Author's response to reviews

Title: Plasmapheresis in a patient with antiphospholipid syndrome before living-donor kidney transplantation: a case report

Authors:

Tadashi Sofue (sofue@med.kagawa-u.ac.jp)
Yushi Hayashida (rinda@med.kagawa-u.ac.jp)
Taiga Hara (taiga@med.kagawa-u.ac.jp)
Kazuyo Kawakami (seahorse@med.kagawa-u.ac.jp)
Nobufumi Ueda (nob@med.kagawa-u.ac.jp)
Yoshio Kushida (ykushida@med.kagawa-u.ac.jp)
Masashi Inui (minui@tymc.twmu.ac.jp)
Hiroaki Dobashi (hdobashi@med.kagawa-u.ac.jp)
Yoshiyuki Kakehi (kakehi@med.kagawa-u.ac.jp)
Masakazu Kohno (mkohno@kms.ac.jp)

Version: 3  Date: 17 September 2014

Author's response to reviews: see over
Dear Dr. Pani:

Thank you very much for your letter regarding our manuscript (reference number 1720270747129230). While we were disappointed that our paper was not acceptable in its original form, we are grateful for your positive comments and appreciate the opportunity to submit our revised manuscript to *BMC Nephrology*. Therefore, please find attached our revised manuscript, titled “Plasmapheresis in a patient with antiphospholipid syndrome before living-donor kidney transplantation: a case report”. We have attached a list of the changes made, and our detailed point-by-point responses to the reviewers’ comments below.

We appreciate the issues raised by the reviewers, and have endeavored to address all of their concerns. We have added additional information in response to the reviewers’ comments, and the text and figures have been modified as necessary. Finally, we have reviewed the entire manuscript to correct any errors and improve the presentation. The revisions made to the text are shown in red font.
We believe that the revisions have much improved our manuscript, and we hope that the revised version will be accepted for publication in *BMC Nephrology*.

Yours sincerely,

Tadashi Sofue, MD, PhD;
Department of Cardiorenal and Cerebrovascular Medicine,
Faculty of Medicine, Kagawa University
1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan
Tel: +81-(87)-898-5111 (Ext. 2623); Fax: +81-(87)-891-2149
E-mail: sofue@med.kagawa-u.ac.jp
Summary of revisions

We have carefully revised the manuscript based on the comments provided by the reviewers. The major revisions are as follows:

1. We have reviewed the entire manuscript to correct any errors and to improve its presentation.

2. We have added information about lupus anticoagulant to the Case presentation (Page 7, Line 18 – Page 8, Line 2, Page 8, Line 15, and Page 11, Line 8) and Discussion (Page 12, Lines 9–12) sections.

3. We have included the “two hit” model in the Background (Page 5, Lines 13–17) and Discussion (Page 12, Lines 14–18) sections.

4. We have replaced “recurrent lupus nephritis” with “lupus nephritis” throughout the Case Presentation (Page 11, Line 3) and in the Figure Legend.

5. We have added information about anticoagulation at the time of recurrence (Page 7, Lines 16–18).

6. We have added information about permanent immunity and total IgG before and after plasmapheresis to the Discussion section (Page 12, Lines 1–9).

7. We have described the technical details of aCL IgG, anti-β2GPI IgG and lupus anticoagulant tests (Page 7, Line 18 – Page 8, Line 2 and Page 8, Lines 12–15).

8. We have cited seven additional references (references 7–9, 18–21).
Responses to Reviewer #1

This is an interesting case reporting the use of plasmapheresis in a patient with antiphospholipid syndrome before living-donor kidney transplantation.

Major points

1. No information is provided about the presence of Lupus Anticoagulant (LA). Among the aPL, LA has been proven to be the strongest risk factor for thrombosis and this should be taken into account when reporting this case.

We completely agree that the results of lupus anticoagulant (LA) test should be included, because the presence of aCL and/or anti-β2GPI IgG is not sufficient for a diagnosis of APS. Unfortunately, the results of the dilute Russell’s viper venom time (dRVVT) test at the time of diagnosis (age 25) were not available. However, at the time of recurrence, we confirmed that the patient was positive for LA (ratio 1.3; normal, <1.3), as determined by the Gradipore-LA dRVVT test (Medical & Biological Laboratories Co., Ltd., Nagoya, Japan). Her dRVVT test was also positive just before and 1 year after kidney transplantation (ratios of 1.3 at both times).

We have added this information to the Case presentation (Page 7, Line 18 – Page 8, Line 2, Page 8, Line 15, and Page 11, Line 8).

2. aPL are known to be "a second hit" for thrombosis (e.g. Giannakopoulos B NEJM). Usually they are necessary but not sufficient for clotting. The Authors should justify more in details the rationale behind their approach. Which was their aim for the temporary aPL removal from blood stream?
We agree that aPL are necessary but not sufficient for the onset of thrombosis. The “two hit” model of thrombosis in APS patients states that an initiating “first hit” injury disrupts the endothelium, with a “second hit” potentiating thrombus formation [Giannakopoulos B, Krilis SA: *N Engl J Med* 2013, **368:**1033-1044]. The “first hit” endothelium injury can result from trauma, infection or drugs [Sciascia S et al: *Nat Rev Nephrol* 2014, **10:**279-289]. In patients with catastrophic APS, infection and recent surgery are also recognized precipitants of endothelial injury [Asherson RA: *Lupus* 1998, **7:**Suppl 2: S55-S62].

During kidney transplantation, the donated kidney is perfused by organ preservation solution, anastomosed to the recipient iliac artery and vein, and then re-perfused (ischemia/reperfusion injury). Therefore, kidney transplant surgery is considered a major risk factor for endothelium injury as a “first hit”. Because these endothelial injuries after kidney transplantation are considered temporary, we believed that temporary prophylactic aPL removal would reduce the risk of thrombosis during the early post-operative period.

In accordance with this comment, we have added three references and included this information in the Background (Page 5, Lines 13–17) and Discussion (Page 12, Lines 14–18) sections.

3. *in the discussion, the authors referred to "recurrent lupus nephritis" but no information is provided about the previous history of LN.*

An original kidney biopsy was not performed on this patient. Moreover, she did not show apparent proteinuria or hematuria prior to diagnosis with end stage kidney disease. Thus, we could not determine whether lupus nephritis was present in her original kidney.
Therefore, we removed the word “recurrent” from the Case Presentation (Page 11, Line 3) and Figure Legend.

4. Anticoagulation at the time of the recurrences should be reported. Was the INR within therapeutic range?

At the time of recurrent thrombosis, the patient’s INR was 1.5, lower than target therapeutic range (2.0 to 3.0) despite anti-coagulant treatment with 3.0 mg/day warfarin. We have added this information to the Case Presentation (Page 7, Lines 16–18).

5. aPL are known to fluctuate over the time. aPL levels before and after plasmapheresis should be compared with permanent immunity (e.g IgG tetanus).

Unfortunately, we did not measure permanent immunity before and after plasmapheresis. Because of the retrospective nature of this case report, we could not determine whether plasmapheresis altered permanent immunity. Although product information for Plasmacure PE-05 stated that 95.7 % of total IgG were removed by PE [http://www.kawasumi.jp/english/hp/medical/plas/p_plas.html], only an average of 26.3 % of total IgG was reduced by PE with Plasmacure PE-05 in patients treated with the same procedure in our hospital, due to replacement by FFP including IgG. However, PE with Plasmacure PE-05 removed 55.5 % of the aCL IgG and 55.6 % of the αβ2GPI IgG from our patient. A previous study reported that plasmapheresis (plus bortezomib treatment) reduced anti-HLA antibodies while leaving tetanus IgG intact in kidney transplant recipients [Everly MJ et al: Transplantation 2010, 90:1493-1498]. Replacement with FFP should be effective in maintaining permanent immunity. These
findings suggested that multiple rounds of plasmapheresis could specifically remove disease-specific IgG while leaving permanent immunity intact.

We have added one reference and included this information in the Discussion section (Page 12, Lines 1–9).
Response to Reviewer #2

The case report is very interesting because in the APS syndrome primary and secondary early graft thrombosis remains the most frequent cause of renal graft failure in this patients. Still what prophylaxis of thrombosis to utilize in APS patients undergoing Kidney Transplantation, especially if they are living-donor recipients, is unclear. On the basis of these findings in this single case it is impossible to conclude that Plasmapheresis is useful as prophylaxis for thrombosis in APS patients undergoing renal transplantation.

We agree that, based on findings in our patient alone, it is impossible to conclude that plasmapheresis is as useful as prophylaxis for thrombosis in APS patients undergoing renal transplantation. Our results, however, suggest that further studies are warranted to verify our conclusion. We have added this as a study limitation to the Conclusions.
Responses to Reviewer #3

Major points

1. It is not clear to this Reviewer if patients have also been tested for Lupus Anticoagulant. If yes, was LA testing affected by plasmapheresis? Which methods for LA testing were used? Do they have all the same behavior?

We completely agree that the results of lupus anticoagulant (LA) test should be included, because the presence of aCL and/or anti-β2GPI IgG is not sufficient for a diagnosis of APS. Unfortunately, the results of the dilute Russell’s viper venom time (dRVVT) test at the time of diagnosis (age 25) were not available. However, at the time of recurrence, we confirmed that the patient was positive for LA (ratio 1.3; normal, <1.3), as determined by the Gradipore-LA dRVVT test (Medical & Biological Laboratories Co., Ltd., Nagoya, Japan). Her dRVVT test was also positive just before and 1 year after kidney transplantation (ratios of 1.3 at both times). All dRVVT tests during the study period were performed in the same laboratory using the same protocol.

Unfortunately, we did not have sufficient data to determine whether plasmapheresis affected the results of the dRVVT test. A previous case report showed that plasmapheresis decreased dRVVT test results from a ratio of 1.8 to 1.2 [van Wissen S et al: Lupus 2008, 17:586-589]. Thus, IgG removal with plasmapheresis may have decreased LA test results as well as aCL IgG and anti-β2GPI IgG.

We have added this information to the Case presentation (Page 7, Line 18 – Page 8, Line 2, Page 8, Line 15, and Page 11, Line 8) and Discussion (Page 12, Lines 9–12) sections.
2. Has the patient a previous history of Lupus Nephritis? This should be clarified

An original kidney biopsy was not performed on this patient. Moreover, she did not show apparent proteinuria or hematuria prior to diagnosis with end stage kidney disease. Thus, we could not determine whether lupus nephritis was present in her original kidney. We therefore removed the word “recurrent” from the Case Presentation (Page 11, Line 3) and Figure Legend.

3. Data about IgG/M/A before and after plasmapheresis should be provided and their trend should be compared with aCL and aβ2GPI.

Unfortunately, we could not obtain sufficient data about total IgG/M/A before and after plasmapheresis. Her pre-treatment IgG, IgM, and IgA concentrations were 1261 mg/dl, 146 mg/dl, and 168 mg/dl, respectively. Because of the retrospective nature of this case report, we could not determine IgG/M/A before and after plasmapheresis and their trend compared with aCL and aβ2GPI.

Product information for Evaflux 2A20 states that around 20% of total IgG is removed by DFPP [Sueoka A: Ther Apher 2000, 4:211-212]. Data on patients treated with the same procedure in our hospital found that DFPP with Evaflux 2A20 removed a mean 60.1% of total IgG, whereas, in our patient, DFPP removed 70.0% of aCL IgG and 71.5% of aβ2GPI IgG. In contrast, product information for Plasmacure PE-05 stated that 95.7% of total IgG were removed by PE [http://www.kawasumi.jp/english/hp/medical/plas/p_plas.html]. Data of patients treated with the same procedure in our hospital found that PE with Plasmacure PE-05 removed a mean 26.3% of total IgG because of replacement by FFP including IgG. In our patient, 55.5% of aCL IgG and 55.6% of aβ2GPI IgG were removed by PE with Plasmacure
PE-05. Replacement with FFP should be effective in maintaining permanent immunity. Multiple steps of plasmapheresis may therefore specifically remove disease-specific IgG while leaving other IgG intact.

We have added two references and included this information in the Discussion section (Page 12, Lines 1–9).

4. Further technical details about aCL and b2GPI testing should be provided. Was the reduction in titer proved in different techniques?

Plasma aCL (MESACUP cardiolipin test, Medical & Biological Laboratories Co, Ltd, Nagoya, Japan) and anti-β2GPI (Yamasa Co, Choshi, Japan) IgGs were measured using enzyme-linked immunosorbent assays. All tests of aCL and anti-β2GPI IgGs were performed using the same procedure in the same laboratory. Reductions in titer were therefore likely not due to the use of different techniques.

We have added this information to the Case Presentation (Page 8, Line 12–15).
Response to Reviewer #4

I do not have remarks to do, and I would accept this case report without any revisions.

We wish to thank you for your careful reading of our manuscript and for the favorable comments.