Author's response to reviews

Title: Elevated soluble cellular adhesion molecules are associated with increased mortality in a prospective cohort of renal transplant recipients

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Author's response to reviews: see over
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Dear Professor Patel

Re: MS: 5816406453954893

Elevated soluble cellular adhesion molecules are associated with increased mortality in a prospective cohort of renal transplant recipients

Thank you for the opportunity to respond to the many helpful comments from the reviewers of the above manuscript. We have carefully considered the points made by the reviewers and revised our manuscript accordingly. The changes in the revised paper are highlighted by the track changes mode in MS word. Detailed individual responses to the expert reviewers’ comments are outlined in the accompanying pages to this letter.

We hope that the revised manuscript will now be acceptable for publication in BMC Nephrology.

Yours sincerely

Dr Grainne M Connolly

on behalf of the authors
Reviewer 1: banu sis

1. We undertook further Kaplan-Meier analyses as requested by Reviewer 1. After excluding those patients with a history of cardiovascular disease at enrolment and those patients who died due to a cardiovascular cause we have determined that VCAM, but not ICAM, remained a significant predictor of survival (p=0.001 and p=0.106 respectively). However we would like to highlight that by excluding these participants (as requested) it does reduce the population studied to a total of 283. Furthermore there were only 29 deaths in this group of 283 during the period of follow-up.

Also of note, as included in the manuscript, there was no significant difference in VCAM or ICAM concentration in those renal transplant recipients who had died of a cardiovascular cause as compared to those who had died of a non-cardiovascular cause (VCAM 2055 ng/ml (1422, 2759) vs. 2478 ng/ml (1340, 3329) p=0.54 and ICAM 985 mg/ml (797, 1300) vs. 1151mg/ml (935, 1634) p=0.10). Similarly, as shown in Table 3 and Table 4, in multivariate survival analysis VCAM and ICAM remained significant predictors of outcome following adjustment for history of vascular disease at enrolment.

These findings suggest that the results of survival analyses are not driven by the sub-group of patients with cardiovascular disease-related deaths. We cannot however exclude the possibility that some of the renal transplant recipients who subsequently died had occult cardiovascular disease at the time of enrolment.

2. We certainly acknowledge that the cross-sectional design of our study meant that testing of ICAM and VCAM in relation to time from renal transplantation is variable within our study population. However, the question this study sought to answer was "would measurement of VCAM and ICAM at any time in the post transplant period, in patients who were clinically well with stable graft function, be predictive of subsequent mortality". The results of our study suggest that measurement of these cellular adhesion molecules in such a group of unselected renal transplant recipients, at varying times post renal transplant, is indeed a significant predictor of subsequent mortality.

If our study is published in BMC Nephrology it might stimulate other clinical researchers, with access to sera at carefully defined time points post renal transplant, to further explore the hypothesis that elevated cell adhesion molecule levels are predictive of premature mortality in renal transplant recipients.

3. All patients at the time of their recruitment to the study were clinically well, attending the transplant centre as an outpatient and were at least 3 months post-
renal transplant, with stable graft function and were on stable maintenance immunosuppressive regimens. We were careful to exclude from recruitment any patient with current or recent infection and any patients with current or recent allograft rejection. A statement to this effect has been included in the manuscript.

4. Individual survival curves for VCAM and ICAM (Figures 1 and 2) are included in the manuscript. The individual survival curves for VCAM and ICAM are broadly similar in that the patients banded into the lowest thirds for these cellular adhesion molecules had the best survival whereas those banded in the highest thirds for VCAM or ICAM had the worst survival.

5. Data on individual renal transplant recipient renal function and graft survival were not collected during the period of follow-up so we are unable to answer this particular discretionary revision.

6. Table 4 has been reformatted as requested in the revised manuscript.

Reviewer 2: Sabine Zitta

1. Discussion of additional factors associated with altered expression of CAMs as suggested by the reviewer has been included in the revised manuscript.

Reviewer 3: Daniela Kniepeiss

1. As stated in the manuscript, no formal exclusion criteria were imposed for recruitment to this study. However, patients who were clinically unwell e.g. current or recent infection or patients who had current or recent signs of allograft rejection at initial assessment were deferred until a subsequent clinic reassessment at least 3 months initial contact. The median and interquartile range in years from time of renal transplantation to recruitment to this study is now included in Table 1 of this manuscript.

2. At enrolment to this prospective study, the participants (as shown in Table 1) were at variable time points after renal transplantation. However, there was no significant difference in time from renal transplant in those participants who had died at follow-up as compared to those who were still alive at follow-up: 9 (4-14) years vs. 7 (3-12.5) respectively, p=0.056, This data has now been included in the revised manuscript.

3. Time on dialysis prior to renal transplantation has been included in Table 1 of the revised manuscript. As included in the revised manuscript, 97 % participants had been on dialysis prior to renal transplantation and 3 % had received a preemptive renal transplant. Given the large majority of patients had been on
dialysis prior to transplantation and consequently, the lack of power to detect a significant difference, a sub-group analysis was not performed comparing differences in these 2 patient groups.

4. The serum creatinine detection range quoted in the manuscript is the detection range of the laboratory assay to analyse creatinine, as stated in the Methods section, 4-1238 µmol/l. This is not the range of serum creatinine values actually measured in the recruited patients. We apologise for any confusion caused to this reviewer.

5. Serum creatinine concentrations have been included in the revised Tables.

6. Data on renal function was not collected during the period of follow-up. We are therefore unable to provide information on stability of renal function at follow-up.

7. We agree that measurement of cardiac parameters such as nt-pro BNP, OPG, sRANKL and ANP may provide useful additional prognostic information to that reported in our study and would be an area for further study in our population group. We do not however have the ability at present to return to the original stored sera to measure these substances and cannot therefore add these additional analyses to the current manuscript. The focus of our study was to address the question "would measurement of VCAM and ICAM at any time in the post transplant period, in patients who were clinically well with stable graft function, be predictive of subsequent mortality". We believe our data does show a significant correlation between elevated ICAM and VCAM levels and mortality in unselected renal transplant recipients.