PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (http://bmjopen.bmj.com/site/about/resources/checklist.pdf) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

| TITLE (PROVISIONAL) | Humoral Response to SARS-CoV-2 Vaccination in Haemodialysis Patients and a Matched Cohort |
|---------------------|--------------------------------------------------------------------------------------------|
| AUTHORS             | Zhao, Tianchen; Nishiuchi, Takamitsu; Omata, Fumiya; Takita, Morihito; Kawashima, Moe; Nishikawa, Yoshitaka; Yamamoto, Chika; Kobashi, Yuri; Kawamura, Takeshi; Shibuya, Kenji; Kazama, Junichiro; Shineha, Ryuzaburo; Tsubokura, Masaharu |

VERSION 1 – REVIEW

| REVIEWER           | Yau , Kevin                                        |
|--------------------|-----------------------------------------------------|
| University of Toronto, Nephrology   |
| REVIEW RETURNED   | 22-Jul-2022                                       |

GENERAL COMMENTS

This is an observational study of hemodialysis (n=65) and generally healthy controls (n=500) which compared serologic measurements for SARS-CoV-2 S1 IgG and SARS-CoV-2 neutralizing antibodies using commercial assays. The main finding of this study is that antibody levels after two COVID-19 vaccine doses were lower in hemodialysis patients in comparison to controls. A strength of this study is the larger control group size which have been smaller in other studies. However, there was significant imbalance in baseline characteristics between study groups requiring adjustment using coarsened exact matching. Even after coarsened matching there remained some imbalance between the groups (e.g. sex) and therefore some residual confounding is likely present. While the finding of this study is consistent with previous studies, it is already established that dialysis patients have lower antibody levels than controls.

I have several comments/questions for the authors:
1) Could exact dates be provided for the study period?
2) It would be helpful to have additional information regarding the neutralizing antibody assay as there are multiple types of assays available (e.g. pseudovirus neutralization assays)
3) How were the cut-offs values for these assays determined? A major challenge of serology studies is comparison of units between studies, can the units in this study be converted to BAU/mL (WHO International Units?)
4) For the very few participants with unknown vaccine type – what types of vaccines were available in Japan other than BNT162b2 at the time?
5) Is there information on timing between 1st and 2nd dose, as this is known to affect serologic response?
6) Did any patients in the study receive third doses at blood collection, I am assuming all the results are time in days post second dose?
7) Can you confirm that assumptions for the linear regression were
met?

8) It seems surprising how few patients had baseline COVID-19 infection, however I recognize that this was earlier in the pandemic. An issue with the assay used is that it measures both the spike protein and nucleocapsid antibody. Normally nucleocapsid antibody seroconversion could be used as a marker of natural COVID-19 infection but I don’t believe that is possible here. Were the baseline characteristics of COVID-19 infection determined at the same time as blood collection?

9) In the discussion, it would make sense to include a discussion of the relevance/correlation between SARS-CoV-2 antibody levels and actual COVID-19 infections/severity of illness.

REVIEWER
Hsu, Caroline
Tufts Medical Center

REVIEW RETURNED
02-Aug-2022

GENERAL COMMENTS
In this study, Zhao and colleagues used anti-SARS-CoV-2 S1 IgG antibodies and neutralizing antibodies to compare the immune response to vaccination between patients receiving dialysis and matched controls. The study confirms previous findings that dialysis patients have a lesser immune response to vaccination. However, some issues remain.

Major issues:
1. The matching methodology needs to be expanded upon. Matching is typically done in a ratio, such a 1:1 or 1:2, and it is not clear how this matched cohort was ultimately generated. I would also expect the baseline characteristics used for matching to be balanced between the dialysis patients and the controls, but sex in particular is skewed
2. Furthermore, I think the matching process is a strength of the study in an attempt to adjust for confounders; therefore the authors should de-emphasize the analyses with the entire cohort and focus on the results of the matched cohort.
3. The study has issues of small cohort size, short follow-up, lack of detailed clinical data, possible recall bias, and no establishment of association with hard clinical outcomes (such as SARS-CoV-2 infection). To the authors’ credit, they acknowledge all of these in the second-to-last paragraph.

Minor issues:
4. The study does not add much new information and largely confirms existing knowledge, but in my opinion, confirmatory studies are still meaningful. If possible, it would strengthen the study to compare these antibody levels with those published in studies correlating antibody levels with hard clinical outcomes (such as SARS-CoV-2 infection).
5. In this vein, the statement on page 4 line 22 “immune response to vaccination in dialysis patients was reported to be poor and short” is exaggerated; multiple studies have showed that the vaccine-induced immune response in dialysis patients is moderate – less than healthy adults, but greater than that of patients receiving immunosuppression (such as transplant patients). If immune response truly was poor, vaccination would be futile
6. Page 4 line 9, “immunosuppression” implies taking medication to suppress the immune system; this should be changed to “immunocompromise”
7. Page 4, lines 10-12: “Regular visits to the dialysis center could increase the risk of SARS-CoV-2 infection due to limited capacity for
I would add due to close proximity, limited ability to physically distance. In many countries, living in a nursing facility was a major risk factor for COVID among dialysis patients, and this may be relevant to Japanese dialysis patients as well.

8. Page 4, line 47-48: Please clarify, were the dialysis patients hospitalized at the time of the study, or were they outpatients receiving treatment at a clinic associated with a hospital?

9. Did any patient have a booster or additional vaccine dose prior to antibody assessment?

10. Table 3: Personally, I prefer reporting a 95% confidence interval rather than the SD

11. Page 9 line 12, the sentence about Adjusted R square values – please interpret this for the reader

12. I feel that the authors make recommendations in the Discussion which are only weakly supported by their results, due to the limitations of the study (see comment 3). More citations from the literature are needed to support these recommendations

13. The language throughout the manuscript needs some improvement

**VERSION 1 – AUTHOR RESPONSE**

Reviewer: 1
Dr. Kevin Yau, University of Toronto

Comments to the Author:
This is an observational study of hemodialysis (n=65) and generally healthy controls (n=500) which compared serologic measurements for SARS-CoV-2 S1 IgG and SARS-CoV-2 neutralizing antibodies using commercial assays. The main finding of this study is that antibody levels after two COVID-19 vaccine doses were lower in hemodialysis patients in comparison to controls. A strength of this study is the larger control group size which have been smaller in other studies. However, there was significant imbalance in baseline characteristics between study groups requiring adjustment using coarsened exact matching. Even after coarsened matching there remained some imbalance between the groups (e.g. sex) and therefore some residual confounding is likely present. While the finding of this study is consistent with previous studies, it is already established that dialysis patients have lower antibody levels than controls.

RESPONSE: Thank you for these constructive comments and careful review. We prepared a point-by-point response to the reviewer comments below.

I have several comments/questions for the authors:
1) Could exact dates be provided for the study period?

Thank you for this comment. We have added an information on the exact dates of the study period. “The blood collection of all the participants was performed between September 14 and September 25, 2021.” (Page 5, line 4)

2) It would be helpful to have additional information regarding the neutralizing antibody assay as there are multiple types of assays available (e.g. pseudovirus neutralization assays)

We used SARS-CoV-2 chemiluminescent immunoassays (CLIA) for IgG(S) and neutralizing antibody assay: iFlash 3000 (YHLO Biotech, Shenzhen, China) and iFlash-2019-nCoV series (YHLO Biotech) reagents. We have added the following sentence.

“All serological assays were performed using the CLIA assay with iFlash 3000 (YHLO Biotech, Shenzhen, China) and iFlash-2019-nCoV series (YHLO Biotech, Shenzhen, China) as reagents.” (Page 5, line 7)
3) How were the cut-offs values for these assays determined? A major challenge of serology studies is comparison of units between studies, can the units in this study be converted to BAU/mL (WHO International Units?)

According to the manufacturer’s insert, the cutoff value for the detection of IgG is 10 AU/mL. As to conversion to WHO international units. For neutralizing activity, AU/mL×2.4 was used to convert to International Units (IU/mL). For IgG, AU/mL×1.0 was used to convert to binding antibody units (BAU/mL). We have added the following sentence.

“The cut-off values of anti-S1 and N antibodies and neutralizing activity were 10 arbitrary units per milliliter (AU/mL), which were the official cut-off values by the manufacturer. For neutralizing activity, AU/mL×2.4 was used to convert to International Units (IU/mL). For IgG, AU/mL×1.0 was used to convert to binding antibody units (BAU/mL).” (Page 5, line 11)

4) For the very few participants with unknown vaccine type – what types of vaccines were available in Japan other than BNT162b2 at the time?

In Japan, BNT162b2 was provided by municipality, and mRNA-1273 (Moderna) was provided for the occupational vaccination in December 2021. AZD1222 (Astra Zeneca) was also available for candidate, but the number was limited. In this study cohort area, most people had BNT162b, and few had mRNA-1273 nor AZD1222. We have added the following sentence.

“While all participants in the present study received 2 doses of vaccination (most patients received BNT162b2), no participants received third doses at blood collection.” (Page 6, line 17)

5) Is there information on timing between 1st and 2nd dose, as this is known to affect serologic response?

Thank you for pointing this out. We agree with the reviewer, and an information on the timing between 1st and 2nd dose were inserted in the Table 1. We did not incorporate days between 1st and 2nd vaccination into the regression variable since the variation of this value was small in the present study. We have added the following sentence.

“Days range between first and second vaccination was not included in the regression variable, since the majority of participants in both the matched cohort of dialysis patients and the control group had 21 days between the first and second vaccination, and the variation in values was small.” (Page 9, line 16)

6) Did any patients in the study receive third doses at blood collection, I am assuming all the results are time in days post second dose?

As you pointed out, no patients in the present study received third doses at blood collection. We have added the following sentence.

“No participants in the present study received third doses at blood collection.” (Page 6, line 18)

7) Can you confirm that assumptions for the linear regression were met?

Thank you for pointing this out. Linearity and heteroscedasticity was confirmed by plotting the residual against the fitted values. Normality on residuals were confirmed by QQ plot. Cook’s distance was less than 0.5 for all points. We have added the following sentence.

“Linearity and heteroscedasticity was confirmed by plotting the residual against the fitted values. Normality on residuals were confirmed by QQ plot. Cook’s distance was less than 0.5 for all points.” (Page 5, line 31)

8) It seems surprising how few patients had baseline COVID-19 infection, however I recognize that this was earlier in the pandemic. An issue with the assay used is that it measures both the spike protein and nucleocapsid antibody. Normally nucleocapsid antibody seroconversion could be used as a marker of natural COVID-19 infection but I don’t believe that is possible here. Were the baseline characteristics of COVID-19 infection determined at the same time as blood collection?
Thank you for pointing this out. In the present study, IgG antibody titers against the SARS-CoV-2 N-protein was used to determine the past COVID-19 infection status, and none and two study participants showed the SARS-CoV-2 N-IgG antibody above the cut-off value in the dialysis and control groups, respectively. We have added the following sentence in the methods section. The results of antibody titers against the SARS-CoV-2 N-protein was explained in the result section.

“IgG antibody titers against the SARS-CoV-2 N-protein was used to determine the past COVID-19 infection status.” (Page 5, line 3)

“No participants with the SARS-CoV-2 N-IgG antibody above the cut-off value were included in the matched cohort of the dialysis and control groups.” (Page 8, line 17)

9) In the discussion, it would make sense to include a discussion of the relevance/correlation between SARS-CoV-2 antibody levels and actual COVID-19 infections/severity of illness.

Thank you for pointing this out. We have measured IgG antibody titers against the SARS-CoV-2 N-protein to determine the past COVID-19 infection status, and none and two study participants showed the SARS-CoV-2 N-IgG antibody above the cut-off value in the dialysis and control groups, respectively. These two participants with positive IgG antibody titers against the SARS-CoV-2 N-protein were not included in the matched cohort.

“Sixth, there were no participants in the dialysis group who showed positive IgG antibody titers against the SARS-CoV-2 N-protein, thus the present study could not assess the correlation between SARS-CoV-2 antibody levels and actual COVID-19 infections/severity of illness.” (Page 10, line 30)

Reviewer: 2
Dr. Caroline Hsu, Tufts Medical Center
Comments to the Author:
In this study, Zhao and colleagues used anti-SARS-CoV-2 S1 IgG antibodies and neutralizing antibodies to compare the immune response to vaccination between patients receiving dialysis and matched controls. The study confirms previous findings that dialysis patients have a lesser immune response to vaccination. However, some issues remain.

Major issues:
1. The matching methodology needs to be expanded upon. Matching is typically done in a ratio, such a 1:1 or 1:2, and it is not clear how this matched cohort was ultimately generated. I would also expect the baseline characteristics used for matching to be balanced between the dialysis patients and the controls, but sex in particular is skewed.

Thank you for pointing this out. CEM was performed using the MatchIt package (Ho, Imai, King, & Stuart, 2011). Sex, age and days passed since 2nd dose were binned by Sturge’s method if the variable were continuous. Thirteen subclasses were obtained with both hemodialysis patients and healthy controls. Weights were assigned to each subclass of hemodialysis patients or healthy controls to ensure the same ratio of hemodialysis patients and healthy controls is maintained within each subclass to the overall matched cohort. These weights were also applied when performing linear regression. Standardized mean difference were less than 0.1 on all variables which have been matched.

To explain the details of the matching methodology, we have added the following sentence.
“CEM was performed using the MatchIt package. Sex, age and days passed since 2nd dose were binned by Sturge’s method if the variable were continuous. Thirteen subclasses were obtained with both hemodialysis patients and healthy controls. Weights were assigned to each subclass of hemodialysis patients or healthy controls to ensure the same ratio of hemodialysis patients and healthy controls is maintained within each subclass to the overall matched cohort. These weights were also applied when performing linear regression. Standardized mean difference were less than 0.1 on all variables which have been matched.” (Page 5, line 22)

2. Furthermore, I think the matching process is a strength of the study in an attempt to adjust for confounders; therefore the authors should de-emphasize the analyses with the entire cohort and
focus on the results of the matched cohort.

Thank you for pointing this out. We agree with the reviewer, and to focus on the results of the matched cohort, we have restructured the results section.

3. The study has issues of small cohort size, short follow-up, lack of detailed clinical data, possible recall bias, and no establishment of association with hard clinical outcomes (such as SARS-CoV-2 infection). To the authors’ credit, they acknowledge all of these in the second-to-last paragraph.

Thank you for pointing out of this critical issue. We have emphasized the limitations of the present study of small cohort size, short follow-up, lack of detailed clinical data, possible recall bias, and no establishment of association with hard clinical outcomes at the beginning of the discussion section.

“The findings suggest that the hemodialysis patients should be carefully monitored for their immunization status although there are several limitations including a small cohort size, short follow-up, lack of detailed clinical data, possible recall bias, and no establishment of association with hard clinical outcomes.” (Page 10, line 4)

Minor issues:
4. The study does not add much new information and largely confirms existing knowledge, but in my opinion, confirmatory studies are still meaningful. If possible, it would strengthen the study to compare these antibody levels with those published in studies correlating antibody levels with hard clinical outcomes (such as SARS-CoV-2 infection).

Thank you for pointing this important issue. There were only two participants with positive IgG antibody titers against the SARS-CoV-2 N-protein, thus the present study could not assess the correlation between SARS-CoV-2 antibody levels and actual COVID-19 infections/severity of illness. We have added references of studies correlating antibody levels with hard clinical outcomes.

“Further studies are warranted to assess the correlation between the antibody titers and hard clinical outcomes as reported in the healthy adults.” (Page 10, line 34)

5. In this vein, the statement on page 4 line 22 “immune response to vaccination in dialysis patients was reported to be poor and short” is exaggerated; multiple studies have showed that the vaccine-induced immune response in dialysis patients is moderate – less than healthy adults, but greater than that of patients receiving immunosuppression (such as transplant patients). If immune response truly was poor, vaccination would be futile

Thank you for this comment. We agree with the reviewer, and changed the sentence as follows.

“immune response to vaccination in dialysis patients was reported to be moderate – less than healthy adults” (Page 4, line 11)

6. Page 4 line 9, “immunosuppression” implies taking medication to suppress the immune system; this should be changed to “immunocompromise”

We have changed the words. (Page 4, line 4)

7. Page 4, lines 10-12: “Regular visits to the dialysis center could increase the risk of SARS-CoV-2 infection due to limited capacity for air ventilation”; I would add due to close proximity, limited ability to physically distance. In many countries, living in a nursing facility was a major risk factor for COVID among dialysis patients, and this may be relevant to Japanese dialysis patients as well.

Thank you for this comment. We have added the words as follows.

“Regular visits to the dialysis center could increase the risk of SARS-CoV-2 infection due to limited capacity for air ventilation, close proximity, and limited ability to physically distance.” (Page 4, line 6)

8. Page 4, line 47-48: Please clarify, were the dialysis patients hospitalized at the time of the study, or were they outpatients receiving treatment at a clinic associated with a hospital?

Thank you for pointing this out. All dialysis patients were outpatients receiving treatment at a clinic
associated with a hospital. We have added the following sentence. 
“All dialysis patients were outpatients receiving treatment at a clinic associated with a hospital.” (Page 4, line 27)

9. Did any patient have a booster or additional vaccine dose prior to antibody assessment?

Thank you for pointing this out. We have added the following sentence for the explanation. 
“While all patients in the present study received 2 doses of vaccination (most patients received BNT162b2), no patients received third doses at blood collection.” (Page 6, line 17)

10. Table 3: Personally, I prefer reporting a 95% confidence interval rather than the SD

Thank you for pointing this out. We have added a 95% confidence interval in the Table 3.

11. Page 9 line 12, the sentence about Adjusted R square values – please interpret this for the reader

We have added the words for the explanation. (Page 9, line 13)

12. I feel that the authors make recommendations in the Discussion which are only weakly supported by their results, due to the limitations of the study (see comment 3). More citations from the literature are needed to support these recommendations

Thank you for this comment. We agree with the reviewer, and have added citations.

13. The language throughout the manuscript needs some improvement

We have asked the language editing service for the improvement.

**VERSION 2 – REVIEW**

**REVIEWER**
Yau, Kevin
University of Toronto, Nephrology

**REVIEW RETURNED**
06-Oct-2022

**GENERAL COMMENTS**
Thank you very much for the revision of your article which have addressed my questions. I just have a few minor remaining comments:

1. “The dialysis patients showed a significantly higher proportion for hypertension, diabetes, cardiovascular disease, respiratory disease, and medication of anti-histamine agents and acetaminophen when compared to the control group (p <0.001, <0.001, <0.01, <0.01, and <0.01, respectively).”

   I would probably not include this as the emphasis should be on the matched cohort which are described later. In addition, in general I would not include the p-values comparing baseline characteristics from an observational study.

2. Could the authors clarify if the linear regression is "multivariable" or "multivariate" as it seems to me that perhaps there are two separate linear regression models for "Log10 SARS-CoV-2 S1 IgG titer" and "Log10 Neutralizing antibodies titer".

3. Some improvements are still needed to the language throughout to make this manuscript suitable for publication.
**Reviewer:** 1  
**Dr. Kevin Yau, University of Toronto**  
**Comments to the Author:**  
Thank you very much for the revision of your article which have addressed my questions. I just have a few minor remaining comments:

**RESPONSE:** Thank you for these constructive comments and for the careful review. We have prepared a point-by-point response to the your comments below.

1. "The dialysis patients showed a significantly higher proportion for hypertension, diabetes, cardiovascular disease, respiratory disease, and medication of anti-histamine agents and acetaminophen when compared to the control group (p <0.001, <0.001, <0.001, <0.01, and <0.01, respectively)."

I would probably not include this as the emphasis should be on the matched cohort which are described later. In addition, in general I would not include the p-values comparing baseline characteristics from an observational study.

**RESPONSE:** Thank you for your suggestion. We fully agree and have deleted the indicated sentence. In addition, we have deleted the statistical comparison of baseline characteristics, including p-values, in the entire cohort.

2. Could the authors clarify if the linear regression is "multivariable" or "multivariate" as it seems to me that perhaps there are two separate linear regression models for "Log10 SARS-CoV-2 S1 IgG titer" and "Log10 Neutralizing antibodies titer".

**RESPONSE:** Thank you for this important comment. There are two separate linear regressions for "Log10 SARS-CoV-2 S1 IgG titre" and "Log10 Neutralizing antibodies titre" respectively. Therefore, the linear regression is "multivariable". We have changed the sentence as follows.

"Multivariable linear regression models were employed to predict log 10 of anti-SARS-CoV-2 S1 IgG and neutralizing antibodies with participant characteristics of the participant group, such as sex, age, and days between blood collection and second vaccination for the matched cohort."

3. Some improvements are still needed to the language throughout to make this manuscript suitable for publication.

**RESPONSE:** Thank you for this. We have asked an English proofreading company to improve the readability of the manuscript.