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ORIGINAL ARTICLE

# Inflammation and endothelial activation are linked to renal function in long-term kidney transplantation

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## Summary

The aim of this study was to investigate the relationships between inflammation and adhesion molecules in long-term kidney transplantation. We measured serum concentrations of tumor necrosis factor-alpha (TNFα) and intercellular and vascular cell adhesion molecules (ICAM-1 and VCAM-1) in 35 renal transplant recipients (mean age of transplantation 5 ± 3 years) and in 35 chronic renal insufficiency (CRI) patients; twenty-six healthy subjects were enrolled as controls. Transplanted showed higher values than controls of TNFα ($P < 0.0001$), ICAM-1 ($P < 0.0001$), and VCAM-1 ($P < 0.0001$). CRI group as well exhibited higher concentrations than controls of TNFα ($P < 0.0001$), ICAM-1 ($P < 0.0001$), and VCAM-1 ($P < 0.0001$). Transplanted and CRI patients had similar blood pressure and renal function levels, and TNFα, ICAM-1, and VCAM-1 were not significantly different in the two groups. In transplanted group ICAM-1, VCAM-1, and TNFα correlated negatively and independently with glomerular filtration rate (GFR) – $P < 0.00001$ for all. TNFα as well correlated with ICAM-1 and VCAM-1 ($P < 0.001$, respectively). In CRI group, TNFα correlated with serum creatinine, ICAM-1, and VCAM-1 ($P = 0.01$ for all). In conclusion, in long-term renal transplantation, the level of kidney function and both inflammation and endothelial activation are closely related. In fact, the multiple regression analysis demonstrated that the level of kidney insufficiency and the levels of the studied molecules were independently associated.

## Introduction

Renal transplantation, the treatment of choice for end stage renal disease (ESRD), has significantly improved the long-term survival of nephropathic patients [1], for whom dialysis represents an indispensable therapy to avoid death caused by uremia. Nevertheless, dialysis itself is associated with a very high risk of cardiovascular death, about 30-fold greater than in the general population [2]. Although transplanted patients have a better cardiovascular outcome than dialysis patients, it remains worse than in the general population [2].

While acute rejection is now less common than in the past, chronic allograft nephropathy remains the first reason of graft loss [3]; this disease is clinically characterized as chronically deteriorating renal allograft function. Indeed, recent studies confirm that even a moderate reduction in kidney function is associated with increased cardiovascular risk, and the level of kidney function itself is an independent predictor of cardiovascular outcome and all-cause mortality [4]. The poor cardiovascular outcome of patients with Chronic Renal Failure (CRF) depends on an 'accelerated atherogenesis' [5]. One key event in the initiation and progression of atherosclerosis

is the adhesion of circulating leukocytes to the vascular wall. In large part, this process is mediated by a group of cellular adhesion molecules, which are expressed on the surface of vascular endothelial cells in response to pro-inflammatory cytokines. Several studies have shown that the alterations of endothelial function – considered as the earliest event in the development of atherosclerotic lesions – can be found in CRF, and that the markers of endothelial activation show a direct correlation with serum creatinine [6–7].

Furthermore, high serum levels of adhesion molecules may predict future cardiovascular events in the general population, in CRF, and in hemodialysis patients [8–10].

More recent studies on the pathogenesis of the atherosclerotic disease emphasize that inflammation plays a central role in atherosclerosis [11], and the markers of inflammation have been demonstrated to be reliable predictors of cardiovascular outcome in ESRD patients and in renal allograft recipients [12–13].

Actually, tumor necrosis factor-alpha (TNFα) and both intercellular and vascular cell adhesion molecules (ICAM-1 and VCAM-1) are considered to be markers of endothelial activation and vascular inflammation [14]. The former promotes expression of adhesion molecules, inflammatory cells recruitment and activation of T- and B cells, and the latter favors leukocytes adhesion to the vascular wall [14]. In recent studies performed in CRF patients, serum concentration of TNFα was shown to be positively correlated with serum concentrations of soluble ICAM-1 and VCAM-1 [6, 9].

In renal transplantation, TNFα is considered to be a useful marker and predictor of acute rejection [15–16]; less is known about its role in the long-term conditions.

The present study was aimed at evaluating inflammation and endothelial activation in a group of transplanted patients, which had a long post-transplantation follow-up and a good renal function. Therefore, we performed a cross-sectional study to evaluate plasma concentrations of TNFα and soluble adhesion molecules ICAM-1 and VCAM-1 in a group of patients with long-term transplanted kidney, in comparison with a group of patients with chronic renal insufficiency (CRI) having similar renal function and blood pressure.

## Materials and methods

In accordance with the Declaration of Helsinki and institutional guidelines, the protocol was approved by the local Ethical Committee and subjects were aware of the investigational nature of the study and agreed to participate after informed consent.

We considered 35 Caucasian male cadaver-donor kidney-transplanted hypertensive patients, having mean serum creatinine 1.56 ± 0.57 mg/dl; the mean age of transplantation was 5 ± 3 years. We also enrolled 35 Caucasian male affected by CRI matched to the transplanted patients for renal function and blood pressure. None of the patients with CRI was undergoing or underwent dialysis. The anamnestic mean duration of CRI was of 1.5 years. As diabetics had been excluded (to avoid a confounding influence on the levels of the studied molecules), CRI was due to nephroangiosclerosis in 21 subjects, autosomal dominant polycystic kidney disease (ADPKD) in five subjects, chronic glomerulonephritis in four patients, and was due to unknown causes in five subjects.

Twenty-six healthy subjects, recruited among our medical and paramedical staff, were enrolled as controls.

Glomerular filtration rate (GFR) was estimated by modification of diet in renal disease (MDRD) equation [17]. It resulted in 60.4 ± 22.63 ml/min for transplanted patients and in 58.3 ± 13.94 ml/min for CRI subjects. Patients were defined as hypertensives when clinic systolic/diastolic blood pressure (SBP/DBP) was >140/90 mmHg, and the severity of hypertension was defined according to the 2003 European Society of Hypertension Guidelines [18]. Clinic blood pressure was considered as the average of three consecutive measurements using a mercury sphygmomanometer after the subject had been supine for 5 min.

Exclusion criteria were: age <30 years and >65 years, apparent urinary tract or systemic infections, diabetes mellitus type 1 or 2, history of TIA or stroke, history of coronary artery disease or myocardial infarction, heart failure, abnormalities of cardiac rhythm, or conduction under pharmacological treatment. All transplanted patients were on maintenance immunosuppression with a triple-drug therapy, including cyclosporine-A (2 mg/kg/day), prednisone (8 mg/day), and azathioprine (1 mg/kg/day). Antihypertensive treatment was based on diuretics, calcium-channels blockers, and angiotensin receptors antagonists for both CRI and transplanted patients.

On the day of the study, at 9 AM, with the patients in supine position and after an overnight fast, blood samples were obtained from an indwelling forearm venous catheter to assay ICAM-1, VCAM-1, and TNFα.

### Laboratory methods

All endothelium-derived parameters were measured by ELISA using a solid-phase-specific sandwich enzyme-linked immunosorbent assay. Standard curves were constructed by using appropriated concentrations for each factor. Precautions were taken to avoid interference with other serum components [19]. Tumor Necrosis Factor was assayed by Amersham Biosciences kit (Little Chalfont, England); sensitivity was <5 pg/ml; reproducibility of both intra- and between-assay had a coefficient of variation <10%.

Adhesion molecules were measured by the Bender kit (Bender MedSystem Diagnostics GmbH, Vienna, Austria); the assay had a sensitivity of 3.3 ng/ml for ICAM-1 and of 0.9 ng/ml for VCAM-1. Intra- and inter-assay coefficients of variation were 4% and 3.1%, respectively.

### Statistical methods

Results are given as means ± SD.

Differences between groups were evaluated by using ANOVA and Student's *t*-test corrected with Bonferroni correction for multiple comparisons. Simple and multiple stepwise linear regression analyses were used to test the relationships between variables.

The null hypothesis was rejected at a two-tailed $P \leq 0.05$.

The statistical analyses were performed by using the SYSTAT DATA software package, version 5.2 (Systat, Evanston, IL, USA).

## Results

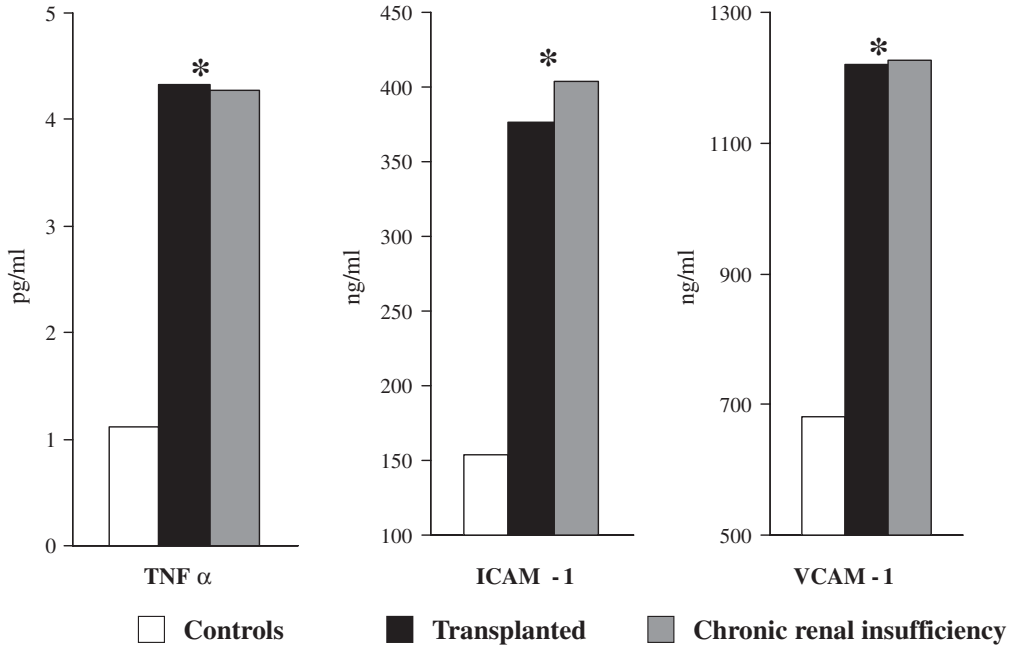
Table 1 shows clinical data of the three groups of subjects. The 'one-way' analysis of variance demonstrated a statistically significant difference between the three groups with regard to tumor necrosis factor-alpha, ICAM-1, and VCAM-1 ($P < 0.0001$, respectively).

**Table 1.** Clinical data of control subjects, of 35 renal transplanted and of 35 chronic renal insufficiency (CRI) patients.

| | Controls ($n = 26$) | Transplanted ($n = 35$) | CRI ($n = 35$) |
|---|---|---|---|
| Age (years) | 37.9 ± 10.7 | 49.3 ± 12.7 | 54.2 ± 15.1 |
| Body mass index (kg/m$^2$) | 23.6 ± 4.8 | 25.7 ± 4.4 | 27.4 ± 4.5 |
| Serum creatinine (mg/dl) | 0.93 ± 0.12 | 1.56 ± 0.57 | 1.54 ± 0.29 |
| Glomerular filtration rate (ml/min) | 105.6 ± 7.2 | 60.4 ± 22.63 | 58.3 ± 13.94 |
| Serum glucose (mg/dl) | 88.2 ± 5.7 | 91.1 ± 11.2 | 90.8 ± 9.6 |
| Serum cholesterol (mg/dl) | 175.3 ± 22 | 200.4 ± 34 | 188 ± 58 |
| Systolic blood pressure (mmHg) | 108.9 ± 12.9 | 139.7 ± 15.7 | 140.5 ± 22.3 |
| Diastolic blood pressure (mmHg) | 71.4 ± 9.6 | 86 ± 11.2 | 86.5 ± 15.3 |

Serum creatinine and the estimated GFR, as well as SBP and DBP, were similar in transplanted group and in CRI group (Table 1).

The comparison with healthy controls showed in the transplanted group significantly higher concentrations of all studied compounds (Fig. 1): TNFα 4.33 ± 1.12 vs.




*$P < 0.0001$ Transplanted versus controls, and chronic renal insufficiency versus controls.

Transplanted versus chronic renal insufficiency: ns.

**Figure 1** Mean values of plasma levels of tumor necrosis factor-alpha and soluble adhesion molecules ICAM-1 and VCAM-1 (intercellular and vascular cell adhesion molecules) in control subjects, in 35 renal transplanted and in 35 chronic renal insufficiency patients.

1.18 ± 0.28 pg/ml ($P < 0.0001$); ICAM-1: 376.8 ± 86.2 vs. 154.2 ± 26.4 ng/ml ($P < 0.0001$); VCAM-1: 1220.3 ± 268.1 vs. 681.6 ± 62.3 ng/ml ($P < 0.0001$). CRI group exhibited significantly higher concentrations of TNFα (4.27 ± 0.59 vs. 1.18 ± 0.28 pg/ml – $P < 0.0001$), ICAM-1 (403.7 ± 86.7 vs. 154.2 ± 26.4 ng/ml – $P < 0.0001$), and VCAM-1 (1227.4 ± 127.5 vs. 681.6 ± 62.3 ng/ml – $P < 0.0001$) than healthy controls.

Comparing transplanted with CRI patients not significant differences in mean serum concentrations of TNFα, ICAM-1, and VCAM-1 were pointed out (Fig. 1).

The linear analysis of correlation showed in the transplanted group a positive correlation of TNFα with both ICAM-1 ($r = 0.9341$; $P < 0.001$) and VCAM-1 ($r = 0.9269$; $P < 0.001$ – Fig. 2). With regard to renal function TNFα correlated positively with serum creatinine ($r = 0.6898$; $P < 0.001$) and negatively with GFR ($r = -0.6798$; $P < 0.001$ – Fig. 3). Adhesion molecules ICAM-1 and VCAM-1 (Fig. 4) were significantly correlated with serum creatinine ($r = 0.6537$ and 0.7009, respectively; $P < 0.001$ for both), and with GFR ($r = -0.6249$ and $-0.6483$, respectively; $P < 0.001$ for both).

Furthermore, TNFα ($r = 0.343$; $P < 0.05$), ICAM-1 ($r = 0.452$; $P < 0.01$), and VCAM-1 (0.359; $P < 0.05$) were correlated with DBP.

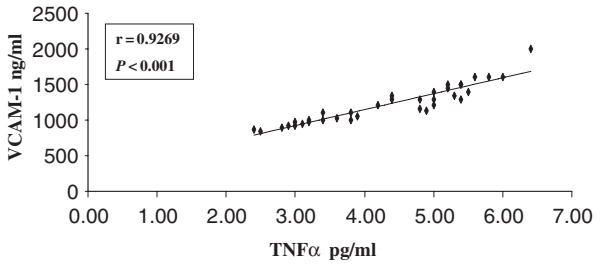



**Figure 2** Correlation of tumor necrosis factor-alpha with soluble VCAM-1 (vascular cell adhesion molecules) in 35 renal transplanted patients.

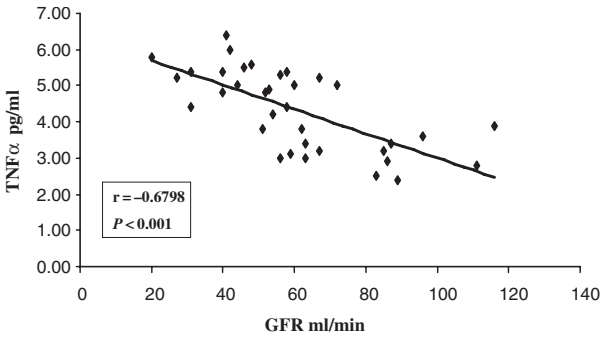



**Figure 3** Correlation of estimated glomerular filtration rate with tumor necrosis factor-alpha in 35 renal transplanted patients.

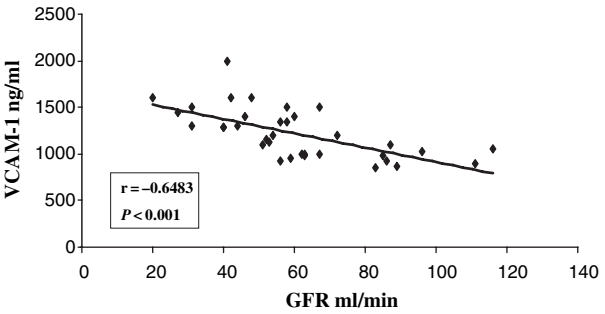



**Figure 4** Correlation of estimated glomerular filtration rate with soluble VCAM-1 (vascular cell adhesion molecules) in 35 renal transplanted patients.

In CRI group, a positive correlation of TNFα with both ICAM-1 and VCAM-1 ($r = 0.4500$ and 0.4692, respectively; $P = 0.01$ for both), and with serum creatinine ($r = 0.4117$; $P = 0.01$) was found. Soluble ICAM-1 correlated with SBP and DBP ($r = 0.537$ and 0.502, respectively; $P < 0.01$ for both), and VCAM-1 was correlated with SBP ($r = 0.413$; $P < 0.05$).

In both groups, the studied variables (TNFα, ICAM-1, and VCAM-1) did not correlate with age or body mass index.

The multiple regression analysis performed in the transplanted group demonstrated that TNFα levels were independently associated with DBP (β 0.276; $P < 0.03$), and with GFR (β −0.651; $P < 0.00001$).

In a multivariate model considering ICAM-1 as dependent variable and including GFR, SBP and DBP, ICAM-1 resulted tightly associated with DBP (β 0.329; $P < 0.03$) and GFR (β −0.583; $P < 0.00001$).

Finally, the multivariate analysis demonstrated that the correlations of VCAM-1 with DBP (β 0.295; $P < 0.03$) and GFR (β −0.618; $P < 0.00001$) were independent.

## Discussion

The aim of this study was to evaluate inflammation and endothelial activation, measured as serum levels of TNFα and soluble adhesion molecules ICAM-1 and VCAM-1, in long-term renal allograft recipients, comparing them with those of a group of CRI patients having similar renal function and BP. A further purpose was to identify the relationships between the studied parameters.

The comparison between transplanted and CRI patients did not show any difference with regard to the concentration of serum TNFα and soluble adhesion molecules ICAM-1 and VCAM-1.

In the transplanted subjects, a strong linkage between TNFα and both soluble adhesion molecules was found. Furthermore, both the former and the latter showed to be

tightly and independently correlated with the level of renal function.

A number of data have shown that after renal transplantation the markers of inflammation acutely predict the development of acute rejection [15–16]. The evolution of the inflammatory phenomenon, however, is less clear in the case of patients with a long-term transplantation.

We studied long-term transplanted subjects who showed increased TNFα plasma levels, which were similar to those of CRI patients. Furthermore, in our transplanted patients, TNFα was correlated with the declining renal function and with the degree of endothelial activation.

To the best of our knowledge, there are only few studies reporting a long post-transplantation follow-up, such as the recent one by Cueto-Manzano *et al.* [20], who demonstrated increased TNFα levels after 12 and 18 months of transplantation in a sample of 37 patients. Our results are in line with those by Cueto-Manzano *et al.* and, interestingly, the mean age of transplantation in our study is of 5 ± 3 years. Moreover, in a very recent study, we obtained similar results in a smaller group of transplant recipients, demonstrating a strong association between inflammation markers and oxidative stress [21].

The results of our cross-sectional study suggest that a 'micro-inflammatory' state can be found in renal allograft recipients with a long post-transplantation follow-up. It is conceivable that although renal transplantation partially restores renal function, in the long-term setting a low grade of inflammation probably remains, despite of the immunosuppressive treatment. Furthermore, the degree of inflammation seems to be similar to that of no-graft patients but with similar degree of kidney insufficiency.

Our findings have also shown a similar grade of endothelial activation in transplanted and in CRI patients, in spite of the immunosuppressive therapy taken by the transplanted subjects. This could explain the accelerated atherosclerosis of CRI subjects as well as the high prevalence of cardiovascular disease mortality in transplanted patients. Actually, it is well known that approximately 15% of renal transplant recipients have coronary artery disease, and the impact of immunosuppressive therapy on the progression of cardiovascular disease is unknown [22].

We can hypothesize that in long-term renal transplantation there is a close relationship between the level of kidney function and both inflammation and endothelial activation, independently of the immunosuppressive treatment. Indeed, the influence of the immunosuppressive drugs on these issues has not sufficiently been understood to date. In fact, the multiple regression analysis demonstrated that GFR was tightly and independently associated with the studied markers of inflammation and endothelial activation.

Finally, all our patients were hypertensives. It is well known that in addition to being an important risk factor for cardiovascular disease, arterial hypertension may accelerate the decline of renal function. In the present study, in the transplanted group, TNFα, ICAM-1 and VCAM-1 were all independently correlated to DBP. Therefore, it is conceivable that blood pressure levels contribute in triggering the inflammatory process even in hypertensives patients who underwent renal transplantation.

The present study has some limitations: the first is the small number of patients. We cannot exclude that the restricted size of the groups could influence the results. Moreover, the blood concentrations of the molecules we studied could not exactly reflect the tissue condition, and it is conceivable that in renal insufficiency a reduced clearance of these molecules could take place, influencing the results. Finally, a bioptic study to be associated with the biomarkers analysis could provide for more deepened conclusions.

In conclusion, in a limited fashion with the findings by our cross-sectional study, the level of kidney insufficiency seems to be strictly linked to the degree of inflammation and of endothelial activation, which therefore could interplay each other in our transplanted patients.

Whether or not inflammation may influence the decline in renal function remains to be elucidated.

Further studies are needed to confirm this hypothesis.

## Acknowledgements

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## Conflict of interest

There is no conflict of interest to declare.

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