CHAPTER 3

ELEVATED LEVELS OF C-REACTIVE PROTEIN INDEPENDENTLY PREDICT ACCELERATED DETERIORATION OF GRAFT FUNCTION IN RENAL TRANSPLANT RECIPIENTS

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CHAPTER 3

ABSTRACT

Background

Chronic transplant dysfunction is characterized by a gradual decline in renal function with slowly rising serum creatinine. The underlying mechanism is thought to include inflammation and atherosclerosis. C-reactive protein (CRP) is a well established marker of both inflammation and atherosclerosis. In this prospective study we investigated whether CRP could be of use as a clinical marker for early identification of renal transplant recipients at increased risk of deterioration of graft function.

Methods

In this prospective study, all participating patients \( n = 606 \) visited the outpatient clinic at least once a year, and serum creatinine was assessed at every visit. Subjects with a follow-up of less \(<1\) year \( n = 31 \) were excluded from analysis.

Results

A total of 575 patients participated at a median (interquartile range) time of 5.9 (2.6-11.3) years post-transplantation. Median time of follow-up was 3.0 (2.4-3.4) years. Changes in serum creatinine during follow-up were \(-0.45 (-4.83-4.76) \) µmol/L/yr in 172 subjects with CRP \(<1.0 \) mg/L, \( 1.04 (-3.36-6.12) \) µmol/L/yr in 184 subjects with CRP 1.0-3.0 mg/L, and \( 2.34 (-3.33-9.07) \) µmol/L/yr in 219 subjects with CRP >3.0 mg/L \( P < 0.001 \) for comparison of the three groups). Proteinuria \( P = 0.003 \), CMV IgG titre \( P = 0.01 \), donor age \( P = 0.01 \), CRP concentration \( P = 0.02 \), recipient age \( P = 0.02 \), and recipient gender \( P = 0.047 \) were independently associated with change in serum creatinine during follow-up in a multivariate analysis.

Conclusions

Elevated levels of CRP independently predict accelerated deterioration of graft function in renal transplant recipients >1 year post-transplantation. Further prospective studies are required to investigate whether early intervention can prevent deterioration of graft function in subjects with elevated levels of CRP.
INTRODUCTION

Chronic transplant dysfunction (CTD) is one of the leading causes of late allograft loss after renal transplantation. CTD is a nonspecific term describing a clinical syndrome which, in most cases, is the functional consequence of chronic allograft nephropathy (CAN). Clinically, CTD is characterized by a gradual decline in renal function with slowly rising serum creatinine, proteinuria, and hypertension of increasing severity. This usually occurs more than one year after transplantation. Once serum creatinine has started to rise, or proteinuria appears, the decline in renal function is usually inevitable. Hence, it is important to identify markers that can predict appearance of CTD as early as possible.

There is an emerging notion in the renal transplant community that the pathogenesis of CTD includes inflammation and intragraft atherosclerosis. C-reactive protein (CRP) is the prototypical marker of the acute phase response of inflammation. CRP has also been shown to be a marker of the inflammatory component of atherosclerosis in the systemic vasculature. As such, it has been shown to be a predictor of cardiovascular morbidity and mortality in the general population.

In this prospective study we aimed to investigate whether CRP could be of use as a clinical marker for early identification of renal transplant recipients at risk of deterioration of graft function.

MATERIALS AND METHODS

Study design and patients

In this longitudinal prospective study, all renal transplant recipients who visited our outpatient clinic between August 2001 and July 2003, who had a functioning graft for at least one year were eligible to participate at their next visit to the outpatient clinic (baseline). A total of 606 out of 847 (72%) eligible renal transplant recipients signed written informed consent. The group that did not sign informed consent was comparable with the group that signed informed consent concerning age, gender, body mass index (BMI), baseline serum creatinine, creatinine clearance, and proteinuria. All participating subjects visited the outpatient clinic at least once a year, and serum creatinine was assessed at every visit. The last known visit to the outpatient clinic was considered as follow-up date, and the serum creatinine assessed at this date was used as outcome variable. Follow-up date for patients who died with a functioning graft (n = 32, median CRP = 3.09 (1.28-7.18) mg/mL) was defined as the last visit to the outpatient clinic prior to death. Follow-up date for patients with graft loss (n = 17, median CRP = 4.83 (0.92-10.5) mg/mL) was defined as the last visit to the outpatient clinic before starting dialysis. Baseline visits were postponed until symptoms had resolved in patients with fever or other signs of infection, and subjects diagnosed with cancer other than cured skin cancer
were not considered eligible for the study. Excluded from analysis were recipients with a follow-up of <1 year \((n=31)\), leaving a total of 575 recipients for analysis.

Details of this study have been published previously.\textsuperscript{7,8} The Institutional Review Board approved the study protocol (METc 01/039) which was in adherence to the Declaration of Helsinki.\textsuperscript{9} Funding sources had neither a role in the collection and analysis of data, nor in the submission and publication of the manuscript.

**Immunosuppressive medication**

Standard immunosuppression consisted of the following: from 1968 until 1989 prednisolone (10 mg/d) and azathioprine (100 mg/d). From January 1989 until February 1993 cyclosporin standard formulation (Sandimmune, Novartis; 10 mg/kg; trough-levels of 175-200 μg/L in first 3 months, 150 μg/L between 3 and 12 months post-transplant, and 100 μg/L thereafter) combined with prednisolone (starting with 20 mg/d, rapidly tapered to 10 mg/d). From March 1993 until May 1996 cyclosporin microemulsion (Neoral, Novartis Pharma b.v., Arnhem, the Netherlands; 10 mg/kg; trough-levels idem) and prednisolone. From May 1996 to date mycophenolate mofetil (MMF) (Cellcept, Roche b.v., Woerden, The Netherlands; 2 g/day) was added. Current medication was extracted from the medical record.

**Baseline measurements**

Body mass index was calculated as weight in kilograms (kg) divided by height in square meters (measured to the nearest 0.5 kg and 0.5 cm respectively). Waist circumference was measured midway between the 10th rib and the iliac crest. Hip circumference was measured at the level of the trochanter major. Blood pressure was measured as the average of three automated (Omron M4; Omron Europe B.V., The Netherlands) measurements with 1-minute intervals after a 6-minute rest in supine position. Diabetes mellitus was diagnosed if the fasting plasma glucose concentration was \(\geq 7.0\) mmol/L and/or anti-diabetic medication was used.

Serum creatinine levels were determined using a modified version of the Jaffé method (MEGA AU 510, Merck Diagnostica, Darmstadt, Germany). High sensitivity CRP was determined once at baseline and measured using a double plated ELISA assay as described before;\textsuperscript{10} the lowest limit of detection was 0.002 mg/L. Cytomegalovirus (CMV) IgG was assessed by routine ELISA assay as described previously.\textsuperscript{11} Plasma glucose was determined by the glucose-oxidase method (YSI 2300 Stat plus, Yellow Springs, OH, USA). Total protein concentration was analysed using the Biuret reaction (MEGA AU 510, Merck Diagnostica, Darmstadt, Germany) and proteinuria was defined as urinary protein excretion \(\geq 0.5\) g/24h.
Statistical analysis

Analyses were performed with SPSS version 12.0 (SPSS Inc. Chicago, IL). Parametric variables are expressed as mean ± standard deviation, whereas non-parametric variables are given as median (interquartile range). Skewed data, such as serum creatinine levels and CRP levels, were normalized by logarithmic transformation in all analyses. A two-sided \( P < 0.05 \) was considered to indicate statistical significance.

For the analyses of CRP as predictor of changes in graft function we primarily applied the cut points of <1.0 mg/L, 1.0-3.0 mg/L, and >3.0 mg/L, which are endorsed as clinical cut points for CRP evaluation by the American Heart Association and the Centers for Disease Control and Prevention. The Kruskal-Wallis test was applied to investigate differences between the three groups. It has been demonstrated that differences between baseline serum creatinine and serum creatinine after prolonged follow-up are more predictive of future graft loss than slopes of creatinine over time. To further investigate whether CRP and other potential risk factors for CTD were associated with an increase in serum creatinine between baseline and follow-up, linear regression analyses were performed with log serum creatinine at follow-up as dependent variable. In order to adjust for the fact that patients started with different baseline allograft function and for the fact that there is different length of follow-up, baseline log serum creatinine and time between baseline and follow-up were included in all analyses (Model 1 in the univariate analyses). The effect of adjustments can be judged by comparing the (standardized) regression coefficients and \( P \)-values of an association before and after adjustment. Strengths of associations of different variables can be compared using standardized regression coefficients. In Model 2 the associations were further adjusted for recipient age at baseline, and for recipient gender. In Model 3 further adjustments for time between transplantation and baseline were applied. All recipient- and transplant-related baseline characteristics listed in Tables 1 and 2 were analysed. The variables that showed at least a trend \( (P \leq 0.2) \) are presented in Tables 3 and 4.

Second, to determine which of the variables from Model 3 were independently associated with log serum creatinine at follow-up, a backward multivariate linear regression procedure was performed. Co-variables with a \( P \)-value \( \leq 0.1 \) in Model 3 were included. As secondary analyses, the procedure was repeated with additional inclusion of variables with a \( P \)-value > 0.1 and \( \leq 0.2 \). Further secondary analyses were performed with (1) additional inclusion of log serum creatinine at one year after transplantation and time between one year after transplantation and baseline assessment for our study and (2) additional inclusion of baseline MDRD estimated glomerular filtration rate (eGFR), and (3) additional exclusion of subjects with CRP ≥ 10 mg/L in all regression models.
RESULTS

Recipient and transplant characteristics are presented in Tables 1 and 2. A total of 575 patients (55% male, aged 51±12 years at baseline, 83% cadaveric transplants) were analysed. Median time between transplantation and baseline measurements was 5.9 (2.6-11.3) years, and the median time of follow-up beyond baseline measurements was 3.0 (2.4-3.4) years.

Median CRP level at baseline was 1.93 (0.76-4.70) mg/l. The distribution of CRP values ranged from 0.07 to 83.7 mg/l. CRP was ≥10 mg/l in 63 subjects. Median serum creatinine concentration was 131.5 (112.0-150.0) μmol/L, 133.5 (111.0-163.8) μmol/L and 134.0 (111.0-174.0) μmol/l in subjects with CRP <1.0 mg/l, 1.0-3.0 mg/l and >3.0 mg/l, respectively. The duration of follow-up was similar for the three groups: 3.01 (2.50-3.30) years for the group with CRP <1.0 mg/l, 2.99 (2.44-3.34) years for the group with CRP 1-3 mg/l, and 3.01 (2.21-3.39) years for the group with CRP >3.0 mg/l. Changes in serum creatinine during follow-up were -0.45 (-4.83;4.76) μmol/l/yr in 172 subjects with CRP <1.0 mg/L, 1.04 (-3.36;6.12) μmol/l/yr in 184 subjects with CRP 1-3 mg/l, and 2.34 (-3.33;9.07) μmol/L/yr in 219 subjects with CRP >3 mg/l (P < 0.05 for comparison of the three groups, Figure 1).

There was a significant correlation between serum creatinine at baseline and CRP (r=0.11, P=0.01). Tables 3 and 4 show the standardized regression coefficients (β) and P-values of analyses of the associations of recipient-related and transplanted kidney-related baseline characteristics with log serum creatinine at follow-up. Smoking (standardized β=0.05, P<0.05), proteinuria (standardized β=0.08, P=0.001), log CMV IgG titre (standardized β=0.05, P=0.02), and log CRP concentration (standardized β=0.05, P=0.05) were associated with higher log serum creatinine at follow-up after adjustment for log baseline serum creatinine and time between baseline and follow-up. Additional adjustment for recipient age and gender (Table 3, Model 2), and further adjustment for time between transplantation and baseline (Table 3, Model 3) did not materially change these associations.

![Figure 1. Change in serum creatinine concentration between baseline and follow-up according to levels of CRP concentration using the cut points <1.0 mg/L, 1.0-3.0 mg/L and >3.0 mg/L. Differences between groups were tested using the Kruskal-Wallis test.](image)
Table 1. Recipient-related baseline characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td></td>
</tr>
<tr>
<td>Transplantation – baseline, years</td>
<td>5.9 (2.6-11.3)</td>
</tr>
<tr>
<td>Baseline – follow-up, years</td>
<td>3.0 (2.4-3.4)</td>
</tr>
<tr>
<td>Recipient demographics</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>51.2±12.1</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>317 (55)</td>
</tr>
<tr>
<td>History of cardiovascular disease</td>
<td></td>
</tr>
<tr>
<td>MI, n (%)</td>
<td>44 (8.0)</td>
</tr>
<tr>
<td>TIA/CVA, n (%)</td>
<td>28 (5.5)</td>
</tr>
<tr>
<td>Ethnicity of recipient</td>
<td></td>
</tr>
<tr>
<td>Caucasian, n (%)</td>
<td>554 (96)</td>
</tr>
<tr>
<td>Body composition measurements</td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.1±4.31</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>97.2±15.8</td>
</tr>
<tr>
<td>Hip circumference, cm</td>
<td>99.8±8.82</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>124 (22)</td>
</tr>
<tr>
<td>Renal allograft function</td>
<td></td>
</tr>
<tr>
<td>Serum creatinine, μmol/L</td>
<td>133 (111-164)</td>
</tr>
<tr>
<td>Creatinine clearance, mL/min</td>
<td>63.2±21.9</td>
</tr>
<tr>
<td>Proteinuria, n (%)</td>
<td>149 (26)</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
</tr>
<tr>
<td>Systolic pressure, mmHg</td>
<td>153±22.6</td>
</tr>
<tr>
<td>Diastolic pressure, mmHg</td>
<td>89.9±9.9</td>
</tr>
<tr>
<td>Antihypertensive therapy, n (%)</td>
<td>500 (87)</td>
</tr>
<tr>
<td>Glycaemia</td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>4.50 (4.10-5.00)</td>
</tr>
<tr>
<td>Posttransplant diabetes, n (%)</td>
<td>100 (17)</td>
</tr>
<tr>
<td>Use of antidiabetic drugs, n (%)</td>
<td>76 (13)</td>
</tr>
<tr>
<td>CMV status</td>
<td></td>
</tr>
<tr>
<td>Seropositivity after transplantation</td>
<td>415 (72)</td>
</tr>
<tr>
<td>CMV IgG titer, U/mL</td>
<td>67.0 (0.0-151)</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>1.93 (0.76-4.70)</td>
</tr>
</tbody>
</table>

Parametric variables are expressed as mean ± SD, whereas non-parametric variables are given as median (interquartile range).

MI, myocardial infarction; TIA/CVA, transient ischaemic attack/cerebrovascular accident.

Table 2. Transplanted kidney-related baseline characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor demographics</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>36.8±15.4</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>312 (55)</td>
</tr>
<tr>
<td>Primary renal disease, n (%)</td>
<td></td>
</tr>
<tr>
<td>Primary glomerular disease</td>
<td>160 (28)</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>37 (6)</td>
</tr>
<tr>
<td>Tubular interstitial disease</td>
<td>89 (15)</td>
</tr>
<tr>
<td>Polycystic renal disease</td>
<td>103 (18)</td>
</tr>
<tr>
<td>Dysplasia and hypoplasia</td>
<td>21 (4)</td>
</tr>
<tr>
<td>Renovascular disease</td>
<td>30 (5)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>22 (4)</td>
</tr>
<tr>
<td>Other or unknown cause</td>
<td>113 (20)</td>
</tr>
<tr>
<td>Ischemia times</td>
<td></td>
</tr>
<tr>
<td>Warm ischemia times, min</td>
<td>35.0 (30.0-40.0)</td>
</tr>
<tr>
<td>Cold ischemia times, hr</td>
<td>22.0 (14.0-27.0)</td>
</tr>
<tr>
<td>HLA mismatches</td>
<td></td>
</tr>
<tr>
<td>HLA-AB, number</td>
<td>1.32±1.05</td>
</tr>
<tr>
<td>HLA-DR, number</td>
<td>0.42±0.58</td>
</tr>
<tr>
<td>Delayed graft function, days</td>
<td>0.0 (0.0-0.0)</td>
</tr>
<tr>
<td>Acute rejection treatment (%)</td>
<td></td>
</tr>
<tr>
<td>High doses corticosteroids</td>
<td>30.6</td>
</tr>
<tr>
<td>Antilymphocyte antibodies</td>
<td>14.4</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td></td>
</tr>
<tr>
<td>Prednisolone dose, mg/day</td>
<td>10.0 (7.5-10.0)</td>
</tr>
<tr>
<td>Cyclosporine, n (%)</td>
<td>372 (65)</td>
</tr>
<tr>
<td>trough-level, μg/L</td>
<td>107 (79.3-139)</td>
</tr>
<tr>
<td>Tacrolimus, n (%)</td>
<td>83 (14)</td>
</tr>
<tr>
<td>trough-level, μg/L</td>
<td>8.70 (6.00-10.7)</td>
</tr>
<tr>
<td>Proliferation inhibitor</td>
<td></td>
</tr>
<tr>
<td>Azathioprine, n (%)</td>
<td>186 (32)</td>
</tr>
<tr>
<td>Mycophenolate mofetil, n (%)</td>
<td>243 (42)</td>
</tr>
</tbody>
</table>

Parametric variables are expressed as mean ± SD, whereas non-parametric variables are given as median (interquartile range).

None of the transplanted kidney-related baseline characteristics was significantly associated with log serum creatinine at follow-up when the associations were only adjusted for log baseline serum creatinine and time between baseline and follow-up. However, the association of donor age with log serum creatinine at follow-up appeared to be confounded by recipient age and gender, because after additional adjustment for these factors, the association of donor age with log serum creatinine at follow-up became significant (standardized β=0.05, P=0.04, Table 4, Model 2). This association became even stronger after further adjustment for time between transplantation and baseline (standardized β=0.07, P=0.01, Table 4, Model 3). Furthermore, after adjustment for time between transplantation and baseline, the association of renovascular disease with log
serum creatinine at follow-up also reached borderline significance (standardized $\beta=0.05$, $P=0.05$, table 4, Model 3).

**Table 3.** Regression analyses of associations between recipient-related baseline characteristics and log serum creatinine at follow up.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Std. $\beta$</td>
<td>$P$-value</td>
<td>Std. $\beta$</td>
</tr>
<tr>
<td>Recipient demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>-0.03</td>
<td>0.2</td>
<td>...</td>
</tr>
<tr>
<td>Gender (female = 0, male = 1)</td>
<td>0.03</td>
<td>0.2</td>
<td>...</td>
</tr>
<tr>
<td>Body composition measurements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
<td>0.02</td>
<td>0.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Smoking at baseline (no = 0, yes = 1)</td>
<td>0.05</td>
<td>0.048</td>
<td>0.04</td>
</tr>
<tr>
<td>Renal allograft function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteinuria baseline (no = 0, yes = 1)</td>
<td>0.08</td>
<td>0.001</td>
<td>0.07</td>
</tr>
<tr>
<td>Blood pressure at baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic pressure, mmHg</td>
<td>0.04</td>
<td>0.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Systolic pressure, mmHg</td>
<td>0.02</td>
<td>0.3</td>
<td>0.04</td>
</tr>
<tr>
<td>Glycaemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes (no = 0, yes = 1)</td>
<td>0.03</td>
<td>0.2</td>
<td>0.04</td>
</tr>
<tr>
<td>Use of antidiabetic drugs (no = 0, yes = 1)</td>
<td>0.02</td>
<td>0.3</td>
<td>0.03</td>
</tr>
<tr>
<td>CMV status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seropositivity after ntx (no = 0, yes = 1)</td>
<td>0.03</td>
<td>0.2</td>
<td>0.04</td>
</tr>
<tr>
<td>CMV IgG titer, U/ml</td>
<td>0.05</td>
<td>0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>0.05</td>
<td>0.05</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Log serum creatinine at follow-up was entered in the regression analyses as dependent variable. Model 1 is adjusted for log serum creatinine at baseline and time between baseline and follow-up; Model 2 is additionally adjusted for recipient age and gender; Model 3 is further adjusted for time between transplantation and baseline. $^a$Std. $\beta$, standardized $\beta$.

**Table 4.** Regression analyses of associations between transplanted kidney-related baseline characteristics and log serum creatinine at follow up.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Std. $\beta$</td>
<td>$P$-value</td>
<td>Std. $\beta$</td>
</tr>
<tr>
<td>Donor demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>0.05</td>
<td>0.06</td>
<td>0.05</td>
</tr>
<tr>
<td>Gender (female = 0, male = 1)</td>
<td>-0.007</td>
<td>0.8</td>
<td>-0.005</td>
</tr>
<tr>
<td>Primary renal disease (no = 0, yes = 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary glomerular disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>0.02</td>
<td>0.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Tubular interstitial disease</td>
<td>0.02</td>
<td>0.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Polycystic renal disease</td>
<td>-0.003</td>
<td>0.9</td>
<td>0.006</td>
</tr>
<tr>
<td>Dysplasia and hypoplasia</td>
<td>0.03</td>
<td>0.3</td>
<td>0.02</td>
</tr>
<tr>
<td>Renovascular disease</td>
<td>0.04</td>
<td>0.07</td>
<td>0.04</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.01</td>
<td>0.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Other or unknown cause</td>
<td>0.01</td>
<td>0.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Ischemia times</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warm ischemia times, min</td>
<td>0.03</td>
<td>0.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Cold ischemia times, hr</td>
<td>-0.006</td>
<td>0.8</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Log serum creatinine at follow-up was entered in the regression analyses as dependent variable. Model 1 is adjusted for log serum creatinine at baseline and time between baseline and follow-up; Model 2 is additionally adjusted for recipient age and gender; Model 3 is further adjusted for time between transplantation and baseline. $^a$Std. $\beta$, standardized $\beta$. 

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To determine which variables were independently associated with serum creatinine at follow-up, we performed a backward linear regression analysis with log serum creatinine at follow-up as dependent variable (Table 5). Co-variables from Model 3 with a $P$-value $\leq 0.1$ were included in the multivariate analysis.

Table 5. Multivariate analysis of determinants and associates of log serum creatinine at follow up in renal transplant recipients.

<table>
<thead>
<tr>
<th></th>
<th>Standardized $\beta$</th>
<th>$\beta$</th>
<th>95% CI of $\beta$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.1</td>
<td>0.1</td>
<td>0.01; 0.3</td>
<td>0.03</td>
</tr>
<tr>
<td>Proteinuria (no = 0, yes = 1)</td>
<td>0.07</td>
<td>0.03</td>
<td>0.01; 0.04</td>
<td>0.003</td>
</tr>
<tr>
<td>Log CMV IgG, U/mL</td>
<td>0.06</td>
<td>0.01</td>
<td>0.002; 0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Donor age, per 10 years</td>
<td>0.06</td>
<td>0.01</td>
<td>0.001; 0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Log CRP concentration, mg/L</td>
<td>0.06</td>
<td>0.02</td>
<td>0.003; 0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Recipient age, per 10 years</td>
<td>-0.05</td>
<td>-0.01</td>
<td>-0.01; -0.001</td>
<td>0.02</td>
</tr>
<tr>
<td>Recipient gender (female = 0, male = 1)</td>
<td>0.05</td>
<td>0.02</td>
<td>0.0002; 0.03</td>
<td>0.047</td>
</tr>
</tbody>
</table>

$R^2 = 0.72$, $F$-statistic=202, total df=571, $P<0.0001$. Log serum creatinine at follow-up was entered in the regression analyses as dependent variable. Recipient age, recipient gender, time between transplantation and baseline, smoking status at baseline, proteinuria, diastolic blood pressure, systolic blood pressure, diabetes, log sVCAM-1 concentration, CMV seropositivity recipient after ntx, log CMV IgG, log CRP concentration, donor age, and renovascular disease were enclosed as independent variables in the multivariate analysis. Log serum creatinine at baseline and time between baseline and follow-up were also enclosed in the multivariate analysis.

Proteinuria (standardized $\beta$=0.07, $P=0.003$), log CMV IgG titre (standardized $\beta$=0.06, $P=0.01$), log CRP concentration (standardized $\beta$=0.06, $P=0.01$), donor age (standardized $\beta$=0.06, $P=0.02$), recipient age (standardized $\beta$=0.05, $P=0.02$), and recipient gender (standardized $\beta$=0.05, $P=0.047$) appeared to be independent determinants of log serum creatinine at follow-up. The outcome of the multivariate analysis did not change when co-variables from Model 3 with a $P$-values $>0.1$ and $\leq 0.2$ were also included. The results of the regression models did also not materially change when log serum creatinine at one year after transplantation and time between one year after transplantation and baseline assessment were included in the analyses. The results did also not materially change if the analyses were repeated with adjustment for baseline eGFR. The results did also not materially change when the 63 subjects with CRP $\geq 10$ mg/L were excluded from analyses.

**DISCUSSION**

In this longitudinal prospective study, we investigated whether CRP could be of clinical use for early identification of renal transplant recipients at risk for deterioration of graft function. The main finding of our study is that relatively high post-transplant levels of CRP, and especially CRP concentrations above the clinically accepted cut-off point of 3 mg/L, independently predict deterioration of graft function.
Leading causes of late allograft loss are patient mortality owing to cardiovascular disease (CVD) and development of CTD.\textsuperscript{1} An emerging notion in transplantation is that CVD and CTD share chronic low-grade inflammation and atherogenesis as pathogenetic factors.\textsuperscript{1,5} CRP is not only the prototypical acute-phase reactant marker of inflammation, but it is also a sensitive marker for the development of CVD and all-cause mortality in the general population.\textsuperscript{6,14} In several cross-sectional studies in healthy subjects and patients with chronic renal insufficiency, it has been shown that there is an association between CRP and impaired renal function.\textsuperscript{15,16} Only a few studies have investigated whether CRP is associated with future decline in renal function. In a case-control study in 15 renal transplant recipients, Fink et al found that pre-transplant CRP concentrations are associated with post-transplant risk of development of CAN in renal transplant recipients.\textsuperscript{17} In another study, it was found that CRP levels 1 month post-transplant predict future occurrence of graft failure.\textsuperscript{18} It was not investigated in that study whether CRP was still predictive if other well-known predictors of graft failure, such as proteinuria, are taken into account. Our study is the first to show that CRP can be used for early identification of subjects at risk for deterioration of graft function, independent of other clinically accepted predictors of graft failure. It remains to be demonstrated whether early intervention is feasible.

Several explanations for the association between CRP and impaired renal function are possible. Atherosclerosis is nowadays considered to be a chronic inflammatory process in the arterial wall.\textsuperscript{19} It may therefore be that atherosclerosis in the renal vasculature is involved. Corroborative for the hypothesis of involvement of atherosclerosis is that we recently found that levels of CRP are associated with several endothelial function markers, such as soluble intercellular adhesion molecule type 1 (sICAM-1), soluble vascular cellular adhesion molecule type 1 (sVCAM-1), and sE-selectin concentrations in renal transplant recipients.\textsuperscript{8} The association between CRP and impaired renal function might also involve uraemia, which has been demonstrated to be a proinflammatory state itself.\textsuperscript{20} Third, it might be that CRP is a marker of a chronic low-grade immune-mediated response to the renal allograft. Another possible explanation could be that CRP itself has an active role in atherogenesis. This hypothesis is supported by recent studies suggesting that CRP is not only a biomarker but also an active mediator in the pathogenesis of atherosclerosis.\textsuperscript{14,21} Fitzgerald et al have furthermore recently shown that elevated levels of pretransplant CRP predict increased intimal thickening and stenosis after arterial allograft transplantation in a primate model.\textsuperscript{22} These results suggest that pretransplantation occult systemic inflammation may lead to inflammatory allograft vessel wall changes after transplantation.

Clinically CTD is characterized by a gradual decline in renal function with a slowly rising serum creatinine level accompanied by proteinuria.\textsuperscript{3,4} Several other studies have
shown that proteinuria is an independent risk factor for a decline in renal function in renal transplant recipients.\textsuperscript{3,23} Our finding of proteinuria as an independent predictor for a future increase in serum creatinine more than one year post-transplantation is therefore in line with existing literature.

CMV IgG was also an independent predictor of a future increase in serum creatinine. CMV is an important pathogen in renal transplant recipients, which remains in a latent state or persists as chronic low-grade inflammation.\textsuperscript{24} The frequency of CMV seropositivity after transplantation varies from 59 to 100%, whereas actual CMV disease is present in 8 to 32% of renal transplant recipients.\textsuperscript{24,25} CMV seropositivity has been associated with atherosclerosis as well as with CTD,\textsuperscript{26} although these associations remain controversial.\textsuperscript{27} Possible mediators of CMV-related CTD include various cytokines and adhesion molecules, including ICAM-1 and VCAM-1, which have been shown to be elevated in the blood of renal transplant recipients with active CMV infection.\textsuperscript{28} In this study there was a significant association of the level of the anti-CMV IgG antibody titer and the level of the adhesion molecules sICAM-1 and sVCAM-1 (data not shown). The increased expression of these endothelial function parameters to the endothelial surface could be an early event in atherogenesis or a sign of chronic low-grade inflammation.\textsuperscript{29} The fact that we found that high anti-CMV IgG antibody titers, rather than seropositivity itself, were associated with a increase in serum creatinine may indicate that high CMV titres counterbalance more active smouldering infection. A possible explanation for the fact that this was independent of CRP might be that CMV (re)activation causes local, intragraft inflammation without notable systemic effects. Our finding that high titres of anti-CMV IgG antibody predict a future increases in serum creatinine needs to be confirmed in other studies on predictors of changes in serum creatinine or graft loss. If so, it needs to be investigated whether titers of anti-CMV antibodies actually reflect the level of activity at which a subclinical CMV infection is smouldering, and whether adjustment of immunosuppressive treatment or treatment with antiviral agents suffice to reduce the level of activity at which the CMV infection is smouldering, and whether this translates in better graft (and recipient) survival.

Several studies have shown that younger age of the recipient, older age of the donor, and male sex of the recipient are risk factors for the development of CTD.\textsuperscript{4,30-32} Our findings of younger age of the recipient, older age of the donor, and male sex of the recipient as independent predictors of a future increase in serum creatinine are therefore in line with existing literature.

Formulas for the calculation of eGFR are neither validated nor intended for assessment or analysis of changes in renal function. If a previously assessed creatinine value is available, calculation and comparison of two eGFRs will not provide more information than comparison of the two creatinine values on which they are based. If one
CHAPTER 3

compares two eGFR’s, one seemingly accounts for individual changes in muscle mass. The reality is that one, in this way, only accounts for average changes in muscle mass. If age increases to the same extent in a group of subjects, change in renal function according to eGFR will be similar in all subjects. Analysis of changes in eGFR instead of changes in creatinine would give the false impression that potential confounding by individual rather than average changes in muscle mass, is taken into account. This can introduce unwanted collinearity and over-adjustment if age, sex, and eGFR are introduced in multivariate analyses at the same time. Results of analyses of age and sex as determinants of changes in serum creatinine can therefore be interpreted much more unambiguously than results from analysis of changes in eGFR. We therefore primarily chose to investigate changes in serum creatinine rather than changes in estimated GFR. The secondary analyses that we performed with GFR estimated by MDRD did not reveal results that were materially different from our primary analysis.

The present study has several limitations. First, because the study population almost entirely consisted of patients of Caucasian ethnicity, the applicability of our results to more racially diverse renal transplant population may be limited. Furthermore, in this renal transplant population there was little variation in the use of immunosuppressive medication or in the use of steroids. Therefore, it cannot be excluded that a higher variation in use of these drugs would influence levels of CRP. Third, our study was a single centre study. The findings of our study are seemingly in discrepancy with the results of the multi-centre study of Meier-Kriesche et al in which it was shown that continuous use of mycophenolate versus azathioprine therapy was associated with a protective effect against decline in renal function beyond 1 year after transplantation. In our analysis there was no trend or significant association between serum creatinine at follow-up and use of MMF. It is, however, difficult to compare the results of a multi-centre study with those of a single-centre cohort like ours, in which subjects that receive azathioprine instead of MMF almost automatically have a longer follow-up after transplantation.

It may be concluded from this study that high levels of CRP and anti-CMV IgG antibodies can be used in addition to existing markers for identification of patients at high risk for CTD. Are there, however, also clinical implications? Increasing the dose of immunosuppressive drugs seems no option, because it is nowadays widely accepted that there is no strategy whatsoever that can beneficially influence the course of CTD, and because it may be expected to upregulate rather than downregulate the activity CMV in CMV positive patients. Statins have been shown to have a CRP lowering effect, but a trial with a statin in renal transplant recipients was disappointing from the perspective of preservation of renal function. Another option would be early intervention with inhibitors of the renin angiotensin system. This class of drugs has also been shown to have
CRP lowering effects,\textsuperscript{35} and has definitely been shown to be renoprotective in various populations, although not yet in the context of CTD.

In conclusion, elevated levels of CRP independently predict accelerated deterioration of graft function in renal transplant recipients more than one year post-transplantation. Further prospective studies are required to investigate whether early intervention can prevent deterioration of graft function in subjects with elevated levels of CRP.

**ACKNOWLEDGEMENTS**

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REFERENCES
