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26th July 2016

Fang-Fang Ji
Scientific Editor
World Journal of Nephrology

Response to reviewers - Manuscript 27353

Outcomes of renal transplant recipients with BK virus infection in the Auckland region from 2006 to 2012.

Editor

A separate Institution review board statement, informed consent statement, conflict of interest statement, data-sharing statement and biostatistics statement has been provided.

A core audio tip is provided.

Comments have been added.

References in the text are now in superscript.

The references have all the authors included, pubmed citations and where available a DOI is also provided.

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Reviewer's code: *02454185*

This is an interesting work, written in fluent English. I really enjoy reading it. I have several minor suggestions and concerns. 1. "eGFR (estimated glomerular filtration rate) was calculated using the CKD-EPI equation."-------- it is better to provide reference here.

An appropriate reference has been added.

2. "Nine patients had biopsy-proven BKV nephropathy equivalent to an incidence of 3.9% of the selected cohort (9/226)."----------it may not be the incidence because only 76 patients underwent BKV test. The incidence can be 9/76.

We agree with this comment and debated how to report this information. We have made changes to the manuscript to highlight this.

3. in investigating Risk factors for BKVN, which cohort do you use? Should the treatment algorithm be included in the Cox model?

We have used the total 226 patients for the cox regression model. The treatment algorithm has not been included in the Cox model mainly because they did not contribute to the model significantly. The number of patients with BKVN is small (only 9) so only those variables contributing to the model substantially were included in the final model.

4. there are varieties of clinical outcomes being used in the present study. The problem of multiple testing should be noted and discussed. Even a positive finding should be hypothesis-generating at best.

The primary outcome in this study is BKVN and there are secondary outcomes such as renal allograft function and graft failure. We acknowledge type I error should be adjusted for multiple outcomes and multiple tests so that the probability of being falsely positive is reduced to a reasonable level.

However, appropriate use of corrections for multiple outcomes and multiplicity is not straightforward and inappropriate use of multiplicity adjustment may obscure important findings (ref: Tyler, K. M., S.-L. T. Normand, et al. (2011). "The use and abuse of multiple outcomes in randomized controlled depression trials." Contemp Clin Trials 32; Rothman, KJ.; Greenland, S. Modern Epidemiology. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 1998). There was also a correlation

between renal function and BKVN, which can make the commonly used Bonferroni method a little bit problematic.

In the context of a retrospective cohort study we think this cannot be effectively addressed so we've not been able to adjust for the multiplicity due to multiple outcomes, acknowledging it may lead to some level of inflated type I error. We hope there can be more studies to be undertaken with large sample size and number of events to confirm what we have found in this paper.

5. in the discussion the authors concluded that "This retrospective study has demonstrated a low rate of BKVN and only one patient with graft failure in our cohort of patients and is comparable to that of other transplant centres with BKV screening programmes reported in the literature (0.8 to 6.4%)." Such low rate of BKVN may be attributable to the limited number of patients underwent testing.

As discussed earlier, the BKVN rate is probably between 4.0% and 11.8% (point estimates) but possibly closer to 4.0%. We agree with a larger sample size, the incidence rate can be reported more accurately (narrower confidence interval width). This is highlighted in the discussion.

6. Do you think other renal biomarkers can be helpful in assessing renal function? Those include Cystatin C, NGAL and interleukins. These biomarkers should be incorporated into analysis if they were available. Otherwise, discussing some of them can give a full picture of the role of BK virus infection in renal functions. Some reference can be cited (Heart Lung Vessel. 2015;7(1):64-73. Am J Kidney Dis. 2011 Sep;58(3):356-65.)

We did not perform any renal biomarkers. The biomarkers quoted in the reference are not specific for BKVN. These are not used in clinical practice in NZ. Therefore, we would rather not include them as a part of discussion in the manuscript. BK viral capsid protein (VP1) mRNA derived from urinary cells is a non-invasive test that could potentially be used in diagnosing BKVN. Similarly, urine granzyme B mRNA and protease inhibitor-9 mRNA levels have been shown to be predictive of graft dysfunction. The combination of these tests could provide useful diagnostic and prognostic information, but they are required further investigations in a larger scale (Transplantation 2002;74:987-994. Transplantation 2010;90:189-197) and could be useful in a prospective study but fall outside the range of a retrospective cohort study.

7." General linear model univariate analysis was used to evaluate the relationship between BKV infection and eGFR and how BKV infection impacted on graft function."---------since eGFR is a continuous variable, the model can be linear model. If Generalized model is specified, the link function should be explicitly declared. The relationship between BKV and graft function should be detailed. For example, how graft function was evaluated, the type of variable (continuous or dichotomous?).

General linear model is an ANOVA procedure in which the calculations are performed using a least squares regression approach to describe the statistical relationship between one or more predictors and a continuous response variable. Predictors can be factors and covariates' (ref: http://support.minitab.com/en-us/minitab/17/topic-library/modeling-statistics/anova/basics/what-is-a-general-linear-model/). As BKV infection is a categorical variable and eGFR is a continuous variable, it is appropriate to use the general linear model.

eGFR is a non-normally distributed continuous variable, and we used non-parametric Kruskal-Wallist test to evaluate it initially. "eGFR in 2013" was a continuous response variable in univariate GLM when analysing graft function. Age at transplant and eGFR at 1 month after transplant are covariates. Comorbidities, donor source, HLA mismatch, use of Basiliximab, type of calcineurin inhibitor, use of thymoglobulin, acute rejection and BKV category are fixed categorical factors. Full factorial model was used, and interaction was not found between the positive BKVN group and the other variables. Leven's test of equality of error of variances has a significance value of 0.580, suggesting that the equal variances assumption is not violated. When comparing graft functions among BKV groups, negative BK viraemia was chosen as the reference category. The covariates in this model were evaluated at the following values: age at transplant in years was 45.7 and eGFR at 1 month after transplant was 56.97 ml/min/1.73m^2. The parameter estimate for graft function was 17.01 ml/min/1.73m^2 lower than the reference category (95% CI: -32.53 to -1.49, *p=0.032*) in the positive BKVN group; -4.05 ml/min/1.73m^2 lower than the reference category (95% CI: -14.96 to 6.85, *p=0.464*) in the positive BKV group; and -1.108 ml/min/1.73m^2 lower than the reference category (95% CI: -8.06 to 5.85, *p=0.754*).

8. In describing univatiate analysis, more details should be given on when to use median (interquartile range Q1 - Q3) and mean (±standard deviation). They have different applications. The reference (Ann Transl Med. 2016 Mar;4(5):91. doi: 10.21037/atm.2016.02.11.) can be cited in the place.

The mean and SD are used for continuous variables following normal distribution, while the median and inter-quartile range are used for non-normally distributed continuous variables. Age and eGFR are non-normally distributed variables.

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Reviewer's code: 03290767

This is an interesting and valuable paper. Results: It is not appropriate to give the incidence of BK viraemia as 12.4% when only 76 of the 226 patients were tested. Only 76 patients were tested, so the incidence of viraemia in the group tested was 36.8%. It is not appropriate to quote a biopsy proven rate of BKVN for the whole group - it is only appropriate to quote the incidence of BKVN as a proportion of all patients biopsied. Discussion: It is necessary to review the comments about incidence in light of re-analysis of the figures obtained. Figures: Figures 3 and 4 are not referenced in the text

We agree. This is a similar point to that made by Reviewer 02454185. We have made changes to address this point as outline *supra vide*.

Good point! We have added the reference to Figure 3 and 4.

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Reviewer's code: *00505314*

This retrospective study looked at the incidence of BK viremia , BK nephropathy and graft outcomes among kidney transplant recipients in Auckland region. Study is well conducted and written clearly. Comments: 1. As suggested by the authors, the incidences of BK Viremia and Nephropathy were likely under estimated since many patients did not undergo testing.

We agree. Also refer to our earlier discussions.

2. Study is likely under powered to fully realize the impact of BK virus infection on graft outcomes. Even though statistically not significant, figure 2 shows a decrease in eGFR from BKV (-) to unknown BKV (some of those likely are BKV positive) to BKV (+) to BK nephropathy. A larger sample size would likely have shown increasing differences among these groups.

This is a good point. Our study is likely under-powered so 'A larger sample size would likely have shown increasing differences among these groups'. We have highlighted this in our discussion.

3.Incidence of BK viremia is around 10-15% based on previous studies. There is a latent period from BK viremia to the development of BK nephropathy. Idea behind prospective screening for BK viremia is to intervene (reduction in immunosuppression) before progression to BK nephropathy since there is no good treatment once nephropathy establishes.

This is highlighted in the discussion.

5. I agree with authors that any screening program should take into account economic impact.

This is highlighted in the discussion.

6. Study is small to make any definite recommendations.

We acknowledge the sample size and the number of events are small. This is highlighted in the discussion and will be a point well recognised by the target audience. We have managed to find something significant. However, we agree there are potential selection bias and the study is likely to be under-powered. We hope our findings will precipitate thought towards a larger and better designed studies or analysis by units with a comprehensive screening programme.

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Reviewer's code: 00289581

Outcomes of renal transplant recipients with BK virus infection and BK virus surveillance in the Aukland region from 2006 to 2012. This is a descriptive retrospective review of a series of 226 patients with renal transplant. There are multiple variables from with the population studied. There were three renal units that are included, one screened, one trialed but stopped due to cost, the third checks if there is an indication. Did this affect who was diagnosed with BK virus?

The variations in testing BK virus among the three units likely had an effect on the analysis of those with BK viraemia; therefore we didn't do any detailed analysis on this as it was commented in the manuscript. However, we think that the variations among the three units had minimal impact on those diagnosed with BKVN, as our strategy in performing allograft biopsies is identical among the three units.

The induction therapy was with Basiliximab from 2010, but subjects are included before this time (since 2006) and this may well affect the incidence of BK virus infection. Of 226 patients included in the study, 76 were tested, what was the clinical indication for testing? When in the time course of the renal transplant were the subjects diagnosed? 28 had BK viremia, but of these only 16 had biopsies, 9 had BK nephropathy. There is then a statistical analysis done which show significance for ethnic groups, donor source and cold ischemia time. With so few patients in the BKVN group (n=9) it is difficult to assume the conclusions are valid in this study. Overall, this is a moderate group of renal transplant recipients studied, but without a comprehensive screening program, the conclusions cannot be reached.

We acknowledge the sample size and the number of events are small. This is highlighted in the discussion and will be a point well recognised by the target audience. We have managed to find something significant. However, we agree there are potential selection bias and the study is likely to be under-powered. We hope our findings will precipitate thought towards a larger and better designed studies or analysis by units with a comprehensive screening programme.

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Reviewer's code: 00504167

Name of Journal: World Journal of Nephrology ESPS Manuscript NO: 02889305 Manuscript Type: Retrospective Cohort Study Full Title: Outcomes of renal transplant recipients with BK virus infection and BK virus surveillance in the Auckland region from 2006 to 2012. With this retrospective study, the authors evaluated the outcomes of kidney transplant patients with BKV infection from 2006 to 2012. Of the patients included in the study, only 76/226 were tested for BKV viral load and 9 had a biopsy-proven BKVN. Among the 9 patients with BKVN, one patients lost the graft. The authors concluded that despite a surveillance program for BKV infection was not in place in their centre, they had a quite low incidence of BKVN similar to those centres with surveillance programmes, and therefore surveillance "should be tailored specifically to that transplant centre based on its epidemiology and outcomes of BKVN, particularly in centres with limited resources". I disagree with this conclusion. To date, BKV viral load by real-time PCR can be determined at a very affordable price and, I think, any transplant centres can afford it. The major limit of this study is that we do not know the real incidence of BKVN in this centre since only a fraction of the transplant patients have been tested for BKV viremia. So, the conclusion of the authors is quite misleading, and I would not recommend this approach.

We agree that it is hard to determine the real incidence of BKVN in any centre as practically no one would perform allograft biopsies in recipients with stable graft function even if they had BK viraemia. This point is highlighted in the discussion. We don't think being tested for BK viraemia has a significant impact on determining the incidence of BKVN as BKVN is diagnosed by biopsies if there is a clinical indication.

The cost of performing a BKV viral load test is NZ$173 in our laboratory. It is considered quite expensive compared with other standard tests in this country. Please note the comments by Reviewer's code: *00505314*.