Author’s response to reviews

Title: Treatment of Chronic Active Antibody-mediated Rejection in Renal Transplant Recipients – A single center retrospective study

Authors:

Hsien-Fu Chiu (hsienfuchiu@gmail.com)
Mei-Chin Wen (mewen@vghtc.gov.tw)
Ming-Ju Wu (wmj530@gmail.com)
Cheng-Hsu Chen (cschen@vghtc.gov.tw)
Tung-Min Yu (yu5523@gmail.com)
Ya-Wen Chuang (colaladr@yahoo.com.tw)
Shih-Ting Huang (kitheroborn@hotmail.com)
Shang-Feng Tsai (acde0324@vghtc.gov.tw)
Ying-Chih Lo (yclo@vghtc.gov.tw)
Hao-Chung Ho (brainhcho@gmail.com)
Kuo Hsiung Shu (khshudr@gmail.com)

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Author’s response to reviews:

14-Nov-2019

Dear Editors-in-Chief,

Re: Manuscript ID BNEP-D-19-00663R1

We are very much thankful to the reviewers for the deep and thorough review. Your comments were highly insightful and enabled us to greatly improve the quality of our manuscript.

The manuscript had been revised extensively, addressing all of the issues raised by the editors and reviewers. In the following pages are our point-by-point responses to each of the reviewer’s comments.
Response to comment from Assistant Editor

1. Title page

Please remove the List of Abbreviations section from the title page in your manuscript.

Response: Thanks for your comment and advice. We had removed the List of Abbreviations section from the title page.

2. Conclusions

We note that your manuscript does not include a ‘Conclusions’ section.

In order to be in line with journal requirements, please ensure that you include the following sections within your manuscript: Background, Methods, Results, Discussion, Conclusions. This section should state clearly the main conclusions of the research article and provide an explanation of the importance and relevance of the study reported.

Response: Sorry for our mistake. We had added the “Conclusion” section in our Manuscript: “In conclusion, although there have been no consensus on treatment strategies on CAMR, aggressive treatment before advanced tissue injury is still associated with better graft outcome in our series. However, higher incidence of adverse events cannot be overlooked. To mitigate potential life-threatening infections, longer duration of trimethoprim-sulfamethoxazole and valganciclovir prophylaxis should be considered after aggressive treatment for rejection.”

3. Remove sections

Please remove the sections entitled ‘Acknowledgement’ and ‘Disclosure’ from page 15 of your manuscript.

Response: Thanks for your suggestion. We removed the sections entitled “Acknowledgement” and “Disclosure” from page 15 of our manuscript.

4. Authors’ contributions
Please clarify whether all of the authors of this manuscript have read and approved the final manuscript in the Authors’ contributions section of your manuscript.

If so, please include the statement “All authors read and approved the final manuscript.” in the Authors’ contributions section of your manuscript.

Response: Thanks for your comment. We had confirmed that all of our authors had read and approved the final manuscript. We included the statement “All authors read and approved the final manuscript.”

5. Remove attachments

Please remove the additional file entitled ‘Cover Letter-BMC Nephrology.doc’ from your manuscript and from the file inventory as it is no longer needed at this stage.

Response: Thanks for your comment. We had removed the additional file entitled ‘Cover Letter-BMC Nephrology.doc’ from the manuscript.

6. Overlap

We note that the current submission contains some textual overlap with other previously published works, in particular: ‘Clinicopathological Correlation of Antibody-mediated Rejection in Renal Transplant Recipients’.

This overlap mainly exists in the Methods section in your manuscript.

While we understand that you may wish to express some of the same ideas contained in these publications, please be aware that we cannot condone the use of text from previously published work.

Please be informed that we cannot proceed with handling your manuscript before this issue is resolved, and the sections of text in question have been reformulated.

Response: Thanks for your important comment. We had revised our Methods section to avoid textual overlap. The revised manuscript is listed below:
Patients and graft biopsies

Computerized records from Taichung Veterans General Hospital were collected to identify the renal transplant biopsies performed in the past 7 years with the diagnosis of CAMR. The first biopsy was used for statistical analysis if the patient had multiple biopsies. All biopsies were performed for cause and reviewed by a renal pathologist. Biopsies with ABO-incompatible grafts and those with recurrent or de novo glomerulonephritis (GN) and DM nephropathy were excluded. All the patients had negative T and B cell complement-dependent cytotoxicity cross-match (CDC-CMX) result before kidney transplantation.

Thymoglobulin or basiliximab may be prescribed for induction therapy. Maintenance immunosuppression included calcineurin inhibitors (CNIs) tacrolimus or cyclosporine A, mycophenolate, and prednisone. mTOR inhibitor, either sirolimus or everolimus, was prescribed in few patients depending on the discretion of the physician.

One or more of the following treatment strategies were selected for CAMR treatment according to the patient’s clinical condition and decision of the individual practitioners: no treatment, methylprednisolone (MP) pulse therapy (usually 500mg of MP for 3 days), double filtration plasmapheresis (DFPP), rituximab intravenous bolus (375 mg/m2), intravenous immunoglobulin (IVIG) (2 g/kg), rabbit antithymocyte globulin (ATG) (Thymoglobulin 1-1.5mg/kg for 3-5 days). DFPP was performed using a Evaflux 4A as the plasma fractionator. The exchange volume was set at 1~1.5 times of plasma volume. Estimated plasma volume was 0.07 x weight(kg) x (1-hematocrit [Hct]). 300–500 mL saline solution was infused as the replacement fluid. In a few patients, bortezomib (1.3 mg/m2) were also used. Multiple treatments, usually yearly, were performed if follow-up graft biopsy revealed persistent lesions. The patients were divided into two groups according to treatment strategy. Group 1: aggressive treatment (DFPP and one of the followings: rituximab, IVIG, ATG, bortezomib, or MP pulse therapy); and group 2: supportive treatment. In group 1, patients were usually treated annually with DFPP plus one of the 5 drugs, but different in each year in order to accomplish a wide blockade of the alloimmunity. In group 2 (and also group 1), patients were involved a medical care for chronic kidney disease, including ideal blood pressure control, blood sugar control, hyperuricemia control, and preventing further kidney damage by avoiding drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs). Antihypertensive agents (ACEI or ARB), oral hypoglycemic agents or insulin, and urate-lowering therapy (allopurinol/febuxostat) were prescribed according to each patient’s clinical condition. Oral sodium bicarbonate were prescribed if the patient had metabolic acidosis (serum bicarbonate less than 22 mEq/L).

All of our colleagues are familiar with the care for chronic kidney disease patients. The control of lipids with statin/fibrate and blood pressure with ACEI/ARB are standard-of-care in our transplant team.
End points

The patients were followed up until graft loss or death or the end of 2017. The definition of graft loss included: returned to dialysis, re-transplant, or patient death. Primary end point was graft survival after treatment in the 2 groups. Secondary outcome included patient survival and the occurrence of major adverse events. Major adverse event was defined by any event that was associated with death, admission to hospital, prolongation of a hospital stay, persistent or significant disability or incapacity, or was otherwise life-threatening in connection with specific treatment, according to World Health Organization Good Clinical Practice guidelines.

Histopathology and diagnosis of CAMR

All renal graft biopsies were performed using ultrasound-guided percutaneous technique (two~three cores per biopsy; 16~18 gauge needle). Graft biopsies were examined by light microscopy using silver methenamine and periodic acid-Schiff (PAS) stains, immunofluorescence studies for IgG, IgA, IgM, C3, C4d, C1q, kappa, and lambda light chains, and electron microscopy.

The same pathologist evaluated and graded graft biopsies according to Banff 2017 criteria (8). Glomerulitis (g), peritubular capillaritis (ptc), transplant glomerulopathy (cg), interstitial fibrosis (ci), tubular atrophy (ct), mesangial matrix (mm) scores were assigned in each case according to Banff parameters (1, 9). C4d staining was performed on all biopsies by direct immunofluorescence on frozen sections.

For CAMR, all 3 criteria in the following were met for diagnosis according to Banff 2017 criteria: (1) morphologic evidence of chronic tissue injury, (2) evidence of current/recent antibody interaction with vascular endothelium, (3) serologic evidence of donor-specific antibodies (DSA, to HLA or other antigens). C4d staining in the biopsy tissue or expression of validated transcripts/classifiers may substitute for DSA (8). Determination of HLA antibody by Luminex○R method is expensive in Taiwan and is not affordable to every patient. Gene expression is not performed routinely. For those who didn’t perform DSA, C4d staining should be positive for the definite diagnosis of CAMR.
Data analysis

Normal distribution of the data was evaluated using the Kolmogorov-Smirnov test. Normally distributed data were expressed as mean ± standard deviations (SD), non-normally distributed data as median and interquartile ranges (IQR). Categorical variables were shown as frequency (%). Fisher exact test was used to compare categorical data, and the Mann–Whitney U test was used for comparison of continuous data. Kaplan–Meier analysis was applied for calculation of graft and patient survival or adverse events free survival. Log-rank test was used for comparison of survival between groups. To identify the predictors of graft loss in CAMR patients, we conducted univariate and multivariable analysis using the Cox proportional hazards regression model. A P-value of less than 0.05 was considered as statistically significant. All statistical analyses were performed by using SPSS software (version 21.0, Chicago, IL, USA).

7. At this stage, please upload your manuscript as a single, final, clean version that does not contain any tracked changes, comments, highlights, strikethroughs or text in different colours. All relevant tables/figures/additional files should also be clean versions. Should you wish to respond to these revision requests, please put your responses to the reviewers'/editors’ comments in the Response to Reviewers box in Editorial Manager. Please do not upload a separate letter.

Response: We had uploaded our manuscript as a single, final, clean version that didn’t contain tracked changes, comments, highlights, or text in different colours. Tables are also revised to be clean version.

Again, we appreciate all your insightful comments. We worked hard to responsive to them. We hope that these revisions improve the manuscript such that you and reviewers now deem it worthy of publication in BMC nephrology.