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Inflammation-associated graft loss in renal transplant recipients

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This is the overview page

Inflammation-associated graft loss in renal transplant recipients

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Prof. Dr. N. Lameire

Editor-in-Chief

Nephrology Dialysis and Transplantation

RE: " Inflammation-associated graft loss in renal transplant recipients".

Dear Dr. Lameire

Please find attached a revised version of the manuscript " Inflammation-associated graft loss in renal transplant recipients " for consideration for publication in Nephrology Dialysis Transplantation.

Thank you again for reviewing a previous version of the manuscript. In preparing this revision, we performed extensive additional analyses based on advice and helpful comments from the reviewers.

Reviewer 2 raises some interesting aspects about inflammation. He focuses on smoking and pulmonal function (bronchitis) as potential causes for elevated inflammation markers in renal transplant patients. Close to 20% of the patients included in the ALERT trial were smokers. Although smoking was an independent risk factor for renal graft loss, there was no interaction or confounding with smoking and the inflammation markers IL-6 and hs-CRP. We have included a separate point-by-point answer to the reviewer's comments.

We have prepared the manuscript in accordance with your instruction for authors.

The results presented in this paper have not been published previously and is not being considered for publication elsewhere in whole or in part in any language except as an abstract.

Thank you for considering this revised manuscript for publication. We believe this work will be of interest to your readership, and are thus eager to resolve your concerns and proceed to publication. Please contact us if you have any questions.

Yours Sincerely

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Lead author   Senior author

Inflammation-associated graft loss in renal transplant recipients

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Abbreviations: Assessment of Lescol in Renal Transplant (ALERT) study. Chronic transplant

dysfunction (CTD). High-sensitivity C-reactive protein (hsCRP). Interleukin 6 (IL-6). Estimated

glomerular filtration rate (eGFR).

Abstract

Background. Although short-term graft survival has improved substantially in renal transplant

recipients, long-term graft survival has not improved over the last decades. The lack of knowledge

of specific causes and risk factors has hampered improvements in long-term allograft survival.

There is an uncertainty if inflammation is associated with late graft loss.

Methods. We examined in a large prospective trial the inflammation markers high-sensitivity C-

reactive protein (hsCRP) and interleukin 6 (IL-6) and their association with chronic graft

dysfunction. We collected data from Assessment of Lescol in Renal Transplant (ALERT) trial

which recruited 2102 maintenance renal transplant recipients.

Results. Baseline values were hsCRP 3.8 +/- 6.7 mg/l and IL-6 2.9 +/- 1.9 pg/ml. Adjusted for

traditional risk factors, hsCRP and IL-6 were independently associated with death censored graft

loss, the composite endpoints graft loss or death, and doubling of serum creatinine, graft loss or

death.

Conclusions: The inflammation markers hsCRP and IL-6 are associated with long term graft

outcomes in renal transplant recipients.

Key words: ALERT, high-sensitivity CRP, inflammation, interleukin-6, renal allograft survival.

Short summary: This post-hoc analysis of of maintenance renal transplant recipients (n=2102) from

the Assessment of Lescol in Renal Transplantation (ALERT) study demonstrates associations of the

inflammatory markers high-sensitivity C-reactive protein and interleukin 6 with chronic transplant

dysfunction.

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Introduction

Advances in immunosuppression, histocompatibility testing, surgery and medical management have allowed transplantation to become favoured treatment for patients with end stage renal disease, with a low rate of acute rejection and excellent short term results. The paradox is that long-term graft survival has barely improved over the last two decades despite short term improvements in graft survival [1]. Annual graft loss is still 2-7% [2-4] and the average deceased donor kidney transplant functions for about ten years [5]. Clinically, we observe a progressive transplant dysfunction characterised by a slowly rising serum creatinine, proteinuria and hypertension [3]. Early detection of chronic transplant dysfunction is recognised to be important, although current interventions have a limited effect (eg "creeping creatinine study"). Current recommendations are to follow serum creatinine, estimated glomerular filtration rate (eGFR), proteinuria and to consider performance of protocol (surveillance) biopsies or to have a low threshold for early diagnostic biopsy [4]. Once serum creatinine rises, or proteinuria appears, the decline in renal function is usually inevitable. Inflammation is present in the biopsies of patients with chronic graft dysfunction[6], but little is known about the role of markers of inflammation on graft outcome. The association of inflammation with atherosclerosis and macrovascular disease is well documented in other populations [7;8], specifically C-reactive protein (CRP). Cytokines induce production of interleukin 6 (IL-6) from various tissues, including lungs in smokers, and increases downstream mediators such as CRP [9;10]. Renal transplant recipients, by the process of receiving an allograft, have an additional activation of their inflammation status [11-13]. We documented recently that the inflammation markers IL-6 and high sensitivity CRP (hsCRP) were independently associated with cardiovascular events and all-cause mortality in renal transplant recipients [14]. Whether IL-6 predicts graft survival in renal transplant recipients has not yet been documented in a prospective study. The aim of the present study is therefore to investigate the baseline role of inflammatory biomarkers on progressive graft dysfunction and graft failure.

Subjects and methods

Study design

The design of the ALERT has been described previously [15]. In short ALERT was a randomized double-blinded placebo-controlled trial of fluvastatin 40 to 80 mg in renal transplant patients with follow-up for 5 to 6 years. Thereafter participants were offered open-label fluvastatin 80 mg in a 2 year extension study. In all, 2102 renal transplant recipients were included with a mean duration of follow-up for 6.7 years[15;16]. Endpoints were cardiovascular and renal events, recorded by an independent critical events committee. Recruitment was undertaken June 1996 to October 1997. Eligible patients were renal transplant recipients 30-75 yr old, with stable graft function, transplanted more than 6 months before enrolment. The patients were recruited to the study at a mean of 5.1 years after transplantation with a stable graft function. Patients were seen 1.5 months after enrolment and at 6 months intervals, recording clinical status and blood biochemistry including lipids, creatinine, creatine kinase and liver enzymes analyzed at a central laboratory (CRL, Medinet, Breda, The Netherlands). IL-6 was measured by human IL-6 immunoassay (R&D Systems Inc., Minneapolis, MN, USA) and hsCRP by immunoturbometric analysis (Roche Diagnostics GmbH, Mannheim, Germany). IL-6 and hsCRP were measured at baseline. The study adhered to the International Conference on Harmonization guidelines for Good Clinical Practice and in accordance with the Declaration of Helsinki. All participants providerd written informed consent, and the ethics committee at each participating center approved the trial (no S-02169).

Statistical analysis

In the initial analysis, treatment arm and placebo arm were analyzed separately for renal endpoints.

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Since the two arms showed no significant heterogeneity in relation between the inflammation

markers hsCRP and IL-6, subsequent analyses were performed on the pooled patient population.

HsCRP and IL-6 were not normally distributed and logarithmic transformation of these factors was

used in the analysis. HsCRP and IL-6 were analysed in separate models due to confounding (not

shown) and the close etiological relationship. Risk factors were evaluated using uni- and

multivariate Cox proportional hazard model. Covariates were chosen in a stepwise approach,

including covariates with univariate significance and previously reported factors associated with

graft outcomes, and then excluding most variables that did not retain statistical significance in the

multivariate analysis. SPSS version 17.0 (SPSS Inc., Chicago, IL) was used for statistical analysis.

Results

Baseline characteristics in ALERT and ALERT extension were similar in both treatment arms [15;16].

Baseline characteristics in relation to renal endpoints in the placebo group have been reported

separately [2]. The present study reports treatment and placebo group together. Baseline

characteristics are shown in table 1. Mean age at study entry was 50 years, 66% were male and all

received cyclosporine-based immunosuppression at entry. The main causes of renal failure leading

to transplantation were glomerulonephritis and polycystic kidney disease. Frequencies of graft

failure endpoints are shown in table 2.

HsCRP and IL-6 were available in 1910 and 1751 patients, respectively. Mean (SD) and median

(interquartile range) values were for hsCRP 3.8 (6.7) mg/l and 1.5 (3.1) mg/l and for IL-6 2.9 (1.9)

pg/ml and 2.4 (2.2) pg/ml. The hsCRP and IL-6 cohort had almost identical baseline characteristic

as reported earlier [14], with no differences in proportions with diabetes, coronary heart disease,

current smokers or live donor.

Uni- and multivariate hazard ratios for the hsCRP and IL-6 cohorts are shown in tables 3 and 4.

Missing data excluded 450 (21.4%) and 576 (27.4%) patients in the hsCRP and IL-6 multivariate models, respectively. Both hsCRP and IL-6 were in separate multivariate models significantly associated with the renal endpoints shown, ie death-censored graft loss, graft loss including death, and graft loss including death and doubling of serum creatinine. Due to a surprisingly high percentage of current smokers we performed separate analysis (not shown) to examine a potential interaction with smoking and inflammation. All interaction covariates were non-significant p>0.20, and the inflammation markers were equally strong in smokers and non-smoker, i.e. no interaction. Excluding smoking as a covariate (not shown) changed the regression coefficients for the inflammation markers less than 15% and the hazard ratios less than 5 %, indicating no confounding effect.

In multivariate analysis for death-censored graft loss the results were similar in the hsCRP and IL-6 cohorts. We found significant negative associations of age and BMI. Siginificant positive associations were seen for current smoking, systolic blood pressure, creatinine, time since last transplantation, two HLA-DR mismatches, previous treatment for rejection and proteinuria.

When including death in the endpoint the association of age became significantly positive, diabetes became significant and previous treatment for rejection lost significance. HLA-DR mismatch was significant when including doubling of serum creatinine in the endpoint. We still found significant negative association of BMI and positive associations of current smoking, systolic blood pressure, creatinine, time since last transplantation and proteinuria.

Discussion

In the present post hoc analysis, we have shown that inflammation, adjusted for traditional risk factors, is associated with death-censored renal graft loss, and the composite endpoints; graft loss or death, and graft loss or death or doubling of serum creatinine. To our knowledge this is the first

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prospective cohort in renal transplant patients documenting an association of IL-6 with graft loss.

HsCRP and IL-6 are close participants in the inflammatory cascade, and the observation that both

markers were predictive of outcome parameters, strengthens the perception of inflammation as an

important risk factor for graft related outcomes in renal transplant patients.

The baseline levels of inflammatory markers in our patients seem moderately elevated compared to

a non-transplanted background population, as seen in most studies with renal transplant recipients

[14]. No universally accepted cutoff values for inflammatory markers in renal transplant recipients

exists, but regarding cardiovascular outcomes in the general population CRP < 1 mg/dL confers low

risk, CRP 1 to 3 mg/dL average risk and CRP > 3 mg/dL high risk [17], and the same cutoff values

were described by van Ree et al. as predicting a rising creatinine in renal transplant recipients [18].

This leaves n=1203 (63%) of our hsCRP cohort with hsCRP > 1 mg/dL in the moderate to high risk

group. IL-6 levels are harder to compare with other studies due to different measurement

methodology. A study by Karczewski et al. found significantly higher IL-6 levels in chronic

rejection (1.64 +/- 0.8 pg/mL) than in patients with stable graft function (0.42 +/- 0.3 pg/mL,

p<0.001) or acute rejection (0.93 +/- 1.7 pg/dL, p=0.001) [19].

Our findings supports the concept that inflammation might be a component of the pathogenesis of

chronic graft dysfunction, and is in line with the report on hsCRP and graft deterioration by van Ree

et al.[18]. CRP and IL-6 has been related to renal function in predialytic chronic renal failure [20].

However, the exact pathogenetic role of hsCRP and IL-6 in graft failure is yet elusive. Indeed,

genetic elevation in CRP was not associated with post-transplant morbidity and mortality [21], and

lowering of hsCRP with statin in dialysis patients did not improve cardiovascular outcome [22],

neither did statin treatment improve graft outcome in the ALERT [15]. IL-6 is upstream to CRP in the

inflammatory cascade and seems to play an etiological role in other inflammatory disease states

including rheumatoid arthritis [23], where tocilizumab, a monoclonal antibody against IL-6-receptor,

has a proven effect. A murine model of cardiac transplantation demonstrated a tripled survival time

of IL-6 deficient grafts [24], but allogeneic cardiac transplants into IL-6 deficient mice did not show

prolonged graft survival indicating that the graft was the relevant source of IL-6. In human heart

transplant recipients IL-6 is associated with low grade cellular rejection [25]. In renal transplant

recipients elevations in serum and urine IL-6 levels are documented in acute rejection episodes [26],

and increased soluble IL-6 receptor was found in urine twelve and 6 months before late graft failure

[27]. A murine model of calcineurin-inhibitor (CNI) nephrotoxicity showed a protective effect of an

IL-6 neutralizing antibody [28], another animal study found a preventive effect of 13-cis-retinoic acid

on chronic allograft nephropathy possibly mediated through decreased secretion of inflammatory

cytokines including tumor necrosis factor-alpha and IL-6 [29]. Some genetic polymorphism studies in

man has shown a protective role on renal graft function of a high IL-6 gene expression in renal

transplant recipients, and low IL-6 production donor genotype was associated with increased

prevalence of chronic graft dysfunction [30;31], whereas other studies failed to show this association

[32;33]. A small retrospective study by Cueto-Manzano et al. found no difference in inflammation

markers in patients with or without CTD [34]. A pilot randomized placebo-controlled trial with an

inflammation antagonist demonstrated reduced inflammation in maintenance hemodialysis patients

[35]. A human randomized trial of IL-6 suppressive therapy might give an answer to whether

inflammation is causative in chronic graft dysfunction, but is probably still premature awaiting

further evidence from mechanistic and correlation studies. Indeed, neutralizing IL-6 antibody

treatment in a murine model of acute reperfusion injury was deleterious [36].

Our finding of protective effect of older age of recipient for death-censored graft loss is in line with

other studies [18;37;38], and might represent higher immunoreactivity in younger subjects.

Hypertension, previous rejection and HLA-DR mismatch were independent risk factors in the

present study is in line with current reviews [4;39]. Creatinine and proteinuria were significant

independent risk factors for graft loss as described previously [2;40], as was time since transplantation.

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Creatinine and proteinuria are used as biomarkers of CTD, although they are unspecific of the underlying pathology and not ideal [41]. Smoking may be a risk factor for reduced patient and graft survival after transplantation[42]. Smoking may by itself increase inflammation markers[9]. Due to a surprisingly high percentage of current smokers we performed separate analysis to examine a potential interaction with smoking and inflammation. Although smoking was an independent risk marker there was no interaction with smoking and inflammation.

A limitation of this study was having only one analysis at baseline for hsCRP and IL-6, and the difference measured between groups is small and of limited prognostic value for individual renal transplant recipients. Our method of choosing covariates increases the possibility of type 1 error and should be regarded as tentative. The strength of the study is the many patients followed for an extended period and an independent adjudication of predefined outcome parameters.

In conclusion we found that the inflammatory biomarkers hsCRP and IL-6 are significantly associated with graft related outcomes in renal transplant recipients, independent of the other examined risk factors for graft loss.

Disclosure

The authors of this manuscript have conflicts of interest to disclose as described by Nephrology, Dialysis and Transplantation. The ALERT trial was sponsored by Novartis AG. Otherwise, the authors report no conflict of interest.

Conflict of interest

This paper have not been published previously in whole or part.

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Table 1. Baseline data. Results are expressed as means (SD) and frequencies (% of valid) where appropriate.

Variable	Available data	Total n=2102	HS-CRP cohort n=1910	IL-6 cohort n=1751
Age at baseline, years	2102	49.7 (10.9)	49.7 (11.0)	49.5 (11.0)
Male gender	2102	1387 (66.0)	1257 (65.8)	1150 (65.7)
Current smoker	2100	389 (18.5)	358 (18.8)	326 (18.6)
Body mass index, kg/m2	2051	25.8 (4.5)	25.7 (4.3)	25.6 (4.2)
Diabetes mellitus	2101	396 (18.8)	362 (19.0)	327 (18.7)
Hypertension	2102	1575 (74.9)	1424 (74.6)	1305 (74.5)
Systolic blood pressure, mmHg	2094	143.9 (18.9)	144.4 (18.9)	144.6 (18.9)
Diastolic blood pressure, mmHg	2093	85.6 (10.0)	85.9 (10.0)	86.0 (9.9)
Coronary heart disease	2101	201 (9.6)	175 (9.2)	145 (8.3)
Left ventricular hypertrophy	2076	311 (15.0)	279 (14.8)	256 (14.8)
ST-T ECG abnormalities	2077	405 (19.5)	367 (19.4)	329 (19.0)
Serum creatinine, μmol/L	2028	145.4 (53.0)	144.8 (53.3)	144.5 (52.9)
Number of transplantations	2101	1.2 (0.4)	1.2 (0.4)	1.2 (0.4)
Time since last tx, years	2101	5.1 (3.4)	5.1 (3.4)	5.2 (3.4)
Time on dialysis, months	2100	27.5 (41.6)	27.7 (42.4)	27.5 (42.5)
Live donor	2100	469 (22.3)	429 (22.5)	400 (22.8)
Donor age, years	2058	40.5 (15.3)	40.9 (15.4)	40.7 (15.4)
Cold ischemia time, hours	1520	19.8 (7.7)	19.7 (7.6)	19.7 (7.7)
Panel reactive antibodies	1845	327 (17.7)	288 (17.1)	258 (16.7)
No HLA-DR mismatch	2029	662 (32.6)	623 (33.7)	560 (32.9)
One HLA-DR mismatch	2029	1039 (51.2)	930 (50.3)	866 (50.9)
Two HLA-DR mismatch	2029	328 (16.2)	297 (16.1)	274 (16.1)
Delayed graft function	2063	365 (17.7)	321 (17.1)	294 (17.1)
Treatment for rejections	2076	902 (43.4)	818 (43.3)	759 (43.8)
Treatment for CMV	2030	286 (14.1)	252 (13.6)	231 (13.6)
Derived proteinuria g/24 hr	1981	0.4 (1.0)	0.4 (1.0)	0.4 (1.0)
Hs-CRP, mg/L	1910	3.8 (6.7)	3.8 (6.7)	
IL-6, pg/mL	1751	2.9 (1.9)		2.9 (1.9)

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Table 2 Occurrence of endpoints in relation to hsCRP and IL-6 cohorts at 7yr followup

Variable	All n=2102	hsCRP cohort n=1910	IL-6 cohort n=1751
Death-censored graft loss (%)	362 (17.2)	346 (18.1)	308 (17.6)
Graft loss, doubling of s-creatinine or death	701 (33.3)	662 (34.7)	590 (33.7)
Graft loss or death (%)	644 (30.6)	606 (31.7)	539 (30.8)
All death (%)	383 (18.2)	354 (18.5)	309 (17.6)

Table 3. High-sensitive C-reactive protein cohort, n=1652*

Risk factor	Graft loss (death-cencored), n=288			Graft loss, doubling of s-creatinine or death, n=567			Graft loss or death, n=515		
	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	P value	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	P value	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	P value
Age	0.98 (0.97-0.99)	0.99 (0.98-1.00)	0.040	1.02 (1.01-1.02)	1.02 (1.01-1.03)	<0.001	1.02 (1.01-1.03)	1.03 (1.02-1.04)	<0.001
Female gender	0.79 (0.63-0.99)	1.02 (0.78-1.34)	0.88	0.90 (0.77-1.06)	1.10 (0.92-1.32)	0.30	0.84 (0.71-1.00)	1.04 (0.86-1.26)	0.70
Current smoking	1.90 (1.51-2.39)	1.68 (1.29-2.19)	<0.001	1.74 (1.47-2.06)	1.71 (1.42-2.08)	<0.001	1.73 (1.45-2.06)	1.72 (1.41-2.10)	<0.001
BMI	0.97 (0.94-0.99)	0.94 (0.91-0.97)	<0.001	1.00 (0.98-1.02)	0.98 (0.96-1.00)	0.017	0.99 (0.98-1.01)	0.97 (0.95-0.99)	0.003
Systolic BP	1.02 (1.01-1.02)	1.01 (1.00-1.01)	0.017	1.02 (1.01-1.02)	1.01 (1.00-1.01)	0.023	1.02 (1.01-1.02)	1.01 (1.00-1.01)	0.042
Diabetes mellitus	1.28 (0.99-1.64)	1.30 (0.96-1.75)	0.09	1.57 (1.32-1.86)	1.49 (1.22-1.82)	<0.001	1.58 (1.32-1.89)	1.55 (1.26-1.90)	<0.001
Creatinine	1.01 (1.01-1.02)	1.02 (1.02-1.02)	<0.001	1.01 (1.01-1.01)	1.01 (1.01-1.01)	<0.001	1.01 (1.01-1.01)	1.01 (1.01-1.01)	<0.001
Time since last tx	1.05 (1.02-1.08)	1.06 (1.02-1.10)	0.001	1.03 (1.01-1.06)	1.04 (1.02-1.07)	0.001	1.04 (1.02-1.06)	1.05 (1.02-1.08)	<0.001
HLA-DR mismatch, one	0.86 (0.70-1.06)	1.17 (0.88-1.55)	0.28	1.01 (0.87-1.17)	1.22 (1.00-1.48)	0.045	0.95 (0.81-1.11)	1.13 (0.92-1.39)	0.23
HLA-DR mismatch, two	1.64 (1.28-2.11)	1.59 (1.14-2.22)	0.006	1.33 (1.10-1.61)	1.35 (1.06-1.73)	0.017	1.34 (1.10-1.64)	1.29 (1.00-1.67)	0.051
Treatment for rejection	1.50 (1.22-1.84)	1.29 (1.01-1.64)	0.045	1.23 (1.06-1.43)	1.13 (0.96-1.35)	0.15	1.22 (1.04-1.42)	1.11 (0.93-1.33)	0.25
Proteinuria	1.38 (1.33-1.44)	1.36 (1.29-1.44)	<0.001	1.33 (1.29-1.37)	1.26 (1.20-1.32)	<0.001	1.33 (1.29-1.38)	1.26 (1.20-1.32)	<0.001
Ln hs-CRP	1.13 (1.05-1.21)	1.18 (1.09-1.27)	<0.001	1.17 (1.11-.1.23)	1.13 (1.07-1.20)	<0.001	1.18 (1.11-1.25)	1.15 (1.08-1.22)	<0.001

*Multivariate Cox regression analysis for hsCRP cohort. P values for the adjusted hazard ratios are shown. All n are for cases with no missing values for risk factors in the multivariate analysis, ie n=450 (21.4%) cases with missing values were excluded when estimating adjusted hazard ratios.
Abbreviation: Ln - natural logarithmic transformed.

Table 4. Interleukin 6 cohort, n=1526*

Risk factor	Graft loss (death-cencored), n=259			Graft loss, doubling of s-creatinine or death, n=509			Graft loss or death, n=461		
	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	P value	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	P value	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	P value
Age	0.98 (0.97-0.99)	0.99 (0.97-1.00)	0.045	1.02 (1.01-1.02)	1.02 (1.01-1.03)	<0.001	1.02 (1.01-1.03)	1.03 (1.02-1.04)	<0.001
Female gender	0.79 (0.63-0.99)	1.03 (0.78-1.37)	0.82	0.90 (0.77-1.06)	1.13 (0.93-1.37)	0.23	0.84 (0.71-1.00)	1.07 (0.87-1.31)	0.54
Current smoking	1.90 (1.51-2.39)	1.65 (1.24-2.19)	0.001	1.74 (1.47-2.06)	1.63 (1.33-2.01)	<0.001	1.73 (1.45-2.06)	1.62 (1.30-2.01)	<0.001
BMI	0.97 (0.94-0.99)	0.95 (0.92-0.98)	<0.001	1.00 (0.98-1.02)	0.98 (0.96-1.00)	0.034	0.99 (0.98-1.01)	0.97 (0.95-0.99)	0.009
Systolic BP	1.02 (1.01-1.02)	1.01 (1.00-1.02)	0.006	1.02 (1.01-1.02)	1.01 (1.00-1.01)	0.008	1.02 (1.01-1.02)	1.01 (1.00-1.01)	0.016
Diabetes mellitus	1.28 (0.99-1.64)	1.32 (0.96-1.81)	0.09	1.57 (1.32-1.86)	1.52 (1.23-1.87)	<0.001	1.58 (1.32-1.89)	1.54 (1.24-1.93)	<0.001
Creatinine	1.01 (1.01-1.02)	1.02 (1.01-1.02)	<0.001	1.01 (1.01-1.01)	1.01 (1.01-1.01)	<0.001	1.01 (1.01-1.01)	1.01 (1.01-1.01)	<0.001
Time since last tx	1.05 (1.02-1.08)	1.05 (1.02-1.09)	0.005	1.03 (1.01-1.06)	1.04 (1.01-1.07)	0.003	1.04 (1.02-1.06)	1.05 (1.02-1.08)	<0.001
HLA-DR mismatch, one	0.86 (0.70-1.06)	1.18 (0.87-1.60)	0.28	1.01 (0.87-1.17)	1.23 (0.99-1.51)	0.06	0.95 (0.81-1.11)	1.10 (0.88-1.37)	0.39
HLA-DR mismatch, two	1.64 (1.28-2.11)	1.61 (1.13-2.30)	0.009	1.33 (1.10-1.61)	1.39 (1.07-1.81)	0.014	1.34 (1.10-1.64)	1.30 (0.99-1.71)	0.06
Treatment for rejection	1.50 (1.22-1.84)	1.36 (1.04-1.77)	0.023	1.23 (1.06-1.43)	1.17 (0.97-1.40)	0.10	1.22 (1.04-1.42)	1.14 (0.94-1.38)	0.18
Proteinuria	1.38 (1.33-1.44)	1.39 (1.31-1.47)	<0.001	1.33 (1.29-1.37)	1.30 (1.24-1.36)	<0.001	1.33 (1.29-1.38)	1.29 (1.23-1.36)	<0.001
Ln IL-6	1.53 (1.27-1.83)	1.26 (1.01-1.56)	0.038	1.75 (1.53-2.00)	1.29 (1.10-1.50)	0.001	1.85 (1.61-2.13)	1.32 (1.12-1.56)	0.001

*Multivariate Cox regression analysis for IL-6 cohort. P values for the adjusted hazard ratios are shown. All n are for cases with no missing values for risk factors in the multivariate analysis, ie n=576 (27.4%) cases with missing values were excluded when estimating adjusted hazard ratios.
Abbreviation: Ln - natural logarithmic transformed.

Your paper has been evaluated by a subject editor, 2 external reviewers and at the editorial office. Although of interest to the readership of NDT, both the subject editor and I feel that major modifications are necessary before your paper can be considered for publication. We would like to ask you to submit a revised version, taking into account the reviewers' remarks below.

As reviewer 2 points out in a very careful evaluation of your paper, there are some flaws and the conclusion that hsCRP and IL-6 are independent risk factors is most likely overstated or may even be wrong (for more details, please see below).

We have detailed this issue in response to reviewer 2

We ask you to address these concerns of the reviewers below and already look forward to receiving your revised paper.

If you do decide to resubmit, we would be pleased to review your modified version for publication. Please submit both a corrected version with additions underlined in red and deletions crossed out in blue, and a clean version of your revision without the corrections still marked in the text. Please also include a cover letter to the Editor-in-Chief, and a separate point-by-point answer to the reviewers' comments. Note that a final decision on your manuscript will only be taken after evaluation of the revised version.

IMPORTANT! You must resubmit via the website and under the same manuscript ID. For instructions on this procedure, please click on 'Instructions and Forms' in your author centre.

Figures: When submitting your revised version, please upload your final publication quality figures to the Manuscript Central website.

These figures should meet the following conditions:
- Line drawings (black and white) should be 600 dpi minimum
- Colour and greyscale figures should be 300 dpi minimum
- Colour figures should be supplied in CMYK colours, not RGB colours
- Each figure must be clearly labelled with the figure/scheme number

Please also note that we require all authors to complete a Conflict of Interest Form (see 'Instructions to Authors'), which should be inserted at the end of the manuscript.

We look forward to hearing from you. Please take into account that the deadline for resubmission is six months, after which your paper will be considered as a new submission.

Yours sincerely,
Prof. dr. N. Lameire Editor In Chief, Nephrology Dialysis Transplantation

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3 _____
4 Reviewer: 1
5 Comments to the Author
6 Many papers of the ALERT-study group have been already published during the last decade. The
7 current paper is obviously another analysis of the huge ALERT database (n=2102 renal transplant
8 recipients) investigating the association between the inflammatory markers high-sensitivity CRP and
9 interleukin-6 with graft outcomes.
10 The aim of the present study is therefore to investigate the baseline role of inflammatory biomarkers
11 on progressive graft dysfunction and failure.
12 The authors came out with the result that adjusted for traditional risk factors, hsCRP and IL-6 were
13 independently associated with death censored graft loss, the composite endpoints graft loss or death,
14 and doubling of serum creatinine, graft loss or death.
15 Despite some flaws of the paper like the fact that only baseline levels of hsCRP and IL-6 were
16 available etc., the large number of patients and the longterm observational period merits the
17 publication of the data. There will be only little chance to wait for a human randomized controlled trial.
18 It is also not possible to recruit more data from the ALERT database. There might be the risk that the
19 data are only tentative however for me it is worthwhile to read it.
20
21 We understand reviewer's concern regarding the lack of longitudinal data for Il-6 and hsCRP. That
22 was one of the flaws we regret in the original study design of the trial. The strength as the reviewer
23 points out; large number of patients, long follow-up time and not least an endpoint committee
24 adjudicating clinical events.
25 We do agree with the reviewer that the data may be tentative. In our discussion we have stated:
26 "Our method of choosing covariates increases the possibility of type 1 error and should be
27 regarded as tentative."
28 We appreciate the reviewer's opinion that our data are worthwhile to read.
29
30 Reviewer: 2
31 Comments to the Author
32 Inflammation-associated graft loss in renal transplant recipients
33 Dahle et al.
34
35 The authors describe an association of hs-CRP and IL-6 blood levels with graft loss and conclude that
36 graft loss might be due to inflammation-induced chronic graft rejection. During a randomized double-
37 blinded placebo-controlled trial of fluvastatin in a total of 2102 patients (ALERT study), hs-CRP and IL-
38 6 blood levels were determined in 1910 and 1751 renal transplant recipients, respectively. Both
39 parameters were determined once at baseline. Because placebo- and verum-treated patients showed
40 similar hsCRP and IL-6 blood levels, statistical analysis was performed on the pooled patient
41 population. Patients were followed for at least 5 years with a mean follow up of 6.7 years. Enrolled
42 patients were 30-75 years old (mean 50 years), showed stable graft function at enrollment (mean
43 creatinine 145 µmol/l), and had been transplanted more than 6 months before enrollment (mean 5.1
44 years). Patients were seen at 6 month intervals during the study period.
45
46 Based on 1910 patients in the hsCRP cohort and 1751 patients in the IL-6 cohort, approximately 18%
47 (18.1% and 17.6%) of the patients in the two groups showed death-censored graft loss, approximately
48 34% (34.7% and 33.7%) graft loss, doubling of serum creatinine or death, and approximately 31%
49 (31.7% and 30.8%) graft loss or death after 7 years of follow up (Table 2).
50
51 Due to missing data, 450 (21.4%) patients in the hsCRP and 576 (27.4%) patients in the IL-6 cohort
52 were excluded from analysis, leaving 1652 patients in the hsCRP cohort and 1526 patients in the IL-6
53 cohort (Tables 3 and 4). In multivariate models, both hs-CRP and IL-6 were significantly associated
54 with the renal endpoints death-censored graft loss, graft loss including death, and graft loss including
55 death and doubling of serum creatinine. In multivariate analysis for death-censored graft loss, there
56 were negative associations of age and BMI and positive associations of current smoking, systolic
57 blood pressure, creatinine, time since last transplantation, 2 HLA-DR mismatches, and previous
58 treatment for rejection and proteinuria.
59
60 Major concerns:
• IL-6 is the major inducer of CRP in the liver (J Immunol. 2008 Mar 1;180(5):3492-501.
Transcriptional complex formation of c-Fos, STAT3, and hepatocyte NF-1 alpha is essential for

cytokine-driven C-reactive protein gene expression. Nishikawa T, Hagihara K, Serada S, Isobe T, Matsumura A, Song J, Tanaka T, Kawase I, Naka T, Yoshizaki K.). Induction of CRP is strongly IL-6-dependent and blood levels of the two parameters would be expected to behave similarly in patients with strong IL-6 induction. Both parameters are sensitive indicators of inflammation.

The reviewer highlights an important point by commenting that hsCRP is strongly IL-6 dependent. Indeed, we do agree. We also share the reviewer's point that the two parameters would be expected to behave similarly, and they did indeed in our trial. We performed a comprehensive statistical re-run for hsCRP with and without IL-6. IL-6 is probably by a large extent a precipitator for hsCRP. In the regression analysis running both inflammation markers included, versus separately, there was a confounding effect indicating a causal relation. We have added a comment on this in the article.

In the present study, current smoking was strongly associated with death-censored graft loss, graft loss, doubling of serum creatinine or death, and graft loss or death in the hsCRP as well as the IL-6 cohort (p<0.001) (Tables 3 and 4). It can be assumed that "current smoker" includes many strong smokers with associated chronic bronchitis at study baseline. Chronic bronchitis might have been responsible - at least in part - for the increased hsCRP and IL-6 blood levels at baseline (Chest. 2009 Oct;136(4):1039-46. The metabolic syndrome in patients with chronic bronchitis and COPD: frequency and associated consequences for systemic inflammation and physical inactivity. Watz H, Waschki B, Kirsten A, Müller KC, Kretschmar G, Meyer T, Holz O, Magnussen H.). To minimize the effect of chronic bronchitis, the authors should analyze hs-CRP and IL-6 blood levels patients excluding "current smokers".

We tested for a possible confounding effect of smoking on the effect of inflammation by running the multivariate analyses with and without current smoking as a covariate, and found no significant difference in the regression coeffisient of hsCRP or IL6 (ie all cange <15%) nor in the hazard ratios of hsCRP or IL6 (ie all change <5%). We also tested for interaction between smoking and inflammation by adding a multiplication covariate (ie smoking*Ln_hsCRP or smoking*Ln_IL6, respectively), with all p>0.20, indicating no interaction. An analysis excluding current smokers reduces the study population, but multivariate hazard ratios did not change substantially, also indicating no interaction between smoking and inflammation. HsCRP remains highly significant among non-smokers for all endpoints (p<=0.001), whereas IL6 remains significant for graftloss or death, graft loss doubling creatinine or death. The multivariate HR of IL6 for death-cencored graftloss in nonsmokers is 1.20 (95% CI 0.93-1.55) p=0.16. We added a comment in the article on the multivariate tests for interaction and confounding, but not for the stratified analysis, as it refers to a much smaller study cohort This lack of difference might indicate that other factors for elevated inflammation is more prominent in transplant patients than smoke status. The inflammation caused by the graft itself is probably an important factor (Vazquez et al Curr Opin Nephrol Hypertens 2000; 9:643-48, Cottone et al Transplant Int 2007;20:82-87, Gotsman et al. Circulation 2006; 114: 2047-55, Martinez-Castelao et al Transplant Proc 2005;37:3788-90, Sadollah et al. cJASN 2009; 4: 1246-54).

• It would be interesting to study whether hs-CRP and IL-6 blood levels were stronger associated with current smoking than with creatinine levels or proteinuria.

As proposed by the reviewer we performed this analysis. As smoking is a dichotomized endpoint we also dichotomized creatinine and protein excretion by median in regard to blood levels of the inflammation markers. IL-6 in smokers 3.27 (2.05) pg/ml and in patients with "high" creatinine 3.08 (1.98) pg/ml.. Corresponding values for hsCRP were 4.89 (8.189) mg/L and 4.01 (7.39) mg/L smokers and "high" creatinine patients. But as stated above, there was no interaction with current smoking. We thus observed small but statistically significant higher baseline levels (Mann-Whitney U-test) of both inflammatory markers in smokers than non-smokers. We also observed significantly higher level of IL-6 in the "high" creatinine group than the "low" creatinine group, and significantly higher level of IL-6 in the "high" proteinuria group than the "low" proteinuria group, while hsCRP was not different in the creatinine or proteinuria groups. Among non-smokers the baseline s-IL6 was significantly higher in the "high" creatinine group than the "low" creatinine group. For non-smokers we observed a trend (p=0.09) for higher IL-6 in the "high" proteinuria-group than the "low" proteinuria group. Among the smokers the baseline IL-6 and hs-CRP levels were not significantly different between creatinine or proteinuria groups. As expected baseline inflammatory levels seem related both to smoking status and to serum creatinine levels and proteinuria levels. These results are not added in the article.

• There are reports in the literature indicating that history of smoking before transplantation is associated with impaired transplant and patient survival and an increased risk of early rejection (Am J Kidney Dis. 2010 May;55(5):907-15. Cigarette smoking, kidney function, and mortality after live donor kidney transplant. Nogueira JM, Haririan A, Jacobs SC, Cooper M, Weir MR).
With smokers included and not considered separately, and given the association of chronic bronchitis with inflamation markers, it is difficult to draw the conclusion from the data that increased hsCRP and IL-6 blood levels are early predictive markers of inflammation-induced graft loss. The sentence at the end of the discussion section, saying that hsCRP and IL-6 increases are risk factors independent of other recognized risk factors for graft loss, seems unsustainable and needs to be substantiated.

The reviewer addresses the point if smoking pre-transplantation is a possible confounder or an effect modifier. There was no interaction or confounding for smoking (see above). We also believe that smoking for several reasons is an important issue also in renal transplant, but as we have outlined above the inflammation status in transplanted patients might be driven by other factors than smoking. We have nuanced the sentence at the end of discussion to "examined risk factors." It might be to ambitious to include all "recognizable risk factors".

Minor concerns:

• In Table 1: "live donor ??????"

Typing error, the question marks are deleted

• In Tables 3 and 4: "Ln hsCRP" and "Ln IL-6". What is the meaning of Ln?

Ln is abbreviation for natural logarithm, we add explanation in the tables.

Inflammation-associated graft loss in renal transplant recipients

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Abbreviations: Assessment of Lescol in Renal Transplant (ALERT) study. Chronic transplant dysfunction (CTD). High-sensitivity C-reactive protein (hsCRP). Interleukin 6 (IL-6). Estimated glomerular filtration rate (eGFR).

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Abstract

Background. Although short-term graft survival has improved substantially in renal transplant recipients, long-term graft survival has not improved over the last decades. The lack of knowledge of specific causes and risk factors has hampered improvements in long-term allograft survival. There is an uncertainty if inflammation is associated with late graft loss.

Methods. We examined in a large prospective trial the inflammation markers high-sensitivity C-reactive protein (hsCRP) and interleukin 6 (IL-6) and their association with chronic graft dysfunction. We collected data from Assessment of Lescol in Renal Transplant (ALERT) trial which recruited 2102 maintenance renal transplant recipients.

Results. Baseline values were hsCRP 3.8 +/- 6.7 mg/l and IL-6 2.9 +/- 1.9 pg/ml. Adjusted for traditional risk factors, hsCRP and IL-6 were independently associated with death censored graft loss, the composite endpoints graft loss or death, and doubling of serum creatinine, graft loss or death.

Conclusions: The inflammation markers hsCRP and IL-6 are associated with long term graft outcomes in renal transplant recipients.

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Key words: ALERT, high-sensitivity CRP, inflammation, interleukin-6, renal allograft survival.

Short summary: This post-hoc analysis of of maintenance renal transplant recipients (n=2102) from the Assessment of Lescol in Renal Transplantation (ALERT) study demonstrates associations of the inflammatory markers high-sensitivity C-reactive protein and interleukin 6 with chronic transplant dysfunction.

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Introduction

Advances in immunosuppression, histocompatibility testing, surgery and medical management have allowed transplantation to become favoured treatment for patients with end stage renal disease, with a low rate of acute rejection and excellent short term results. The paradox is that long-term graft survival has barely improved over the last two decades despite short term improvements in graft survival [1]. Annual graft loss is still 2-7% [2-4] and the average deceased donor kidney transplant functions for about ten years [5]. Clinically, we observe a progressive transplant dysfunction characterised by a slowly rising serum creatinine, proteinuria and hypertension [3]. Early detection of chronic transplant dysfunction is recognised to be important, although current interventions have a limited effect (eg "creeping creatinine study"). Current recommendations are to follow serum creatinine, estimated glomerular filtration rate (eGFR), proteinuria and to consider performance of protocol (surveillance) biopsies or to have a low threshold for early diagnostic biopsy [4]. Once serum creatinine rises, or proteinuria appears, the decline in renal function is usually inevitable. Inflammation is present in the biopsies of patients with chronic graft dysfunction[6], but little is known about the role of markers of inflammation on graft outcome. The association of inflammation with atherosclerosis and macrovascular disease is well documented in other populations [7;8], specifically C-reactive protein (CRP). Cytokines induce production of interleukin 6 (IL-6) from various tissues, including lungs in smokers, and increases downstream mediators such as CRP [9;10]. Renal transplant recipients, by the process of receiving an allograft, have an additional activation of their inflammation status [11-13]. We documented recently that the inflammation markers IL-6 and high sensitivity CRP (hsCRP) were independently associated with cardiovascular events and all-cause mortality in renal transplant recipients [14]. Whether IL-6 predicts graft survival in renal transplant recipients has not yet been documented in a prospective study. The aim of the present study is therefore to investigate the baseline role of inflammatory biomarkers on progressive graft dysfunction and graft failure.

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Subjects and methods

Study design

The design of the ALERT has been described previously [15]. In short ALERT was a randomized

double-blinded placebo-controlled trial of fluvastatin 40 to 80 mg in renal transplant patients with

follow-up for 5 to 6 years. Thereafter participants were offered open-label fluvastatin 80 mg in a 2

year extension study. In all, 2102 renal transplant recipients were included with a mean duration of

follow-up for 6.7 years[15;16]. Endpoints were cardiovascular and renal events, recorded by an

independent critical events committee. Recruitment was undertaken June 1996 to October 1997.

Eligible patients were renal transplant recipients 30-75 yr old, with stable graft function,

transplanted more than 6 months before enrolment. The patients were recruited to the study at a

mean of 5.1 years after transplantation with a stable graft function. Patients were seen 1.5 months

after enrolment and at 6 months intervals, recording clinical status and blood biochemistry

including lipids, creatinine, creatine kinase and liver enzymes analyzed at a central laboratory

(CRL, Medinet, Breda, The Netherlands). IL-6 was measured by human IL-6 immunoassay (R&D

Systems Inc., Minneapolis, MN, USA) and hsCRP by immunoturbometric analysis (Roche

Diagnostics GmbH, Mannheim, Germany). IL-6 and hsCRP were measured at baseline. The study

adhered to the International Conference on Harmonization guidelines for Good Clinical Practice

and in accordance with the Declaration of Helsinki. All participants providerd written informed

consent, and the ethics committee at each participating center approved the trial (no S-02169).

Statistical analysis

In the initial analysis, treatment arm and placebo arm were analyzed separately for renal endpoints.

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Since the two arms showed no significant heterogeneity in relation between the inflammation

markers hsCRP and IL-6, subsequent analyses were performed on the pooled patient population.

HsCRP and IL-6 were not normally distributed and logarithmic transformation of these factors was

used in the analysis. HsCRP and IL-6 were analysed in separate models due to confounding (not

shown) and the close etiological relationship. Risk factors were evaluated using uni- and

multivariate Cox proportional hazard model. Covariates were chosen in a stepwise approach,

including covariates with univariate significance and previously reported factors associated with

graft outcomes, and then excluding most variables that did not retain statistical significance in the

multivariate analysis. SPSS version 17.0 (SPSS Inc., Chicago, IL) was used for statistical analysis.

Results

Baseline characteristics in ALERT and ALERT extension were similar in both treatment arms [15;16].

Baseline characteristics in relation to renal endpoints in the placebo group have been reported

separately [2]. The present study reports treatment and placebo group together. Baseline

characteristics are shown in table 1. Mean age at study entry was 50 years, 66% were male and all

received cyclosporine-based immunosuppression at entry. The main causes of renal failure leading

to transplantation were glomerulonephritis and polycystic kidney disease. Frequencies of graft

failure endpoints are shown in table 2.

HsCRP and IL-6 were available in 1910 and 1751 patients, respectively. Mean (SD) and median

(interquartile range) values were for hsCRP 3.8 (6.7) mg/l and 1.5 (3.1) mg/l and for IL-6 2.9 (1.9)

pg/ml and 2.4 (2.2) pg/ml. The hsCRP and IL-6 cohort had almost identical baseline characteristic

as reported earlier [14], with no differences in proportions with diabetes, coronary heart disease,

current smokers or live donor.

Uni- and multivariate hazard ratios for the hsCRP and IL-6 cohorts are shown in tables 3 and 4.

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Missing data excluded 450 (21.4%) and 576 (27.4%) patients in the hsCRP and IL-6 multivariate models, respectively. Both hsCRP and IL-6 were in separate multivariate models significantly associated with the renal endpoints shown, ie death-censored graft loss, graft loss including death, and graft loss including death and doubling of serum creatinine. Due to a surprisingly high percentage of current smokers we performed separate analysis (not shown) to examine a potential interaction with smoking and inflammation. All interaction covariates were non-significant $p>0.20$, and the inflammation markers were equally strong in smokers and non-smoker, i.e. no interaction. Excluding smoking as a covariate (not shown) changed the regression coefficients for the inflammation markers less than 15% and the hazard ratios less than 5 %, indicating no confounding effect.

In multivariate analysis for death-censored graft loss the results were similar in the hsCRP and IL-6 cohorts. We found significant negative associations of age and BMI. Siginificant positive associations were seen for current smoking, systolic blood pressure, creatinine, time since last transplantation, two HLA-DR mismatches, previous treatment for rejection and proteinuria.

When including death in the endpoint the association of age became significantly positive, diabetes became significant and previous treatment for rejection lost significance. HLA-DR mismatch was significant when including doubling of serum creatinine in the endpoint. We still found significant negative association of BMI and positive associations of current smoking, systolic blood pressure, creatinine, time since last transplantation and proteinuria.

Discussion

In the present post hoc analysis, we have shown that inflammation, adjusted for traditional risk factors, is associated with death-censored renal graft loss, and the composite endpoints; graft loss or death, and graft loss or death or doubling of serum creatinine. To our knowledge this is the first

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prospective cohort in renal transplant patients documenting an association of IL-6 with graft loss. HsCRP and IL-6 are close participants in the inflammatory cascade, and the observation that both markers were predictive of outcome parameters, strengthens the perception of inflammation as an important risk factor for graft related outcomes in renal transplant patients.

The baseline levels of inflammatory markers in our patients seem moderately elevated compared to a non-transplanted background population, as seen in most studies with renal transplant recipients [14]. No universally accepted cutoff values for inflammatory markers in renal transplant recipients exists, but regarding cardiovascular outcomes in the general population CRP < 1 mg/dL confers low risk, CRP 1 to 3 mg/dL average risk and CRP > 3 mg/dL high risk [17], and the same cutoff values were described by van Ree et al. as predicting a rising creatinine in renal transplant recipients [18]. This leaves n=1203 (63%) of our hsCRP cohort with hsCRP > 1 mg/dL in the moderate to high risk group. IL-6 levels are harder to compare with other studies due to different measurement methodology. A study by Karczewski et al. found significantly higher IL-6 levels in chronic rejection (1.64 +/- 0.8 pg/mL) than in patients with stable graft function (0.42 +/- 0.3 pg/mL, p<0.001) or acute rejection (0.93 +/- 1.7 pg/dL, p=0.001) [19].

Our findings supports the concept that inflammation might be a component of the pathogenesis of chronic graft dysfunction, and is in line with the report on hsCRP and graft deterioration by van Ree et al.[18]. CRP and IL-6 has been related to renal function in predialytic chronic renal failure [20]. However, the exact pathogenetic role of hsCRP and IL-6 in graft failure is yet elusive. Indeed, genetic elevation in CRP was not associated with post-transplant morbidity and mortality [21], and lowering of hsCRP with statin in dialysis patients did not improve cardiovascular outcome [22], neither did statin treatment improve graft outcome in the ALERT [15]. IL-6 is upstream to CRP in the inflammatory cascade and seems to play an etiological role in other inflammatory disease states including rheumatoid arthritis [23], where tocilizumab, a monoclonal antibody against IL-6-receptor,

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has a proven effect. A murine model of cardiac transplantation demonstrated a tripled survival time of IL-6 deficient grafts [24], but allogeneic cardiac transplants into IL-6 deficient mice did not show prolonged graft survival indicating that the graft was the relevant source of IL-6. In human heart transplant recipients IL-6 is associated with low grade cellular rejection [25]. In renal transplant recipients elevations in serum and urine IL-6 levels are documented in acute rejection episodes [26], and increased soluble IL-6 receptor was found in urine twelve and 6 months before late graft failure [27]. A murine model of calcineurin-inhibitor (CNI) nephrotoxicity showed a protective effect of an IL-6 neutralizing antibody [28], another animal study found a preventive effect of 13-cis-retinoic acid on chronic allograft nephropathy possibly mediated through decreased secretion of inflammatory cytokines including tumor necrosis factor-alpha and IL-6 [29]. Some genetic polymorphism studies in man has shown a protective role on renal graft function of a high IL-6 gene expression in renal transplant recipients, and low IL-6 production donor genotype was associated with increased prevalence of chronic graft dysfunction [30;31], whereas other studies failed to show this association [32;33]. A small retrospective study by Cueto-Manzano et al. found no difference in inflammation markers in patients with or without CTD [34]. A pilot randomized placebo-controlled trial with an inflammation antagonist demonstrated reduced inflammation in maintenance hemodialysis patients [35]. A human randomized trial of IL-6 suppressive therapy might give an answer to whether inflammation is causative in chronic graft dysfunction, but is probably still premature awaiting further evidence from mechanistic and correlation studies. Indeed, neutralizing IL-6 antibody treatment in a murine model of acute reperfusion injury was deleterious [36].

Our finding of protective effect of older age of recipient for death-censored graft loss is in line with other studies [18;37;38], and might represent higher immunoreactivity in younger subjects. Hypertension, previous rejection and HLA-DR mismatch were independent risk factors in the present study is in line with current reviews [4;39]. Creatinine and proteinuria were significant independent risk factors for graft loss as described previously [2;40], as was time since transplantation.

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Creatinine and proteinuria are used as biomarkers of CTD, although they are unspecific of the underlying pathology and not ideal [41]. Smoking may be a risk factor for reduced patient and graft survival after transplantation[42]. Smoking may by itself increase inflammation markers[9]. Due to a surprisingly high percentage of current smokers we performed separate analysis to examine a potential interaction with smoking and inflammation. Although smoking was an independent risk marker there was no interaction with smoking and inflammation.

A limitation of this study was having only one analysis at baseline for hsCRP and IL-6, and the difference measured between groups is small and of limited prognostic value for individual renal transplant recipients. Our method of choosing covariates increases the possibility of type 1 error and should be regarded as tentative. The strength of the study is the many patients followed for an extended period and an independent adjudication of predefined outcome parameters.

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In conclusion we found that the inflammatory biomarkers hsCRP and IL-6 are significantly associated with graft related outcomes in renal transplant recipients, independent of the other examined risk factors for graft loss.

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Conflict of interest

This paper have not been published previously in whole or part.

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