



# LUND UNIVERSITY

## Inflammation-associated graft loss in renal transplant recipients

Dahle, Dag Olav; Mjoen, Geir; Öqvist, Björn; Scharnagl, Hubert; Weihrauch, Gisela; Grammer, Tanja; Maerz, Winfried; Abedini, Sadollah; Norby, Gudrun E.; Holme, Ingar; Fellstrom, Bengt; Jardine, Alan; Holdaas, Hallvard

*Published in:*  
Nephrology Dialysis Transplantation

*DOI:*  
[10.1093/ndt/gfr163](https://doi.org/10.1093/ndt/gfr163)

2011

[Link to publication](#)

*Citation for published version (APA):*

Dahle, D. O., Mjoen, G., Öqvist, B., Scharnagl, H., Weihrauch, G., Grammer, T., Maerz, W., Abedini, S., Norby, G. E., Holme, I., Fellstrom, B., Jardine, A., & Holdaas, H. (2011). Inflammation-associated graft loss in renal transplant recipients. *Nephrology Dialysis Transplantation*, 26(11), 3756-3761. <https://doi.org/10.1093/ndt/gfr163>

*Total number of authors:*  
13

### General rights

Unless other specific re-use rights are stated the following general rights apply:  
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

### Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117  
221 00 Lund  
+46 46-222 00 00





This is the overview page

## Inflammation-associated graft loss in renal transplant recipients

|                               |   |
|-------------------------------|---|
| Journal:                      | <i>Nephrology Dialysis Transplantation</i>  |
| Manuscript ID:                | NDT-00012-2011.R1   |
| Manuscript Type:              | Original Article  |
| Date Submitted by the Author: | 23-Feb-2011   |
| Complete List of Authors:     | <p>Dahle, Dag Olav; Oslo University Hospital, Ullevaal, Cardiology<br/>         Mjøen, Geir; National Hospital, Nephrology<br/>         Öqvist, Björn; University Hospital of Lund, Department of Nephrology<br/>         Scharnagl, Hubert; Medical University of Graz, Clinical Institute of Medical and Chemical Laboratory Diagnostics<br/>         Grammer, Tanja; Rupertus Carola University Heidelberg, Medical Faculty Mannheim, Mannheim Institute of Public Health, Social and Preventive Medicine<br/>         März, Winfried; Rupertus Carola University Heidelberg, Medical Faculty Mannheim, Mannheim Institute of Public Health, Social and Preventive Medicine; Synlab, Center of Laboratory Diagnostics<br/>         Abedini, Sadollah; Vestfold Hospital, Nephrology<br/>         Norby, Gudrun; National Hospital, Nephrology<br/>         Holme, Ingar; Dept Cardiology, OUS Ulleval<br/>         Fellstrom, Bengt; University Hospital Uppsala, Internal Medicine, Nephrology<br/>         Jardine, Alan; British Heart Foundation, Glasgow Cardiovascular Research Centre<br/>         Holdaas, Hallvard; National Hospital/Medical Dpt B, Nephrology</p> |
| Key Words:                    | ALERT, high-sensitivity CRP, inflammation, interleukin-6, renal allograft survival, chronic transplant dysfunction  |
|                               |   |

Department of Medicine, Oslo University Hospital, Rikshospitalet, Oslo, Norway

February 19, 2011

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 pulmonary 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

Dag Olav Dahle

Lead author

Hallvard Holdaas

Senior author

## Inflammation-associated graft loss in renal transplant recipients

Dag Olav Dahle<sup>a,\*</sup>, Geir Mjøen<sup>a</sup>, Björn Öqvist<sup>b</sup>, Hubert Scharnagl<sup>c</sup>, Gisela Weihrauch<sup>c</sup>, Tanja Grammer<sup>d</sup>, Winfried März<sup>c,d,e</sup>, Sadollah Abedini<sup>f</sup>, Gudrun Norby<sup>a</sup>, Ingar Holme<sup>g</sup>, Bengt Fellström<sup>h</sup>, Alan Jardine<sup>i</sup>, Hallvard Holdaas<sup>a</sup>.

<sup>a</sup>Dept of Nephrology, Oslo University Hospital, Rikshospitalet, Oslo, Norway

<sup>b</sup>Dept of Nephrology, Lunds University, Lund, Sweden

<sup>c</sup>Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria

<sup>d</sup>Institute of Public Health, Social and Preventive Medicine, Medical Faculty Mannheim, University of Heidelberg, Germany

<sup>e</sup>Synlab Center of Laboratory Diagnostics Heidelberg LLC, Eppelheim, Germany

<sup>f</sup>Toensberg County Hospital, Toensborg, Norway

<sup>g</sup>Oslo University Hospital, Ullevaal, Oslo

<sup>h</sup>University Hospital Uppsala, International Medicine, Nephrology, Uppsala, Sweden

<sup>i</sup>British Heart Foundation Glasgow Cardiovascular Research Centre, Glasgow, United Kingdom

\*Corresponding author: Dag Olav Dahle, d\_o\_dahle@yahoo.com. Current adr of Dept cardiology, Oslo University Hospital, Ullevål, Oslo, Norway. Ph.+4722118080

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.

1  
2 Key words: ALERT, high-sensitivity CRP, inflammation, interleukin-6, renal allograft survival.  
3  
4  
5

6  
7 Short summary: This post-hoc analysis of of maintenance renal transplant recipients (n=2102) from  
8  
9 the Assessment of Lescol in Renal Transplantation (ALERT) study demonstrates associations of the  
10  
11 inflammatory markers high-sensitivity C-reactive protein and interleukin 6 with chronic transplant  
12  
13 dysfunction.  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review

## 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<sup>1</sup>. Annual graft loss is still 2-7%<sup>2-4</sup> and the average deceased donor kidney transplant functions for about ten years<sup>5</sup>. Clinically, we observe a progressive transplant dysfunction characterised by a slowly rising serum creatinine, proteinuria and hypertension<sup>3</sup>. 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<sup>4</sup>. 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<sup>6</sup>, 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<sup>7,8</sup>, 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<sup>9,10</sup>. Renal transplant recipients, by the process of receiving an allograft, have an additional activation of their inflammation status<sup>11-13</sup>. 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<sup>14</sup>. 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<sup>15</sup>. 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<sup>15;16</sup>. 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 provided 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.

1  
2 Since the two arms showed no significant heterogeneity in relation between the inflammation  
3  
4 markers hsCRP and IL-6, subsequent analyses were performed on the pooled patient population.  
5  
6 HsCRP and IL-6 were not normally distributed and logarithmic transformation of these factors was  
7  
8 used in the analysis. HsCRP and IL-6 were analysed in separate models due to confounding (not  
9  
10 shown) and the close etiological relationship. Risk factors were evaluated using uni- and  
11  
12 multivariate Cox proportional hazard model. Covariates were chosen in a stepwise approach,  
13  
14 including covariates with univariate significance and previously reported factors associated with  
15  
16 graft outcomes, and then excluding most variables that did not retain statistical significance in the  
17  
18 multivariate analysis. SPSS version 17.0 (SPSS Inc., Chicago, IL) was used for statistical analysis.  
19  
20  
21  
22  
23  
24  
25

## 26 **Results**

27  
28 Baseline characteristics in ALERT and ALERT extension were similar in both treatment arms<sup>15;16</sup>.  
29

30 Baseline characteristics in relation to renal endpoints in the placebo group have been reported  
31  
32 separately<sup>2</sup>. The present study reports treatment and placebo group together. Baseline  
33  
34 characteristics are shown in table 1. Mean age at study entry was 50 years, 66% were male and all  
35  
36 received cyclosporine-based immunosuppression at entry. The main causes of renal failure leading  
37  
38 to transplantation were glomerulonephritis and polycystic kidney disease. Frequencies of graft  
39  
40 failure endpoints are shown in table 2.  
41  
42  
43  
44  
45  
46

47 HsCRP and IL-6 were available in 1910 and 1751 patients, respectively. Mean (SD) and median  
48  
49 (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)  
50  
51 pg/ml and 2.4 (2.2) pg/ml. The hsCRP and IL-6 cohort had almost identical baseline characteristic  
52  
53 as reported earlier<sup>14</sup>, with no differences in proportions with diabetes, coronary heart disease,  
54  
55 current smokers or live donor.  
56  
57  
58  
59  
60

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

1  
2 Missing data excluded 450 (21.4%) and 576 (27.4%) patients in the hsCRP and IL-6 multivariate  
3  
4 models, respectively. Both hsCRP and IL-6 were in separate multivariate models significantly  
5  
6 associated with the renal endpoints shown, ie death-censored graft loss, graft loss including death,  
7  
8 and graft loss including death and doubling of serum creatinine. Due to a surprisingly high  
9  
10 percentage of current smokers we performed separate analysis (not shown) to examine a potential  
11  
12 interaction with smoking and inflammation. All interaction covariates were non-significant  $p>0.20$ ,  
13  
14 and the inflammation markers were equally strong in smokers and non-smoker, i.e. no interaction.  
15  
16 Excluding smoking as a covariate (not shown) changed the regression coefficients for the  
17  
18 inflammation markers less than 15% and the hazard ratios less than 5 %, indicating no confounding  
19  
20 effect.  
21  
22  
23  
24  
25  
26  
27

28 In multivariate analysis for death-censored graft loss the results were similar in the hsCRP and IL-6  
29  
30 cohorts. We found significant negative associations of age and BMI. Significant positive  
31  
32 associations were seen for current smoking, systolic blood pressure, creatinine, time since last  
33  
34 transplantation, two HLA-DR mismatches, previous treatment for rejection and proteinuria.  
35  
36  
37  
38

39 When including death in the endpoint the association of age became significantly positive, diabetes  
40  
41 became significant and previous treatment for rejection lost significance. HLA-DR mismatch was  
42  
43 significant when including doubling of serum creatinine in the endpoint. We still found significant  
44  
45 negative association of BMI and positive associations of current smoking, systolic blood pressure,  
46  
47 creatinine, time since last transplantation and proteinuria.  
48  
49  
50  
51  
52  
53

## 54 Discussion

55  
56 In the present post hoc analysis, we have shown that inflammation, adjusted for traditional risk  
57  
58 factors, is associated with death-censored renal graft loss, and the composite endpoints; graft loss or  
59  
60 death, and graft loss or death or doubling of serum creatinine. To our knowledge this is the first

1  
2 prospective cohort in renal transplant patients documenting an association of IL-6 with graft loss.  
3  
4 HsCRP and IL-6 are close participants in the inflammatory cascade, and the observation that both  
5  
6 markers were predictive of outcome parameters, strengthens the perception of inflammation as an  
7  
8 important risk factor for graft related outcomes in renal transplant patients.  
9

10  
11  
12  
13  
14 The baseline levels of inflammatory markers in our patients seem moderately elevated compared to  
15  
16 a non-transplanted background population, as seen in most studies with renal transplant recipients  
17  
18 <sup>14</sup>. No universally accepted cutoff values for inflammatory markers in renal transplant recipients  
19  
20 exists, but regarding cardiovascular outcomes in the general population CRP < 1 mg/dL confers low  
21  
22 risk, CRP 1 to 3 mg/dL average risk and CRP > 3 mg/dL high risk <sup>17</sup>, and the same cutoff values  
23  
24 were described by van Ree et al. as predicting a rising creatinine in renal transplant recipients <sup>18</sup>.  
25  
26  
27 This leaves n=1203 (63%) of our hsCRP cohort with hsCRP > 1 mg/dL in the moderate to high risk  
28  
29 group. IL-6 levels are harder to compare with other studies due to different measurement  
30  
31 methodology. A study by Karczewski et al. found significantly higher IL-6 levels in chronic  
32  
33 rejection (1.64 +/- 0.8 pg/mL) than in patients with stable graft function (0.42 +/- 0.3 pg/mL,  
34  
35 p<0.001) or acute rejection (0.93 +/- 1.7 pg/dL, p=0.001) <sup>19</sup>.  
36  
37  
38  
39  
40  
41  
42

43  
44 Our findings supports the concept that inflammation might be a component of the pathogenesis of  
45  
46 chronic graft dysfunction, and is in line with the report on hsCRP and graft deterioration by van Ree  
47  
48 et al. <sup>18</sup>. CRP and IL-6 has been related to renal function in predialytic chronic renal failure <sup>20</sup>.

49  
50 However, the exact pathogenetic role of hsCRP and IL-6 in graft failure is yet elusive. Indeed,  
51  
52 genetic elevation in CRP was not associated with post-transplant morbidity and mortality <sup>21</sup>, and  
53  
54 lowering of hsCRP with statin in dialysis patients did not improve cardiovascular outcome <sup>22</sup>,  
55  
56 neither did statin treatment improve graft outcome in the ALERT <sup>15</sup>. IL-6 is upstream to CRP in the  
57  
58 inflammatory cascade and seems to play an etiological role in other inflammatory disease states  
59  
60 including rheumatoid arthritis <sup>23</sup>, where tocilizumab, a monoclonal antibody against IL-6-receptor,

1  
2 has a proven effect. A murine model of cardiac transplantation demonstrated a tripled survival time  
3  
4 of IL-6 deficient grafts <sup>24</sup>, but allogeneic cardiac transplants into IL-6 deficient mice did not show  
5  
6 prolonged graft survival indicating that the graft was the relevant source of IL-6. In human heart  
7  
8 transplant recipients IL-6 is associated with low grade cellular rejection <sup>25</sup>. In renal transplant  
9  
10 recipients elevations in serum and urine IL-6 levels are documented in acute rejection episodes <sup>26</sup>,  
11  
12 and increased soluble IL-6 receptor was found in urine twelve and 6 months before late graft failure  
13  
14 <sup>27</sup>. A murine model of calcineurin-inhibitor (CNI) nephrotoxicity showed a protective effect of an  
15  
16 IL-6 neutralizing antibody <sup>28</sup>, another animal study found a preventive effect of 13-cis-retinoic acid  
17  
18 on chronic allograft nephropathy possibly mediated through decreased secretion of inflammatory  
19  
20 cytokines including tumor necrosis factor-alpha and IL-6 <sup>29</sup>. Some genetic polymorphism studies in  
21  
22 man has shown a protective role on renal graft function of a high IL-6 gene expression in renal  
23  
24 transplant recipients, and low IL-6 production donor genotype was associated with increased  
25  
26 prevalence of chronic graft dysfunction <sup>30;31</sup>, whereas other studies failed to show this association  
27  
28 <sup>32;33</sup>. A small retrospective study by Cueto-Manzano et al. found no difference in inflammation  
29  
30 markers in patients with or without CTD <sup>34</sup>. A pilot randomized placebo-controlled trial with an  
31  
32 inflammation antagonist demonstrated reduced inflammation in maintenance hemodialysis patients  
33  
34 <sup>35</sup>. A human randomized trial of IL-6 suppressive therapy might give an answer to whether  
35  
36 inflammation is causative in chronic graft dysfunction, but is probably still premature awaiting  
37  
38 further evidence from mechanistic and correlation studies. Indeed, neutralizing IL-6 antibody  
39  
40 treatment in a murine model of acute reperfusion injury was deleterious <sup>36</sup>.  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52

53 Our finding of protective effect of older age of recipient for death-censored graft loss is in line with  
54  
55 other studies <sup>18;37;38</sup>, and might represent higher immunoreactivity in younger subjects.

56 Hypertension, previous rejection and HLA-DR mismatch were independent risk factors in the  
57  
58 present study is in line with current reviews <sup>4;39</sup>. Creatinine and proteinuria were significant  
59  
60 independent risk factors for graft loss as described previously <sup>2;40</sup>, as was time since transplantation.

1  
2 Creatinine and proteinuria are used as biomarkers of CTD, although they are unspecific of the  
3  
4 underlying pathology and not ideal<sup>41</sup>. Smoking may be a risk factor for reduced patient and graft  
5  
6 survival after transplantation<sup>42</sup>. Smoking may by itself increase inflammation markers<sup>9</sup>. Due to a  
7  
8 surprisingly high percentage of current smokers we performed separate analysis to examine a  
9  
10 potential interaction with smoking and inflammation. Although smoking was an independent risk  
11  
12 marker there was no interaction with smoking and inflammation.  
13  
14  
15  
16  
17  
18

19 A limitation of this study was having only one analysis at baseline for hsCRP and IL-6, and the  
20  
21 difference measured between groups is small and of limited prognostic value for individual renal  
22  
23 transplant recipients. Our method of choosing covariates increases the possibility of type 1 error and  
24  
25 should be regarded as tentative. The strength of the study is the many patients followed for an  
26  
27 extended period and an independent adjudication of predefined outcome parameters.  
28  
29  
30  
31  
32

33 In conclusion we found that the inflammatory biomarkers hsCRP and IL-6 are significantly  
34  
35 associated with graft related outcomes in renal transplant recipients, independent of the other  
36  
37 examined risk factors for graft loss.  
38  
39  
40  
41  
42  
43  
44

#### 45 **Acknowledgements**

46  
47 The ALERT trial was sponsored by Novartis AG.  
48  
49  
50  
51

#### 52 **Disclosure**

53  
54 The authors of this manuscript have conflicts of interest to disclose as described by Nephrology,  
55  
56 Dialysis and Transplantation. The ALERT trial was sponsored by Novartis AG. Otherwise, the  
57  
58 authors report no conflict of interest.  
59  
60

## Conflict of interest

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

## Reference List

1. Meier-Kriesche HU, Schold JD, Kaplan B. Long-term renal allograft survival: have we made significant progress or is it time to rethink our analytic and therapeutic strategies? *Am J Transplant* 2004; 4: 1289-1295
2. Fellstrom B, Holdaas H, Jardine AG *et al*. Risk factors for reaching renal endpoints in the assessment of Lescol in renal transplantation (ALERT) trial. *Transplantation* 2005; 79: 205-212
3. Pascual M, Theruvath T, Kawai T, Tolkoff-Rubin N, Cosimi AB. Strategies to improve long-term outcomes after renal transplantation. *N Engl J Med* 2002; 346: 580-590
4. Weir MR, Wali RK. Minimizing the risk of chronic allograft nephropathy. *Transplantation* 2009; 87: S14-S18
5. Halloran PF, Melk A, Barth C. Rethinking chronic allograft nephropathy: the concept of accelerated senescence. *J Am Soc Nephrol* 1999; 10: 167-181
6. Mengel M. The kidney transplant: new horizons. *Curr Opin Nephrol Hypertens* 2010; 19: 260-265
7. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997; 336: 973-979
8. Pai JK, Pischon T, Ma J *et al*. Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med* 2004; 351: 2599-2610
9. Watz H, Waschki B, Kirsten A *et al*. The metabolic syndrome in patients with chronic bronchitis and COPD: frequency and associated consequences for systemic inflammation and physical inactivity. *Chest* 2009; 136: 1039-1046
10. Nishikawa T, Hagihara K, Serada S *et al*. Transcriptional complex formation of c-Fos, STAT3, and hepatocyte NF-1 alpha is essential for cytokine-driven C-reactive protein gene expression. *J Immunol* 2008; 180: 3492-3501
11. Vazquez MA, Jeyarajah DR, Kielar ML, Lu CY. Long-term outcomes of renal transplantation: a result of the original endowment of the donor kidney and the inflammatory response to both alloantigens and injury. *Curr Opin Nephrol Hypertens* 2000; 9: 643-648
12. Cottone S, Palermo A, Vaccaro F *et al*. Inflammation and endothelial activation are linked to renal function in long-term kidney transplantation. *Transpl Int* 2007; 20: 82-87
13. Kruger B, Krick S, Dhillon N *et al*. Donor Toll-like receptor 4 contributes to ischemia and reperfusion injury following human kidney transplantation. *Proc Natl Acad Sci U S A* 2009;

- 1  
2 106: 3390-3395  
3  
4 14. Abedini S, Holme I, Marz W *et al.* Inflammation in renal transplantation. *Clin J Am Soc*  
5 *Nephrol* 2009; 4: 1246-1254  
6  
7 15. Holdaas H, Fellstrom B, Jardine AG *et al.* Effect of fluvastatin on cardiac outcomes in renal  
8 transplant recipients: a multicentre, randomised, placebo-controlled trial. *Lancet* 2003; 361:  
9 2024-2031  
10  
11 16. Holdaas H, Fellstrom B, Cole E *et al.* Long-term cardiac outcomes in renal transplant  
12 recipients receiving fluvastatin: the ALERT extension study. *Am J Transplant* 2005; 5: 2929-  
13 2936  
14  
15 17. Pearson TA, Mensah GA, Alexander RW *et al.* Markers of inflammation and cardiovascular  
16 disease: application to clinical and public health practice: A statement for healthcare  
17 professionals from the Centers for Disease Control and Prevention and the American Heart  
18 Association. *Circulation* 2003; 107: 499-511  
19  
20 18. van Ree RM, Oterdoom LH, de Vries AP *et al.* Elevated levels of C-reactive protein  
21 independently predict accelerated deterioration of graft function in renal transplant  
22 recipients. *Nephrol Dial Transplant* 2007; 22: 246-253  
23  
24 19. Karczewski M, Karczewski J, Poniedzialek B, Wiktorowicz K, Smietanska M, Glyda M.  
25 Distinct cytokine patterns in different states of kidney allograft function. *Transplant Proc*  
26 2009; 41: 4147-4149  
27  
28 20. Panichi V, Migliori M, De PS *et al.* C-reactive protein and interleukin-6 levels are related to  
29 renal function in predialytic chronic renal failure. *Nephron* 2002; 91: 594-600  
30  
31 21. Kruger B, Boger CA, Schroppel B *et al.* No effect of C-reactive protein (CRP) haplotypes  
32 on CRP levels and post-transplant morbidity and mortality in renal transplantation. *Transpl*  
33 *Int* 2008; 21: 452-458  
34  
35 22. Fellstrom BC, Jardine AG, Schmieder RE *et al.* Rosuvastatin and cardiovascular events in  
36 patients undergoing hemodialysis. *N Engl J Med* 2009; 360: 1395-1407  
37  
38 23. Nishimoto N. Interleukin-6 as a therapeutic target in candidate inflammatory diseases. *Clin*  
39 *Pharmacol Ther* 2010; 87: 483-487  
40  
41 24. Liang Y, Christopher K, Finn PW, Colson YL, Perkins DL. Graft produced interleukin-6  
42 functions as a danger signal and promotes rejection after transplantation. *Transplantation*  
43 2007; 84: 771-777  
44  
45 25. Perez-Villa F, Benito B, Llancaqueo M, Cuppoletti A, Roig E. Elevated levels of serum  
46 interleukin-6 are associated with low grade cellular rejection in patients with heart  
47 transplantation. *Transplant Proc* 2006; 38: 3012-3015  
48  
49 26. Waiser J, Budde K, Katalinic A, Kuerzdorfer M, Riess R, Neumayer HH. Interleukin-6  
50 expression after renal transplantation. *Nephrol Dial Transplant* 1997; 12: 753-759  
51  
52 27. Sadeghi M, Daniel V, Wiesel M, Hergesell O, Opelz G. High urine sIL-6R as a predictor of  
53 late graft failure in renal transplant recipients. *Transplantation* 2003; 76: 1190-1194  
54  
55 28. LaSpina M, Tripathi S, Gatto LA, Bruch D, Maier KG, Kittur DS. An interleukin-6-

- 1  
2 neutralizing antibody prevents cyclosporine-induced nephrotoxicity in mice. *J Surg Res*  
3 2008; 148: 121-125  
4
- 5  
6 29. Adams J, Kiss E, Arroyo AB *et al.* 13-cis retinoic acid inhibits development and progression  
7 of chronic allograft nephropathy. *Am J Pathol* 2005; 167: 285-298  
8
- 9  
10 30. Muller-Steinhardt M, Hartel C, Muller B, Kirchner H, Fricke L. The interleukin-6 -  
11 174promoter polymorphism is associated with long-term kidney allograft survival. *Kidney*  
12 *Int* 2002; 62: 1824-1827  
13
- 14  
15 31. Kocierz M, Kujawa-Szewieczek A, Kolonko A, Chudek J, Wiecek A. [The influence of  
16 selected cytokine gene polymorphisms on the occurrence of acute and chronic rejection and  
17 on kidney graft survival]. *Postepy Hig Med Dosw (Online )* 2009; 63: 613-626  
18
- 19  
20 32. Kruger B, Walberer A, Farkas S *et al.* The impact of "high-producer" interleukin-6  
21 haplotypes on cardiovascular morbidity and mortality in a kidney transplant population.  
22 *Transplant Proc* 2009; 41: 2539-2543  
23
- 24  
25 33. Sanchez-Velasco P, Rodrigo E, Fernandez-Fresnedo G *et al.* Influence of interleukin-6  
26 promoter polymorphism -174 g/c on kidney graft outcome. *Transplant Proc* 2010; 42: 2854-  
27 2855  
28
- 29  
30 34. Cueto-Manzano AM, Morales-Buenrostro LE, Gonzalez-Espinoza L *et al.* Markers of  
31 inflammation before and after renal transplantation. *Transplantation* 2005; 80: 47-51  
32
- 33  
34 35. Hung AM, Ellis CD, Shintani A, Booker C, Ikizler TA. IL-1{beta} Receptor Antagonist  
35 Reduces Inflammation in Hemodialysis Patients. *J Am Soc Nephrol* 2011;  
36  
37 36. de Vries DK, Lindeman JH, Tsikas D *et al.* Early renal ischemia-reperfusion injury in  
38 humans is dominated by IL-6 release from the allograft. *Am J Transplant* 2009; 9: 1574-  
39 1584  
40
- 41  
42 37. Joosten SA, Sijpkens YW, van KC, Paul LC. Chronic renal allograft rejection:  
43 pathophysiologic considerations. *Kidney Int* 2005; 68: 1-13  
44
- 45  
46 38. Siddiqi N, McBride MA, Hariharan S. Similar risk profiles for post-transplant renal  
47 dysfunction and long-term graft failure: UNOS/OPTN database analysis. *Kidney Int* 2004;  
48 65: 1906-1913  
49
- 50  
51 39. Li C, Yang CW. The pathogenesis and treatment of chronic allograft nephropathy. *Nat Rev*  
52 *Nephrol* 2009; 5: 513-519  
53
- 54  
55 40. Kasiske BL, Andany MA, Danielson B. A thirty percent chronic decline in inverse serum  
56 creatinine is an excellent predictor of late renal allograft failure. *Am J Kidney Dis* 2002; 39:  
57 762-768  
58
- 59  
60 41. Bestard O, Cruzado JM, la FM, Grinyo JM. Biomarkers in renal transplantation. *Curr Opin*  
*Organ Transplant* 2010; 15: 467-473  
61
- 62  
63 42. Nogueira JM, Haririan A, Jacobs SC, Cooper M, Weir MR. Cigarette smoking, kidney  
64 function, and mortality after live donor kidney transplant. *Am J Kidney Dis* 2010; 55: 907-  
65 915  
66

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review

**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/m <sup>2</sup> | 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, $\mu$ mol/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)          |

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

| Variable                                      | All<br>n=2102 | hsCRP cohort<br>n=1910 | IL-6 cohort<br>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)            |

For Peer Review

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

| Risk factor             | Graft loss (death-censored), n=288 |                         |         | Graft loss, doubling of s-creatinine or death, n=567 |                         |         | Graft loss or death, n=515 |                         |         |
|-------------------------|------------------------------------|-------------------------|---------|--|-------------------------|---------|----------------------------|-------------------------|---------|
|                         | Unadjusted HR<br>(95% CI)          | Adjusted HR<br>(95% CI) | P value | Unadjusted HR<br>(95% CI)                            | Adjusted HR<br>(95% CI) | P value | Unadjusted HR<br>(95% CI)  | Adjusted HR<br>(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-censored), n=259 |                         |         | Graft loss, doubling of s-creatinine or death, n=509 |                         |         | Graft loss or death, n=461 |                         |         |
|-------------------------|------------------------------------|-------------------------|---------|--|-------------------------|---------|----------------------------|-------------------------|---------|
|                         | Unadjusted HR<br>(95% CI)          | Adjusted HR<br>(95% CI) | P value | Unadjusted HR<br>(95% CI)                            | Adjusted HR<br>(95% CI) | P value | Unadjusted HR<br>(95% CI)  | Adjusted HR<br>(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.

1  
2  
3  
4 Your paper has been evaluated by a subject editor, 2 external reviewers and at the editorial office.  
5 Although of interest to the readership of NDT, both the subject editor and I feel that major  
6 modifications are necessary before your paper can be considered for publication. We would like to ask  
7 you to submit a revised version, taking into account the reviewers' remarks below.  
8

9 As reviewer 2 points out in a very careful evaluation of your paper, there are some flaws and the  
10 conclusion that hsCRP and IL-6 are independent risk factors is most likely overstated or may even be  
11 wrong (for more details, please see below).  
12

13 [We have detailed this issue in response to reviewer 2](#)  
14

15 We ask you to address these concerns of the reviewers below and already look forward to receiving  
16 your revised paper.  
17

18 If you do decide to resubmit, we would be pleased to review your modified version for publication.  
19 Please submit both a corrected version with additions underlined in red and deletions crossed out in  
20 blue, and a clean version of your revision without the corrections still marked in the text. Please also  
21 include a cover letter to the Editor-in-Chief, and a separate point-by-point answer to the reviewers'  
22 comments. Note that a final decision on your manuscript will only be taken after evaluation of the  
23 revised version.  
24

25 **IMPORTANT!** You must resubmit via the website and under the same manuscript ID. For instructions  
26 on this procedure, please click on 'Instructions and Forms' in your author centre.  
27

28 **Figures:** When submitting your revised version, please upload your final publication quality figures to  
29 the Manuscript Central website.  
30

31 These figures should meet the following conditions:

- 32 - Line drawings (black and white) should be 600 dpi minimum
- 33 - Colour and greyscale figures should be 300 dpi minimum
- 34 - Colour figures should be supplied in CMYK colours, not RGB colours
- 35 - Each figure must be clearly labelled with the figure/scheme number

36 Please also note that we require all authors to complete a Conflict of Interest Form (see 'Instructions to  
37 Authors'), which should be inserted at the end of the manuscript.  
38

39 We look forward to hearing from you. Please take into account that the deadline for resubmission is six  
40 months, after which your paper will be considered as a new submission.  
41

42 Yours sincerely,  
43 Prof. dr. N. Lameire Editor In Chief, Nephrology Dialysis Transplantation  
44  
45

46 —  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

---

Reviewer: 1

Comments to the Author

Many papers of the ALERT-study group have been already published during the last decade. The current paper is obviously another analysis of the huge ALERT database (n=2102 renal transplant recipients) investigating the association between the inflammatory markers high-sensitivity CRP and interleukin-6 with graft outcomes.

The aim of the present study is therefore to investigate the baseline role of inflammatory biomarkers on progressive graft dysfunction and failure.

The authors came out with the result that 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.

Despite some flaws of the paper like the fact that only baseline levels of hsCRP and IL-6 were available etc., the large number of patients and the longterm observational period merrits the publication of the data. There will be only little chance to wait for a human randomized controlled trial. It is also not possible to recruite more data from the ALERT database. There might be the risk that the data are only tentative however for me it is worthwhile to read it.

We understand reviewer's concern regarding the lack of longitudinal data for IL-6 and hsCRP. That was one of the flaws we regret in the original study design of the trial. The strength as the reviewer points out; large number of patients, long follow-up time and not least an endpoint committee adjudicating clinical events.

We do agree with the reviewer that the data may be tentative. In our discussion we have stated:

"Our method of choosing covariates increases the possibility of type 1 error and should be regarded as tentative."

We appreciate the reviewer's opinion that our data are worthwhile to read.

Reviewer: 2

Comments to the Author

Inflammation-associated graft loss in renal transplant recipients  
Dahle et al.

The authors describe an association of hs-CRP and IL-6 blood levels with graft loss and conclude that graft loss might be due to inflammation-induced chronic graft rejection. During a randomized double-blinded placebo-controlled trial of fluvastatin in a total of 2102 patients (ALERT study), hs-CRP and IL-6 blood levels were determined in 1910 and 1751 renal transplant recipients, respectively. Both parameters were determined once at baseline. Because placebo- and verum-treated patients showed similar hsCRP and IL-6 blood levels, statistical analysis was performed on the pooled patient population. Patients were followed for at least 5 years with a mean follow up of 6.7 years. Enrolled patients were 30-75 years old (mean 50 years), showed stable graft function at enrollment (mean creatinine 145  $\mu\text{mol/l}$ ), and had been transplanted more than 6 months before enrollment (mean 5.1 years). Patients were seen at 6 month intervals during the study period.

Based on 1910 patients in the hsCRP cohort and 1751 patients in the IL-6 cohort, approximately 18% (18.1% and 17.6%) of the patients in the two groups showed death-censored graft loss, approximately 34% (34.7% and 33.7%) graft loss, doubling of serum creatinine or death, and approximately 31% (31.7% and 30.8%) graft loss or death after 7 years of follow up (Table 2).

Due to missing data, 450 (21.4%) patients in the hsCRP and 576 (27.4%) patients in the IL-6 cohort were excluded from analysis, leaving 1652 patients in the hsCRP cohort and 1526 patients in the IL-6 cohort (Tables 3 and 4). In multivariate models, both hs-CRP and IL-6 were significantly associated with the renal endpoints death-censored graft loss, graft loss including death, and graft loss including death and doubling of serum creatinine. In multivariate analysis for death-censored graft loss, there were negative associations of age and BMI and positive associations of current smoking, systolic blood pressure, creatinine, time since last transplantation, 2 HLA-DR mismatches, and previous treatment for rejection and proteinuria.

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

1  
2  
3 cytokine-driven C-reactive protein gene expression. Nishikawa T, Hagihara K, Serada S, Isobe T,  
4 Matsumura A, Song J, Tanaka T, Kawase I, Naka T, Yoshizaki K.). Induction of CRP is strongly IL-6-  
5 dependent and blood levels of the two parameters would be expected to behave similarly in patients  
6 with strong IL-6 induction. Both parameters are sensitive indicators of inflammation.  
7

8 The reviewer highlights an important point by commenting that hsCRP is strongly IL-6 dependent.  
9 Indeed, we do agree. We also share the reviewer's point that the two parameters would be expected  
10 to behave similarly, and they did indeed in our trial. We performed a comprehensive statistical re-run  
11 for hsCRP with and without IL-6. IL-6 is probably by a large extent a precipitator for hsCRP. In the  
12 regression analysis running both inflammation markers included, versus separately, there was a  
13 confounding effect indicating a causal relation. We have added a comment on this in the article.  
14

15 In the present study, current smoking was strongly associated with death-censored graft loss, graft  
16 loss, doubling of serum creatinine or death, and graft loss or death in the hsCRP as well as the IL-6  
17 cohort ( $p < 0.001$ ) (Tables 3 and 4). It can be assumed that "current smoker" includes many strong  
18 smokers with associated chronic bronchitis at study baseline. Chronic bronchitis might have been  
19 responsible - at least in part - for the increased hsCRP and IL-6 blood levels at baseline (Chest. 2009  
20 Oct;136(4):1039-46. The metabolic syndrome in patients with chronic bronchitis and COPD: frequency  
21 and associated consequences for systemic inflammation and physical inactivity. Watz H, Waschki B,  
22 Kirsten A, Müller KC, Kretschmar G, Meyer T, Holz O, Magnussen H.). To minimize the effect of  
23 chronic bronchitis, the authors should analyze hs-CRP and IL-6 blood levels patients excluding  
24 "current smokers".  
25

26 We tested for a possible confounding effect of smoking on the effect of inflammation by running the  
27 multivariate analyses with and without current smoking as a covariate, and found no significant  
28 difference in the regression coefficient of hsCRP or IL6 (ie all change  $< 15\%$ ) nor in the hazard ratios of  
29 hsCRP or IL6 (ie all change  $< 5\%$ ). We also tested for interaction between smoking and inflammation  
30 by adding a multiplication covariate (ie smoking\*Ln\_hsCRP or smoking\*Ln\_IL6, respectively), with all  
31  $p > 0.20$ , indicating no interaction. An analysis excluding current smokers reduces the study population,  
32 but multivariate hazard ratios did not change substantially, also indicating no interaction between  
33 smoking and inflammation. HsCRP remains highly significant among non-smokers for all endpoints  
34 ( $p < 0.001$ ), whereas IL6 remains significant for graftloss or death, graft loss doubling creatinine or  
35 death. The multivariate HR of IL6 for death-censored graftloss in nonsmokers is 1.20 (95% CI 0.93-  
36 1.55)  $p = 0.16$ . We added a comment in the article on the multivariate tests for interaction and  
37 confounding, but not for the stratified analysis, as it refers to a much smaller study cohort  
38 This lack of difference might indicate that other factors for elevated inflammation is more prominent in  
39 transplant patients than smoke status. The inflammation caused by the graft itself is probably an  
40 important factor (Vazquez et al Curr Opin Nephrol Hypertens 2000; 9:643-48, Cottone et al Transplant  
41 Int 2007;20:82-87, Gotsman et al. Circulation 2006; 114: 2047-55, Martinez-Castelao et al Transplant  
42 Proc 2005;37:3788-90, Sadollah et al. cJASN 2009; 4: 1246-54).  
43

- 44 • It would be interesting to study whether hs-CRP and IL-6 blood levels were stronger associated  
45 with current smoking than with creatinine levels or proteinuria.  
46

47 As proposed by the reviewer we performed this analysis. As smoking is a dichotomized endpoint we  
48 also dichotomized creatinine and protein excretion by median in regard to blood levels of the  
49 inflammation markers. IL-6 in smokers 3.27 (2.05) pg/ml and in patients with "high" creatinine 3.08  
50 (1.98) pg/ml.. Corresponding values for hsCRP were 4.89 (8.189) mg/L and 4.01 (7.39) mg/L smokers  
51 and "high" creatinine patients. But as stated above, there was no interaction with current smoking. We  
52 thus observed small but statistically significant higher baseline levels (Mann-Whitney U-test) of both  
53 inflammatory markers in smokers than non-smokers. We also observed significantly higher level of IL-  
54 6 in the "high" creatinine group than the "low" creatinine group, and significantly higher level of IL-6 in  
55 the "high" proteinuria group than the "low" proteinuria group, while hsCRP was not different in the  
56 creatinine or proteinuria groups. Among non-smokers the baseline s-IL6 was significantly higher in the  
57 "high" creatinine group than the "low" creatinine group. For non-smokers we observed a trend ( $p = 0.09$ )  
58 for higher IL-6 in the "high" proteinuria-group than the "low" proteinuria group. Among the smokers the  
59 baseline IL-6 and hs-CRP levels were not significantly different between creatinine or proteinuria  
60 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.

1  
2  
3  
4 • There are reports in the literature indicating that history of smoking before transplantation is  
5 associated with impaired transplant and patient survival and an increased risk of early rejection (Am J  
6 Kidney Dis. 2010 May;55(5):907-15. Cigarette smoking, kidney function, and mortality after live donor  
7 kidney transplant. Nogueira JM, Haririan A, Jacobs SC, Cooper M, Weir MR).  
8 With smokers included and not considered separately, and given the association of chronic bronchitis  
9 with inflammation markers, it is difficult to draw the conclusion from the data that increased hsCRP and  
10 IL-6 blood levels are early predictive markers of inflammation-induced graft loss. The sentence at the  
11 end of the discussion section, saying that hsCRP and IL-6 increases are risk factors independent of  
12 other recognized risk factors for graft loss, seems unsustainable and needs to be substantiated.  
13

14 The reviewer addresses the point if smoking pre-transplantation is a possible confounder or an effect  
15 modifier. There was no interaction or confounding for smoking (see above). We also believe that  
16 smoking for several reasons is an important issue also in renal transplant, but as we have outlined  
17 above the inflammation status in transplanted patients might be driven by other factors than smoking.  
18 We have nuanced the sentence at the end of discussion to “examined risk factors.” It might be to  
19 ambitious to include all “recognizable risk factors”.  
20

21  
22 Minor concerns:

23 • In Table 1: “live donor ??????”

24 Typing error, the question marks are deleted  
25

26 • In Tables 3 and 4: “Ln hsCRP” and “Ln IL-6”. What is the meaning of Ln?  
27

28 Ln is abbreviation for natural logarithm, we add explanation in the tables.  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## Inflammation-associated graft loss in renal transplant recipients

Dag Olav Dahle<sup>a,\*</sup>, Geir Mjøen<sup>a</sup>, Björn Öqvist<sup>b</sup>, Hubert Scharnagl<sup>c</sup>, Gisela Weihrauch<sup>c</sup>, Tanja Grammer<sup>d</sup>, Winfried März<sup>c,d,e</sup>, Sadollah Abedini<sup>f</sup>, Gudrun Norby<sup>a</sup>, Ingar Holme<sup>g</sup>, Bengt Fellström<sup>h</sup>, Alan Jardine<sup>i</sup>, Hallvard Holdaas<sup>a</sup>.

<sup>a</sup>Dept of Nephrology, Oslo University Hospital, Rikshospitalet, Oslo, Norway

<sup>b</sup>Dept of Nephrology, Lunds University, Lund, Sweden

Deleted: <sup>b</sup> Dept

<sup>c</sup>Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria

<sup>d</sup>Institute of Public Health, Social and Preventive Medicine, Medical Faculty Mannheim, University of Heidelberg, Germany

<sup>e</sup>Synlab Center of Laboratory Diagnostics Heidelberg LLC, Eppelheim, Germany

<sup>f</sup>Toensberg County Hospital, Toensborg, Norway

<sup>g</sup>Oslo University Hospital, Ullevaal, Oslo

Deleted: Ullevål

<sup>h</sup>University Hospital Uppsala, International Medicine, Nephrology, Uppsala, Sweden

<sup>i</sup>British Heart Foundation Glasgow Cardiovascular Research Centre, Glasgow, United Kingdom

\*Corresponding author: Dag Olav Dahle, [d\\_o\\_dahle@yahoo.com](mailto:d_o_dahle@yahoo.com). Current adr of Dept cardiology, Oslo University Hospital, Ullevål, Oslo, Norway. Ph. [+4722118080](tel:+4722118080)

Deleted: Dr

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  $\pm$  6.7 mg/l and IL-6 2.9  $\pm$  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.

Deleted:  $\pm$ Deleted:  $\pm$

1 Key words: ALERT, high-sensitivity CRP, inflammation, interleukin-6, renal allograft survival.  
2  
3

4 Short summary: This post-hoc analysis of of maintenance renal transplant recipients (n=2102) from  
5  
6 the Assessment of Lescol in Renal Transplantation (ALERT) study demonstrates associations of the  
7  
8 inflammatory markers high-sensitivity C-reactive protein and interleukin 6 with chronic transplant  
9  
10 dysfunction.  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Deleted: participants in

Deleted: LEscol

Deleted: (n=2102)

For Peer Review

## 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<sup>1</sup>. Annual graft loss is still 2-7%<sup>2-4</sup> and the average deceased donor kidney transplant functions for about ten years<sup>5</sup>. Clinically, we observe a progressive transplant dysfunction characterised by a slowly rising serum creatinine, proteinuria and hypertension<sup>3</sup>. 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<sup>4</sup>. 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<sup>6</sup>, 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<sup>7,8</sup>, 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<sup>9,10</sup>. Renal transplant recipients, by the process of receiving an allograft, have an additional activation of their inflammation status<sup>11-13</sup>. 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<sup>14</sup>. 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.

Deleted: interleukin 6 (

Deleted: )

## Subjects and methods

### Study design

The design of the ALERT has been described previously<sup>15</sup>. 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<sup>15;16</sup>. 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 provided written informed consent, and the ethics committee at each participating center approved the trial (no S-02169).

Deleted: .

### Statistical analysis

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

1 Since the two arms showed no significant heterogeneity in relation between the inflammation  
2 markers hsCRP and IL-6, subsequent analyses were performed on the pooled patient population.  
3 HsCRP and IL-6 were not normally distributed and logarithmic transformation of these factors was  
4 used in the analysis. HsCRP and IL-6 were analysed in separate models due to confounding (not  
5 shown) and the close etiological relationship. Risk factors were evaluated using uni- and  
6 multivariate Cox proportional hazard model. Covariates were chosen in a stepwise approach,  
7 including covariates with univariate significance and previously reported factors associated with  
8 graft outcomes, and then excluding most variables that did not retain statistical significance in the  
9 multivariate analysis. SPSS version 17.0 (SPSS Inc., Chicago, IL) was used for statistical analysis.  
10  
11  
12  
13  
14  
15  
16  
17  
18

## 20 Results

21 Baseline characteristics in ALERT and ALERT extension were similar in both treatment arms <sup>15;16</sup>.

22 Baseline characteristics in relation to renal endpoints in the placebo group have been reported  
23 separately <sup>2</sup>. The present study reports treatment and placebo group together. Baseline  
24 characteristics are shown in table 1. Mean age at study entry was 50 years, 66% were male and all  
25 received cyclosporine-based immunosuppression at entry. The main causes of renal failure leading  
26 to transplantation were glomerulonephritis and polycystic kidney disease. Frequencies of graft  
27 failure endpoints are shown in table 2.  
28  
29  
30  
31  
32  
33  
34  
35

36 HsCRP and IL-6 were available in 1910 and 1751 patients, respectively. Mean (SD) and median  
37 (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)  
38 pg/ml and 2.4 (2.2) pg/ml. The hsCRP and IL-6 cohort had almost identical baseline characteristic  
39 as reported earlier <sup>14</sup>, with no differences in proportions with diabetes, coronary heart disease,  
40 current smokers or live donor.  
41  
42  
43  
44  
45  
46  
47  
48

49 Uni- and multivariate hazard ratios for the hsCRP and IL-6 cohorts are shown in tables 3 and 4.  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 Missing data excluded 450 (21.4%) and 576 (27.4%) patients in the hsCRP and IL-6 multivariate  
2  
3 models, respectively. Both hsCRP and IL-6 were in separate multivariate models significantly  
4  
5 associated with the renal endpoints shown, ie death-censored graft loss, graft loss including death,  
6  
7 and graft loss including death and doubling of serum creatinine. Due to a surprisingly high  
8  
9 percentage of current smokers we performed separate analysis (not shown) to examine a potential  
10  
11 interaction with smoking and inflammation. All interaction covariates were non-significant  $p>0.20$ ,  
12  
13 and the inflammation markers were equally strong in smokers and non-smoker, i.e. no interaction.  
14  
15 Excluding smoking as a covariate (not shown) changed the regression coefficients for the  
16  
17 inflammation markers less than 15% and the hazard ratios less than 5 %, indicating no confounding  
18  
19 effect.

20  
21  
22 In multivariate analysis for death-censored graft loss the results were similar in the hsCRP and IL-6  
23  
24 cohorts. We found significant negative associations of age and BMI. Significant positive  
25  
26 associations were seen for current smoking, systolic blood pressure, creatinine, time since last  
27  
28 transplantation, two HLA-DR mismatches, previous treatment for rejection and proteinuria.

29  
30  
31 When including death in the endpoint the association of age became significantly positive, diabetes  
32  
33 became significant and previous treatment for rejection lost significance. HLA-DR mismatch was  
34  
35 significant when including doubling of serum creatinine in the endpoint. We still found significant  
36  
37 negative association of BMI and positive associations of current smoking, systolic blood pressure,  
38  
39 creatinine, time since last transplantation and proteinuria.

## 40 41 42 43 **Discussion**

44  
45 In the present post hoc analysis, we have shown that inflammation, adjusted for traditional risk  
46  
47 factors, is associated with death-censored renal graft loss, and the composite endpoints; graft loss or  
48  
49 death, and graft loss or death or doubling of serum creatinine. To our knowledge this is the first  
50

1 prospective cohort in renal transplant patients documenting an association of IL-6 with graft loss.  
2  
3 HsCRP and IL-6 are close participants in the inflammatory cascade, and the observation that both  
4  
5 markers were predictive of outcome parameters, strengthens the perception of inflammation as an  
6  
7 important risk factor for graft related outcomes in renal transplant patients.  
8  
9

10 The baseline levels of inflammatory markers in our patients seem moderately elevated compared to  
11  
12 a non-transplanted background population, as seen in most studies with renal transplant recipients  
13  
14 <sup>14</sup>. No universally accepted cutoff values for inflammatory markers in renal transplant recipients  
15  
16 exists, but regarding cardiovascular outcomes in the general population CRP < 1 mg/dL confers low  
17  
18 risk, CRP 1 to 3 mg/dL average risk and CRP > 3 mg/dL high risk <sup>17</sup>, and the same cutoff values  
19  
20 were described by van Ree et al. as predicting a rising creatinine in renal transplant recipients <sup>18</sup>.  
21  
22 This leaves n=1203 (63%) of our hsCRP cohort with hsCRP > 1 mg/dL in the moderate to high risk  
23  
24 group. IL-6 levels are harder to compare with other studies due to different measurement  
25  
26 methodology. A study by Karczewski et al. found significantly higher IL-6 levels in chronic  
27  
28 rejection (1.64 +/- 0.8 pg/mL) than in patients with stable graft function (0.42 +/- 0.3 pg/mL,  
29  
30 p<0.001) or acute rejection (0.93 +/- 1.7 pg/dL, p=0.001) <sup>19</sup>.  
31  
32  
33

34 Our findings supports the concept that inflammation might be a component of the pathogenesis of  
35  
36 chronic graft dysfunction, and is in line with the report on hsCRP and graft deterioration by van Ree  
37  
38 et al. <sup>18</sup>. CRP and IL-6 has been related to renal function in predialytic chronic renal failure <sup>20</sup>.  
39  
40 However, the exact pathogenetic role of hsCRP and IL-6 in graft failure is yet elusive. Indeed,  
41  
42 genetic elevation in CRP was not associated with post-transplant morbidity and mortality <sup>21</sup>, and  
43  
44 lowering of hsCRP with statin in dialysis patients did not improve cardiovascular outcome <sup>22</sup>,  
45  
46 neither did statin treatment improve graft outcome in the ALERT <sup>15</sup>. IL-6 is upstream to CRP in the  
47  
48 inflammatory cascade and seems to play an etiological role in other inflammatory disease states  
49  
50 including rheumatoid arthritis <sup>23</sup>, where tocilizumab, a monoclonal antibody against IL-6-receptor,  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 has a proven effect. A murine model of cardiac transplantation demonstrated a tripled survival time  
2 of IL-6 deficient grafts <sup>24</sup>, but allogeneic cardiac transplants into IL-6 deficient mice did not show  
3 prolonged graft survival indicating that the graft was the relevant source of IL-6. In human heart  
4 transplant recipients IL-6 is associated with low grade cellular rejection <sup>25</sup>. In renal transplant  
5 recipients elevations in serum and urine IL-6 levels are documented in acute rejection episodes <sup>26</sup>,  
6 and increased soluble IL-6 receptor was found in urine twelve and 6 months before late graft failure  
7 <sup>27</sup>. A murine model of calcineurin-inhibitor (CNI) nephrotoxicity showed a protective effect of an  
8 IL-6 neutralizing antibody <sup>28</sup>, another animal study found a preventive effect of 13-cis-retinoic acid  
9 on chronic allograft nephropathy possibly mediated through decreased secretion of inflammatory  
10 cytokines including tumor necrosis factor-alpha and IL-6 <sup>29</sup>. Some genetic polymorphism studies in  
11 man has shown a protective role on renal graft function of a high IL-6 gene expression in renal  
12 transplant recipients, and low IL-6 production donor genotype was associated with increased  
13 prevalence of chronic graft dysfunction <sup>30;31</sup>, whereas other studies failed to show this association  
14 <sup>32;33</sup>. A small retrospective study by Cueto-Manzano et al. found no difference in inflammation  
15 markers in patients with or without CTD <sup>34</sup>.

16 [A pilot randomized placebo-controlled trial with an inflammation antagonist demonstrated reduced inflammation in maintenance hemodialysis patients](#)

17 [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.](#) <sup>36</sup>

18 Our finding of protective effect of older age of recipient for death-censored graft loss is in line with  
19 other studies <sup>18;37;38</sup>, and might represent higher immunoreactivity in younger subjects.

20 Hypertension, previous rejection and HLA-DR mismatch were independent risk factors in the  
21 present study is in line with current reviews <sup>4;39</sup>. Creatinine and proteinuria were significant  
22 independent risk factors for graft loss as described previously <sup>2;40</sup>, as was time since transplantation.

Deleted: c

1 Creatinine and proteinuria are used as biomarkers of CTD, although they are unspecific of the  
2  
3 underlying pathology and not ideal <sup>41</sup>. Smoking may be a risk factor for reduced patient and graft  
4 survival after transplantation <sup>42</sup>. Smoking may by itself increase inflammation markers <sup>9</sup>. Due to a  
5 surprisingly high percentage of current smokers we performed separate analysis to examine a  
6 potential interaction with smoking and inflammation. Although smoking was an independent risk  
7 marker there was no interaction with smoking and inflammation.  
8  
9  
10  
11  
12

13  
14 A limitation of this study was having only one analysis at baseline for hsCRP and IL-6, and the  
15 difference measured between groups is small and of limited prognostic value for individual renal  
16 transplant recipients. Our method of choosing covariates increases the possibility of type 1 error and  
17 should be regarded as tentative. The strength of the study is the many patients followed for an  
18 extended period and an independent adjudication of predefined outcome parameters.  
19  
20  
21  
22  
23  
24  
25

**Deleted:** A human randomized trial of IL-6 suppressive therapy might give the answer to whether IL-6 is causative in chronic graft dysfunction, but is probably still premature awaiting further evidence from mechanistic and correlation studies.

26 In conclusion we found that the inflammatory biomarkers hsCRP and IL-6 are significantly  
27 associated with graft related outcomes in renal transplant recipients, independent of the other  
28 examined risk factors for graft loss.  
29  
30  
31  
32  
33  
34  
35

**Deleted:** other recognized

### 36 Acknowledgements

37 The ALERT trial was sponsored by Novartis AG.  
38  
39  
40

### 41 Disclosure

42 The authors of this manuscript have conflicts of interest to disclose as described by Nephrology,  
43 Dialysis and Transplantation. The ALERT trial was sponsored by Novartis AG. Otherwise, the  
44 authors report no conflict of interest.  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## Conflict of interest

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

## Reference List

1. Meier-Kriesche HU, Schold JD, Kaplan B. Long-term renal allograft survival: have we made significant progress or is it time to rethink our analytic and therapeutic strategies? *Am J Transplant* 2004; 4: 1289-1295
2. Fellstrom B, Holdaas H, Jardine AG *et al.* Risk factors for reaching renal endpoints in the assessment of Lescol in renal transplantation (ALERT) trial. *Transplantation* 2005; 79: 205-212
3. Pascual M, Theruvath T, Kawai T, Tolckoff-Rubin N, Cosimi AB. Strategies to improve long-term outcomes after renal transplantation. *N Engl J Med* 2002; 346: 580-590
4. Weir MR, Wali RK. Minimizing the risk of chronic allograft nephropathy. *Transplantation* 2009; 87: S14-S18
5. Halloran PF, Melk A, Barth C. Rethinking chronic allograft nephropathy: the concept of accelerated senescence. *J Am Soc Nephrol* 1999; 10: 167-181
6. Mengel M. The kidney transplant: new horizons. *Curr Opin Nephrol Hypertens* 2010; 19: 260-265
7. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997; 336: 973-979
8. Pai JK, Pischon T, Ma J *et al.* Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med* 2004; 351: 2599-2610
9. Watz H, Waschki B, Kirsten A *et al.* The metabolic syndrome in patients with chronic bronchitis and COPD: frequency and associated consequences for systemic inflammation and physical inactivity. *Chest* 2009; 136: 1039-1046
10. Nishikawa T, Hagihara K, Serada S *et al.* Transcriptional complex formation of c-Fos, STAT3, and hepatocyte NF-1 alpha is essential for cytokine-driven C-reactive protein gene expression. *J Immunol* 2008; 180: 3492-3501
11. Vazquez MA, Jeyarajah DR, Kielar ML, Lu CY. Long-term outcomes of renal transplantation: a result of the original endowment of the donor kidney and the inflammatory response to both alloantigens and injury. *Curr Opin Nephrol Hypertens* 2000; 9: 643-648
12. Cottone S, Palermo A, Vaccaro F *et al.* Inflammation and endothelial activation are linked to renal function in long-term kidney transplantation. *Transpl Int* 2007; 20: 82-87
13. Kruger B, Krick S, Dhillon N *et al.* Donor Toll-like receptor 4 contributes to ischemia and reperfusion injury following human kidney transplantation. *Proc Natl Acad Sci U S A* 2009;

106: 3390-3395

14. [Abedini S, Holme I, Marz W \*et al.\* Inflammation in renal transplantation. \*Clin J Am Soc Nephrol\* 2009; 4: 1246-1254](#)
15. [Holdaas H, Fellstrom B, Jardine AG \*et al.\* Effect of fluvastatin on cardiac outcomes in renal transplant recipients: a multicentre, randomised, placebo-controlled trial. \*Lancet\* 2003; 361: 2024-2031](#)
16. [Holdaas H, Fellstrom B, Cole E \*et al.\* Long-term cardiac outcomes in renal transplant recipients receiving fluvastatin: the ALERT extension study. \*Am J Transplant\* 2005; 5: 2929-2936](#)
17. [Pearson TA, Mensah GA, Alexander RW \*et al.\* Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. \*Circulation\* 2003; 107: 499-511](#)
18. [van Ree RM, Oterdoom LH, de Vries AP \*et al.\* Elevated levels of C-reactive protein independently predict accelerated deterioration of graft function in renal transplant recipients. \*Nephrol Dial Transplant\* 2007; 22: 246-253](#)
19. [Karczewski M, Karczewski J, Poniedzialek B, Wiktorowicz K, Smietanska M, Glyda M. Distinct cytokine patterns in different states of kidney allograft function. \*Transplant Proc\* 2009; 41: 4147-4149](#)
20. [Panichi V, Migliori M, De PS \*et al.\* C-reactive protein and interleukin-6 levels are related to renal function in predialytic chronic renal failure. \*Nephron\* 2002; 91: 594-600](#)
21. [Kruger B, Boger CA, Schroppel B \*et al.\* No effect of C-reactive protein \(CRP\) haplotypes on CRP levels and post-transplant morbidity and mortality in renal transplantation. \*Transpl Int\* 2008; 21: 452-458](#)
22. [Fellstrom BC, Jardine AG, Schmieder RE \*et al.\* Rosuvastatin and cardiovascular events in patients undergoing hemodialysis. \*N Engl J Med\* 2009; 360: 1395-1407](#)
23. [Nishimoto N. Interleukin-6 as a therapeutic target in candidate inflammatory diseases. \*Clin Pharmacol Ther\* 2010; 87: 483-487](#)
24. [Liang Y, Christopher K, Finn PW, Colson YL, Perkins DL. Graft produced interleukin-6 functions as a danger signal and promotes rejection after transplantation. \*Transplantation\* 2007; 84: 771-777](#)
25. [Perez-Villa F, Benito B, Llancaqueo M, Cuppoletti A, Roig E. Elevated levels of serum interleukin-6 are associated with low grade cellular rejection in patients with heart transplantation. \*Transplant Proc\* 2006; 38: 3012-3015](#)
26. [Waiser J, Budde K, Katalinic A, Kuerzdorfer M, Riess R, Neumayer HH. Interleukin-6 expression after renal transplantation. \*Nephrol Dial Transplant\* 1997; 12: 753-759](#)
27. [Sadeghi M, Daniel V, Wiesel M, Hergesell O, Opelz G. High urine sIL-6R as a predictor of late graft failure in renal transplant recipients. \*Transplantation\* 2003; 76: 1190-1194](#)
28. [LaSpina M, Tripathi S, Gatto LA, Bruch D, Maier KG, Kittur DS. An interleukin-6-](#)

1 [neutralizing antibody prevents cyclosporine-induced nephrotoxicity in mice. \*J Surg Res\*](#)  
2 [2008; 148: 121-125](#)

3  
4 [29. Adams J, Kiss E, Arroyo AB \*et al.\* 13-cis retinoic acid inhibits development and progression](#)  
5 [of chronic allograft nephropathy. \*Am J Pathol\* 2005; 167: 285-298](#)

6  
7 [30. Muller-Steinhardt M, Hartel C, Muller B, Kirchner H, Fricke L. The interleukin-6 -](#)  
8 [174promoter polymorphism is associated with long-term kidney allograft survival. \*Kidney\*](#)  
9 [Int 2002; 62: 1824-1827](#)

10  
11 [31. Kocierz M, Kujawa-Szewieczek A, Kolonko A, Chudek J, Wiecek A. \[The influence of](#)  
12 [selected cytokine gene polymorphisms on the occurrence of acute and chronic rejection and](#)  
13 [on kidney graft survival\]. \*Postepy Hig Med Dosw \(Online \)\* 2009; 63: 613-626](#)

14  
15 [32. Kruger B, Walberer A, Farkas S \*et al.\* The impact of "high-producer" interleukin-6](#)  
16 [haplotypes on cardiovascular morbidity and mortality in a kidney transplant population.](#)  
17 [Transplant Proc 2009; 41: 2539-2543](#)

18  
19 [33. Sanchez-Velasco P, Rodrigo E, Fernandez-Fresnedo G \*et al.\* Influence of interleukin-6](#)  
20 [promoter polymorphism -174 g/c on kidney graft outcome. \*Transplant Proc\* 2010; 42: 2854-](#)  
21 [2855](#)

22  
23 [34. Cueto-Manzano AM, Morales-Buenrostro LE, Gonzalez-Espinoza L \*et al.\* Markers of](#)  
24 [inflammation before and after renal transplantation. \*Transplantation\* 2005; 80: 47-51](#)

25  
26 [35. Hung AM, Ellis CD, Shintani A, Booker C, Ikizler TA. IL-1{beta} Receptor Antagonist](#)  
27 [Reduces Inflammation in Hemodialysis Patients. \*J Am Soc Nephrol\* 2011;](#)

28  
29 [36. de Vries DK, Lindeman JH, Tsikas D \*et al.\* Early renal ischemia-reperfusion injury in](#)  
30 [humans is dominated by IL-6 release from the allograft. \*Am J Transplant\* 2009; 9: 1574-](#)  
31 [1584](#)

32  
33 [37. Joosten SA, Sijpkens YW, van KC, Paul LC. Chronic renal allograft rejection:](#)  
34 [pathophysiologic considerations. \*Kidney Int\* 2005; 68: 1-13](#)

35  
36 [38. Siddiqi N, McBride MA, Hariharan S. Similar risk profiles for post-transplant renal](#)  
37 [dysfunction and long-term graft failure: UNOS/OPTN database analysis. \*Kidney Int\* 2004;](#)  
38 [65: 1906-1913](#)

39  
40 [39. Li C, Yang CW. The pathogenesis and treatment of chronic allograft nephropathy. \*Nat Rev\*](#)  
41 [Nephrol 2009; 5: 513-519](#)

42  
43 [40. Kasiske BL, Andany MA, Danielson B. A thirty percent chronic decline in inverse serum](#)  
44 [creatinine is an excellent predictor of late renal allograft failure. \*Am J Kidney Dis\* 2002; 39:](#)  
45 [762-768](#)

46  
47 [41. Bestard O, Cruzado JM, la FM, Grinyo JM. Biomarkers in renal transplantation. \*Curr Opin\*](#)  
48 [Organ Transplant 2010; 15: 467-473](#)

49  
50 [42. Nogueira JM, Haririan A, Jacobs SC, Cooper M, Weir MR. Cigarette smoking, kidney](#)  
51 [function, and mortality after live donor kidney transplant. \*Am J Kidney Dis\* 2010; 55: 907-](#)  
52 [915](#)

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review