Author's response to reviews

Title: Comparison of double filtration plasmapheresis with immunoadsorption therapy in patients with anti-glomerular basement membrane nephritis

Authors:

Yiyan Zhang (yiyanzhang86@hotmail.com)
Zheng Tang (tang_dr@163.com)
Dongmei Chen (245687533@qq.com)
Dehua Gong (gong_doctor@126.com)
Daxi Ji (jdx@jlonline.com)
Zhihong Liu (zhihong--liu@hotmail.com)

Version: 5
Date: 5 March 2014

Author's response to reviews: see over
Author's response to reviews

Title: Comparison of double filtration plasmapheresis with immunoadsorption therapy in patients with anti-glomerular basement membrane nephritis

Authors:

Zhang Yi-yan (viyanzhang86@hotmail.com)
TANG Zheng(tang_dr@163.com)
After reading through your manuscript, we feel that the quality of written English needs to be improved before the manuscript can be considered further. We advise you to seek the assistance of a fluent English speaking colleague, or to have a professional editing service correct your language. Please ensure that particular attention is paid to the abstract.

As the editor advised, we have chose Edanz Editing and made some changes to improve the quality of written English.

1. **Objective:** Compared with immunoadsorption (IA), double filtration plasmapheresis (DFPP) needs only small amount of plasma or albumin. But the clinical efficacy and safety of DFPP in treating anti-glomerular basement membrane (Anti-GBM) disease was unclear. The Objective was changed as below:

   **Background:** Double filtration plasmapheresis (DFPP) requires less plasma or albumin than immunoadsorption (IA). However, the clinical efficacy and safety of DFPP in patients with anti-glomerular basement membrane (anti-GBM) disease are unclear.

2. **Methods:** Twenty eight patients with diagnosis of anti-GBM disease by serological and pathological examinations from 2003 to 2013 were enrolled in this study. All patients were treated with DFPP (DFPP group; n = 16) or IA (IA group; n = 12) therapy as well as immunosuppressive agents. The repeated examination of serum anti-GBM antibodies, IgG, urinary and blood tests were detected pre and post procedures. The renal function and outcome were followed-up. In DFPP treatment, EC50W and EC20W (Asahi Kasei Corporation, Japan) were used as the first filter for plasma separation and the second filter for plasma fractionation respectively. Double volume of plasma was processed, and 30-40g human albumin was supplemented during each session. IA was administered for 10 cycles per session and 8-10 sessions as a course daily or once every other day; a total of 30-60 L of plasma was regenerated in each course.

   We have now adjusted some orders of sentences in the methods section of the abstract as the Edans indicates. Also, some words were changed to be more appropriate. ‘followed-up’ were changed to ‘determined’

3. **Conclusion:** Combined with immunosuppressive therapy, DFPP efficiently and safely removed anti-GBM antibodies in this study. Because of the smaller amount of plasma, less side-effect caused by plasma and the less loss of IgG, the DFPP could be a better choose in treatment of anti-GBM disease, especially in the case of insufficient plasma.

   **Conclusion:** DPPP plus immunosuppressive therapy efficiently and safely removed anti-GBM antibodies. The smaller amount of plasma, fewer plasma-associated side effects and reduced loss of IgG suggest that DFPP may be a better treatment choice for anti-GBM disease, especially in patients with insufficient plasma.

4. **Introduction** was changed to background.
5. Treatment depends on timely immunosuppressive agents and removal of circulating antibodies. Removal of anti-GBM antibody by extracorporeal treatment including plasmapheresis or immunoadsorption (IA) has been considered an effective method in the treatment of Goodpasture’s syndrome.

The sentence in manuscript now appears as:
Treatment consists of immunosuppressive agents and removal of circulating antibodies. Extracorporeal removal of anti-GBM antibody, by, for example, plasmapheresis or immunoadsorption (IA) is an effective treatment for Goodpasture’s syndrome.

6. Double filtration plasmapheresis (DFPP) needs only small amount of plasma or albumin, and has been reported to be performed in many cases of disorders as a means to remove auto-antibodies, particularly those associated with immunologic pathogenesis, such as myasthenia gravis, chronic inflammatory neuropathy, Guillain-Barré syndrome, ANCA associated vasculitides and so on.

“In contrast” was inserted in front of the sentence.
“reported to be performed in many cases of disorders as a means” was changed to be “used”.
“included diseases” were inserted in front of “such as …… and so on.”

7. Recently, some small samples about DFPP used in treatment of anti-GBM nephritis to remove the serum anti-GBM antibodies have been reported. In this study, compared with plasmapheresis and immunoadsorption, the effect of DFPP on serum anti-GBM level was investigated and its clinical efficacy was evaluated in patients with anti-GBM nephritis.

The sentence in manuscript now appears as:
Recently, DFPP was used to in several patients with anti-GBM nephritis to remove serum anti-GBM antibodies. To expand on these findings, we compared the effects of DFPP and immunoadsorption (IA) on serum anti-GBM concentrations, as well as comparing their clinical efficacy in patients with anti-GBM nephritis.

8. Patient
   a) Patients diagnosed anti-GBM nephritis hospitalized from December 2003 to June 2013 in Department of Nephrology, Jinling Hospital met the following conditions were included: (1) renal function decreased rapidly, with persistent hematuria, anuria, and ultrasound showing normal kidney size; (2) serum anti-GBM antibody positive; the renal biopsy showing crescentic glomerulonephritis with IF liner IgG deposition along the glomerular basement membrane(GBM).

   Some grammatical errors have been collected. The numbers have been deleted. The sentence in manuscript has been changed to be more explicit and now appears as follows:
Patients diagnosed with anti-GBM nephritis and hospitalized between December 2003 and June 2013 in the Department of Nephrology, Jinling Hospital were considered eligible. Other inclusion criteria included a rapid decrease in renal function, with persistent
hematuria and anuria, and an ultrasound showing normal kidney size; positivity for serum anti-GBM antibody; the renal biopsy showing crescentic glomerulonephritis with IF liner IgG deposition along the glomerular basement membrane (GBM).

b) A total of 28 patients underwent plasma therapy were included in the study. They were divided into 2 groups: DFPP (the DFPP group; n=16) and staphylococcal protein A immunoadsorption (the IA group; n=12). Rule out the cases with active infection, immunodeficiency, and severe cardiovascular and cerebrovascular diseases. The forms of expression have been changed to be explicit.

c) We performed DFPP, staphylococcal protein A immunoadsorption and renal biopsy for clinical not research purposes. The written informed consent for participation in the study was obtained from participants or, where participants are children, a parent or guardian. In addition, ethical permission was obtained from the ethics committee of our hospital.

The sentences have been changed to be concise:
“Patients underwent DFPP, staphylococcal protein A immunoadsorption and renal biopsy for clinical not research purposes. All patients or their parent/guardian provided written informed consent. The study protocol was approved by the ethics committee of our hospital.”

9. Treatment and fellow-up protocols

a) “fellowed-up” has been change to “fellow-up”

b) The second sentence in this paragraph, “Maintenance hemodialysis therapies were given to the patients with deterioration of renal function” have been changed to “Patients showing with deterioration of renal function were received maintenance hemodialysis therapy.”

c) The protocol has been changed to be more explicit and now appears as follows:
“Each patient in the DFPPs group underwent were performed for 2—4 sessions in each patient once every other day. IA was administered for 10 cycles per session, with and 8—10 sessions performed as a course daily or once every other day; a total of 30—60 L of plasma was regenerated during in each session/course. The repeated examinations of Sserum anti-GBM antibodies and serum IgG were measured followed after every single procedure.”

10. The method of DFPP has been change to be more explicit as follows:

A double volume of plasma was processed during each DFPP session. Two filters were used, an EC50W filter (Asahi Kasei Corporation, Japan) as the first filter for plasma separation and the second and EC20W filter (Asahi Kasei) for plasma fractionation. Using a blood pump, native blood was pumped into the first filter and separated into plasma and cellular components. The second pump was used to pump filtered plasma into the second filter, which separated albumin from larger plasma molecules, including immunoglobulins, immune complexes, and lipoproteins. During each session, each patient received a supplement of 30—40g human albumin. During DFPP, waste plasma was discarded intermittently. When the pressure on the second filter reached the threshold value to
discard plasma, 800 ml normal saline were used to flush the second filter of accumulated plasma proteins, prior to discarding waste plasma.

11. Histopathology
It has been change to be explicit as follows:
For light microscopy, renal biopsy specimens were fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned at 2 μm thickness. Sections were stained with hematoxylin-eosin, periodic acid-Schiff’s reagent, Masson’s trichrome and periodic acid methenamine silver. Glomerular, tubular interstitial and vascular lesions in biopsies were recorded. Biopsy samples were evaluated separately by two pathologists, with each blinded to the other’s results and to patients’ data.

12. Statistical methods
a) In the second sentence, the “according to” has been changed to “depending on”.

b) The third sentence has been change to be explicit as follows:
Between group differences were evaluated statistically using t-tests, whereas rates were compared by using the Kruskal-Wallis H test or the χ2 test. A p-value <0.05 indicated statistical significance.

13. RESULTS
There were much more mistakes in the express of English, as the professional editing service(Edanz) suggested, the written English has been changed to be fluent without changing the meaning of it.

a) The first paragraph has been changed to “The 28 patients included 13 males and 15 females, of median age 44.5 years (range, 9–68 years). The median duration of disease was 5 weeks (range, 1 week–5 years). Six patients had pulmonary hemorrhage, including one diagnosed with diffuse alveolar hemorrhage and five with blood-tinged sputum. Three patients were ANCA positive, 10 had anuria and 18 had a serum creatinine (Scr) concentration > 500 μmol/l and required renal replacement therapy at disease onset. Thirteen patients had hypertension, and 17 had symptoms of gross hematuria. There were no significant differences between the DFPP and IA groups. (Table 1)”

b) The second paragraph has been changed to “Laboratory and pathological data are shown in Table 2. Most patients were anemic upon admission to hospital, 19 (67.9%) had disorders of calcium and phosphorus metabolism, 18 (64.3%) had high C-reactive protein (CRP) concentrations. Except for patients with anuria, all had abnormal findings on urinalysis, including 7 (25.0%) with a nephrotic degree of proteinuria. Upon hospital admission, 18 (64.3%) patients had SCr levels >500 μmol/L, with 26 (92.9%) having SCr levels >500 μmol/L during the course of disease. NAG and RBP were also higher than normal. Except for NAG, which was significantly higher in the DPP than in the IA group (84.4±37.3 vs. 58.1±33.7, p=0.03), none of these data differed significantly in the two groups. At the time of biopsy, a median 73.9% (range, 54.6–95.4%) of glomeruli per patient had a crescent shape, with 26 (92.9%) patients having >50% and 11 (39.3%) having >85% crescent-shaped glomeruli.”

c) The second paragraph has been changed to “Following the DFPP sessions, the concentration of anti-GBM-antibody declined significantly, from 210 to 86.3 RU/ml
(P<0.01), a reduction of 123.7 RU/ml or 61.9%, as did the concentration of IgG, from 12.4 to 4.2 g/L, a reduction of 8.2 g/L or 62.7% (Table 4, Figure 1). Similarly, the concentration of anti-GBM antibody in the IA group declined from 199 to 57.0 RU/ml, a reduction of 142 RU/ml or 70.8%, and the concentration of IgG from 12.7 to 2.1 g/L, a reduction of 10.6 g/L or 82.5%. The change in IgG concentration differed significantly in the two groups (p=0.049), whereas the change in anti-GBM concentration did not (p=0.452)

d) The side effect has been changed to “Four patients experienced fever during DFPP, one had bleeding in the lung and one had ecchymosis in the skin. The adverse effect rates were similar in the DFPP and AI groups (Table 4).”

e) The follow-up has been changed to “Of the 16 patients in the DFPP group, 3 (18.8%) died at presentation or during follow-up; four (25.0%) were lost to follow-up; and nine (59.4%) progressed to end-stage renal disease (ESRD), with a median renal survival of 9 weeks (95% CI, 5–15 weeks). Five patients in this group (31.3%) progressed to CRF, while one returned to normal, being negative for anti-GBM antibody and on urine tests, and an SCr range of 67–86 µmol/L. Outcomes were similar in the two groups.”

f) The name of Table 1 has been changed to “Baseline demographic and clinical characteristics of the DFPP and IA groups”.

g) The name of Table 2 has been changed to “Laboratory and pathological findings in the DFPP and IA groups”.

h) The name of Table 3 has been changed to “Comparative effects of IA and DFPP on changes in anti-GBM antibody and IgG concentrations”

i) The name of Table 4 has been changed to “Adverse effects during DFPP and IA”.

j) The name of Fig.1 has been changed to “Effects of IA and DFPP on concentrations of anti-GBM antibodies and IgG in individual patients”

14. Discussion

We did not change the meaning of the paper during the correction of written English. The changes contained the correction of syntax error, use of conjunctions, use of professional vocabulary and so on. The corrections were made under the communication and suggestion of professional editing service (Edanz). So if the editors need more details, I would like to provide in another file.

The part of discussion now appears as follows:

To our knowledge, this study is the largest to date comparing these two methods of extracorporeal blood purification in patients with anti-GBM disease, including 16 who received DFPP treatment. Although renal dysfunction and abnormal urine analysis did not improve significantly after DFPP, serum anti-GBM-antibody titers declined significantly, demonstrating the effectiveness of DFPP therapy in removing these antibodies. Moreover, the ability of DFFP to remove anti-GBM antibodies was similar to that of IA. The two groups were also quite similar clinically and pathologically, as well as in renal and patient survival outcomes. None of these patients experienced any severe side effects during treatment. DFPP was superior to IA in reducing the loss of useful substances and in requirements for exogenous supplements owing to semi-selective removal. Reductions in IgG concentration were lower with DFPP than with IA, and DFPP was unrestricted, lacking an IA column.

Anti-GBM antibodies initiate the inflammatory destruction of the basement membrane of
the kidney glomeruli, leading to a rapidly progressive glomerulonephritis[3]. Treatments are focused on decreasing inflammation induced by circulating antibodies and on removing these antibodies[4]. Although plasma exchange (PE) was effective in removing these antibodies[5], it unselectively removed all plasma proteins. Thus, these patients required replacement of human proteins. Moreover, PE was associated with inherent risks, including anaphylactic reactions, transfusion-related acute lung injury and transfusion-transmitted infections, including infections with hepatitis B and C virus and human immunodeficiency virus. Furthermore, PE required large volumes of plasma and was therefore costly, limiting use of this method[6,7].

DFPP was designed to selectively remove the immunoglobulin fraction from serum, thus minimizing the volume of substitution fluid[8]. Unlike PE, DFPP selectively removes high-molecular-weight molecules, minimizing albumin loss, the need for substitution fluids, and associated hemodynamic fluctuations[9]. DFPP requires only 10–15% of the plasma volume required during PE, greatly reducing the incidence of adverse reactions. Moreover, using albumin or normal saline instead of plasma as a replacement fluid allows DFPP to be performed even in the absence of plasma.

We found that the concentrations of anti-GBM-antibody decreased significantly after DFPP procedures, with a mean decrement of 56.3±22.7%, indicating that DFPP may be effective at clearing anti-GBM antibodies. In comparison, IgG concentration declined a mean 67.4±12.3%. After the first procedure, IgG decreased 37.8±12.4%, whereas anti-GBM-antibody decreased 24.3±16.0%. Thus, in agreement with previous findings, we found that a simple DFPP procedure was more efficient at removing anti-GBM antibodies than IgG[10]. Moreover, in agreement with previous findings, we found that serum IgG concentration decreased exponentially while serum anti-GBM antibodies decreased linearly[11].

None of the patients in the DFPP group experienced severe complications. Four patients had fever during the course of DFPP, but their temperatures decreased to normal range after antibiotic therapy. Nevertheless, it was difficult to determine whether fever was caused by infection or an allergic reaction. One patient had diffuse alveolar hemorrhage, as confirmed by chest CT, but without severe hemoptysis, with the symptoms improving after symptomatic treatment. One study of 335 patients who underwent a total of 2502 plasmapheresis procedures during 515 courses of plasmapheresis found that complications during DFPP included hypotension, bleeding events, allergic reactions, muscle cramps, catheter-related complications such as vascular trauma and septicemia, and problems associated with membrane filters such as hemolysis[12]. That study found that 67.5% of patients experienced complications, occurring during 26.3% of procedures and 60.0% of courses of plasmapheresis.

Hemolysis may be the most frequent complication of DFPP, reported during 20% of procedures. While only 2 of our patients had visible hemolysis that affected the skin or lungs, slight hemolysis without an obvious decrease in hemoglobin (Hb) concentration may have occurred. The mean decrease in Hb following a course of DFPP was 2 g/L. However, since DFPP could also remove fibrinogen, patients undergoing this procedure may have hypofibrinogenemia. Although this condition is not associated with bleeding tendency, clinically overt bleeding has been occasionally reported, especially after serial
plasmapheresis. Moreover, most of our patients had anemia and were treated with erythropoietin. Because of the difficulty determining whether DFPP increased internal bleeding, invasive procedures should not be performed during the course of DFPP and high-risk patients should be closely monitored during DFPP treatment. The time required for fibrinogen concentration to return to pretreatment levels after a single DFPP session is 3 to 4 days. Therefore, all of our patients were administered fresh plasma infusions after DFPP to restore fibrinogen and prevent bleeding episodes, perhaps explaining why none of these patients experienced severe hemolysis during the course of DFPP.

Outcomes were similar in the DFPP and IA groups. We found that 4/16 (25%) patients in the DFPP group no longer required maintenance renal replacement therapy at the end of follow-up, a lower percentage than previously reported[13]. Renal injury, however, was more severe in our patients. For example, the maximum mean Scr concentration was 826 μmol/l, and the highest Scr levels in 14 of the 16 patients in this group were higher than the level indicative of uremia. These 14 patients therefore required renal replacement therapy at admission. In addition, although the prognosis of patients with anti-GBM nephritis would be improved by earlier diagnosis and treatment[14-17], the time from disease onset to admission in our patients ranged from 10 days to 13 months. A comparison of clinical features showed that patients who progressed to ESRD tended to have a longer course of disease. Therefore, a longer time from disease onset to admission is predictive of poorer prognosis. Thus, if the diagnosis is highly suspected, it may be appropriate to begin plasmapheresis while awaiting confirmation. The titer of circulating anti-GBM antibody is not the only determinant of the degree of renal injury. Complement components and proteases are involved in the process of renal injury following the binding of anti-GBM antibody to GBM.

Compared with patients in a previous study[18], our patients had more severe lesions histopathologically. Seven of the 16 (43.8%) patients had >85% crescent formation, indicative of a poor outcome[19,20]. A comparison of the pathological features in patients with different outcomes showed that patients who progressed to ESRD tended to have more crescent formation, especially fibrocellular/fibrous crescents. Taken together, all of the factors cited above may affect the prognosis of renal function and may explain why outcomes in our DFPP group were not better than previously reported[21]. Moreover, urine increased in three patients after DFPP therapy.

Correlation analysis showed that outcomes were significantly and negatively corrected with formation of crescents \( r = -0.462, P = 0.008 \) and fibrocellular/fibrous crescents \( r = -0.376, P = 0.034 \), with peak Scr concentration \( r = -0.495, P= 0.004 \), and with the course of kidney disease \( r =-0.393, P= 0.015 \). The predictors of kidney survival in patients with anti-GBM GN were found to include the percentage of glomerular crescents and Scr concentration and need for dialysis at presentation. We found that all of our patients with an initial Scr >500 μmol/L and 85–100% glomerular crescents became chronically dialysis-dependent despite aggressive treatment.

We found that patient survival was good and kidney survival moderate, suggesting that, unless there was evidence of pulmonary hemorrhage, which indicates a very high risk of death, patients with high initial Scr and crescent formation do not require DFPP or IA. Patients with a higher percentage of cellular crescents should be treated with DFPP to
remove anti-GBM antibodies, thus avoiding further damage. This is particularly applicable to
patients with high serum concentrations of anti-GBM antibodies.

15. Conclusion

Compared with IA, DFPP could effectively and safely clear anti-GBM antibodies and to some
extent improve renal symptoms. Since DFPP requires smaller volumes of plasma than IA, DFPP
may be a better choice for the treatment of anti-GBM disease, especially if IA columns and
plasma are insufficient. DFPP resulted in good patient survival and moderate kidney survival.
Except for patients with pulmonary hemorrhage requiring active plasma therapy, complications
during DFPP may be decreased by reducing the number of procedures in patients with SCr >500
µmol/L and severe chronic renal histology, especially those with a long course of disease.