admission and 30 and 180 days after transplantation

Biomarkers	Sampling time			
	Admission	30 days	180 days	p
Creatinine (mg/dL)	8.97 ± 4.1	1.5 ± 1.2	1.6 ± 0.7	< 0.0001[a]
Cystatin C (mg/dL)	6.59 ± 1.4	1.6 ± 0.85	1.39 ± 0.72	< 0.0001[a]
IL-2 (pg/mL)	13.47 ± 29.1	8.7 ± 19.0	4.93 ± 7.8	0.0418[b]
IL- 6 (pg/mL)	29.87 ± 51.7	24.50 ± 50.7	50.61 ± 130.8	0.7457
TNF-α (pg/mL)	13.4 ± 7.2	4.96 ± 3.5	10.35 ± 13.1	0.0001[c]

[a] Significant difference (at the 5% level) between admission and 30 days post-transplant and between admission and 180 days post-transplant. [b] Significant difference (at the 5% level) between 30 and 180 days post-transplant. [c] Significant difference (at the 5% level) between admission and 30 days post-transplant.

Table 3 – Mean and standard deviation of the biomarkers in biopsied (n =8) and non-biopsied patients (n = 15) 30 and 180 days after transplantation

	30 days after transplantation			180 days after transplantation		
	Biopsied	Non-biopsied	p	Biopsied	Non-biopsied	p
Creatinine	2.29 ± 4.9	1.12 ± 0.3	0.0040*	2.1 ± 0.9	1.3 ± 0.3	0.0077*
Cystatin C	2.11 ± 1.2	1.32 ± 0.3	0.0417*	1.83 ± 1.0	1.15 ± 0.2	0.0137*
IL-2	15.68 ± 32.1	4.97 ± 2.4	0.5444	8.42 ± 13.0	3.07 ± 0.3	0.5212
IL-6	34.72 ± 63.9	19.05 ± 43.7	0.6055	91.21 ± 208.5	28.95 ± 60.8	0.9484
TNF-α	5.96 ± 4.5	4.41 ± 2.8	0.8399	16.05 ± 21.0	7.30 ± 4.5	0.8461

* Significant at the 5% level.

to healthy volunteers and dialysis patients, as we all as an increase of IL-2 expression in dialysis patients when compared to healthy volunteers. Similarly, Fijter *et al.*[15] showed that kidneys from elderly donors provoke an increase in immunogenicity associated with a higher susceptibility to graft loss due to rejection of the transplant and chronic graft nephropathy.

According to Rostaing *et al.*[16], the dialysis membrane may eventually stimulate T cells and different dialysis membranes have different abilities to stimulate the expression of IL-2. In contrast, van Riemsdijk-Van Overbeeke *et al.*[17] found that the expression of IL-2 and IL-2 receptor on T cells is unaffected in dialysis patients. In agreement with the findings of Rostaing *et al.*[16], the present study also observed elevated IL-2 levels after dialysis. The induction of the proinflammatory cytokine IL-2 during dialysis might be unfavorable for the short-term outcome after kidney transplantation since it sensitizes the recipient's immune system to the graft[14].

In the current study, patients originating from hemodialysis presented elevated serum levels of IL-2, IL-6 and TNF-α. These results agree with Rysz *et al.*[18], in terms of serum IL-6 and TNF-α levels but not IL-2. Rysz *et al.*[18] studied patients on regular hemodialysis therapy at different times (20, 60 and 240 min) of a single session in order to compare the serum levels of a panel of inflammatory cytokines (IL-1β, IL-2, IL-6, IL-8, and TNF-α) with C-reactive protein (CRP) using healthy subjects as controls. The authors observed a higher CRP concentration in hemodialysis patients when compared to healthy controls. The concentrations of IL-1β, IL-6, IL-8 and TNF-α were increased, whereas serum IL-2 levels remained unchanged during the hemodialysis session.

In the present study, we found increased serum concentrations of creatinine, cystatin C, IL-2, IL-6 and TNF-α at admission period. After transplantation, there was a decrease in creatinine, cystatin C and IL-2 levels, whereas IL-6 and TNF-α remained elevated. This fact indicates that a kidney transplant has an important impact on these markers, which is more pronounced in the case of some markers and less pronounced in the case of others. One interesting finding was the gradual decline in IL-2 levels at 30 and 180 days post-transplant, which was not observed for IL-6 and TNF-α whose levels were found to be reduced at 30 days and increased at 180 days post-transplant.

The present results showed a non-significant reduction in serum IL-6 levels, with levels above normal, at 30 days post-transplant and higher levels at 6 months post-transplant. These findings disagree with Simmons *et al.*[19], but are similar when compared to the reduced serum levels of TNF-α 30 days post-transplant. Simmons *et al.*[19] studied 19 patients undergoing living donor kidney transplantation at different time points: 1 week before transplantation and 1 week and 2 months after transplantation. The following biomarkers of inflammation and oxidative stress were assayed: CRP, IL-6, IL-10, TNF-α, protein-associated carbonyl content, and F_2 isoprostanes. The authors observed that pretransplant levels of the proinflammatory proteins IL-6, TNF-α and CRP, as well as the oxidative stress markers plasma protein carbonyl and F_2 isoprostanes, were significantly elevated when compared to control. In addition, there was a rapid and significant decline in all of these biomarkers after transplantation that persisted for 2 months, indicating the restoration of renal function characterized by a reduction of chronic inflammation and oxidative stress associated with uremia.

Cueto-Manzano *et al.*[9] compared serum levels of CRP, IL-6 and TNF-α in 37 patients before and after kidney transplantation. CRP levels tended to be higher in recipients than in donors but this difference was not statistically significant, with the observation of a significant reduction of CRP concentration in recipients immediately after transplantation. Pre-transplant serum levels of TNF-α and IL-6 were significantly higher in recipients than in donors. After an initial reduction at 6 months post-transplant, TNF-α and IL-6 levels increased even further in recipients at 12 and 18 months. In contrast to the results reported by Cueto-Manzano *et al.*[9], the present study found no reduction of TNF-α or IL-6 levels in recipients but rather an increase of these markers at 6 months post-transplant.

Comparison of serum creatinine and cystatin C levels between the different sampling times showed a significant reduction in the two markers between admission and 30 days post-transplant ($p < 0.0001$) and between admission and 180 days post-transplant ($p < 0.0001$), in agreement with the literature[20-22]. This similarity in the creatinine and cystatin C profiles calls attention because mean creatinine levels tend to increase (1.6 mg/dL) in the sixth post-transplant month when compared to 30 days post-transplant (1.5 mg/dL), whereas the opposite is observed for cystatin C (1.6 mg/dL at 30 days post-transplant and 1.39 mg/dL at 180 days). In the study of Harada *et al.*[23], one of the risk factors associated with graft loss was renal function in the sixth post-transplant month evaluated based on creatinine using a level > 1.5 mg/dL as the criterion cut-off.

In a study performed by Geramizadeh *et al.*[24], there was a progressive decline in serum creatinine levels during the first 5 days post transplant, but the levels of serum cystatin C did not decrease in these days. There was also a significant correlation with serum cystatin C levels and steroid dose in the first 5 days post transplant ($r = 0.625$). This study suggests that during the first week, after transplantation, serum creatinine is still a good marker to assess the renal function. According to the authors, the level of cystatin C is dependent on high steroid dose in the first week post renal transplantation and should be used as a marker of renal function afterwards.

Karczewski *et al.*[10], studying 44 kidney transplant patients with and without rejection, evaluated serum concentrations of IL-2, IL-4, IL-5, IL-10, IFN-γ, and TNF-α in samples collected one day before and 2, 7, 14 and 30 days after transplantation.

Eleven of these patients developed episodes of acute rejection, which were associated with higher pretransplant serum levels of IFN-γ and IL-10. No significant difference in plasma levels of IL-2, IL-4, IL-5 or TNF-α were observed between the two groups.

Similar to the study of Karczewski *et al.*[10], no significant difference in serum IL-2 or TNF-α levels was observed at admission and at 30 days post-transplant between biopsied and non-biopsied patients. However, only 4% (1/23) of the patients presented graft rejection, whereas this prevalence was 25% (11/44) in the study of Karczewski *et al.*[10].

Ozdemir *et al.*[12] also studied the demographic, clinical and laboratory data of 141 kidney transplant recipients over a period of 5 years. The patients were divided into three groups based on serum CRP concentration: normal, intermittently high and consistently high. Renal graft survival rates were 90%, 72.6% and 11.1% in these groups, respectively. In addition, Cox regression analysis revealed that acute rejection, advanced recipient age and consistently high serum concentrations of CRP were associated with an elevated risk of renal graft loss. Intermittent elevations in serum CRP levels were not associated with an increased risk of graft loss. The authors concluded that consistently high post-transplant concentrations of CRP have a high negative predictive value for graft survival. According to the authors, kidney transplant recipients presenting an ongoing inflammatory process during the 5-year post-transplant period require additional efforts to control inflammation and to prolong graft survival. These considerations suggest the long-term investigation of other inflammatory markers which, if also consistently elevated, may compromise renal graft survival.

Recent studies demonstrate that novel kidney markers such as cystatin C, IL-18, kidney injury molecule 1, and neutrophil gelatinase-associated lipocalin serve as more accurate markers for acute kidney injury as compared with the more traditional marker, creatinine. Each individual kidney marker possesses its own strengths and weaknesses in determining the onset and severity of acute kidney injury. However, in combination, a panel of kidney markers may serve as powerful tools in diagnosing kidney injury with high accuracy[25].

Some limitations of the present study should be mentioned. The inclusion criteria did not permit to obtain a larger sample of patients. Serum samples were not obtained from donors at transplantation. Inflammation markers (cystatin C, IL-2, IL-6, and TNF-α) were investigated over a short period of time (180 days) and not during episodes of graft dysfunction.

Conclusion

Numerous studies are investigating markers of early renal graft dysfunction that would be able to detect the onset of renal leukocyte infiltration since the best marker has yet to be defined. The current study showed no significant correlations between CysC, IL-2, IL-6 and TNF-α serum levels in kidney transplant recipients at short-term follow-up. Moreover, CysC levels were very similar to creatinine levels in contrast to other inflammatory markers studied in biopsied and non-biopsied patients. When compared to the other inflammatory biomarkers, serum creatinine and cystatin C seem to be good predictors of early graft dysfunction in transplant patients and can be used to establish actions that prevent functional loss of the graft. Our results showed an impact of kidney transplant on the concentrations of the inflammatory markers studied, with IL-2, IL-6 and TNF-α representing an option for the long-term monitoring of renal grafts. However, further studies evaluating pre- and post-transplant inflammatory markers are necessary to confirm the findings of the present study.

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