Author’s response to reviews

Title: Clinical effects of direct hemoperfusion using a polymyxin B-immobilized fiber column in clinically amyopathic dermatomyositis-associated rapidly progressive interstitial pneumonias

Authors:

Hiroko Okabayashi (hirokokaba@hotmail.co.jp)
Hidenori Ichiyasu (ichiyasu@kumamoto-u.ac.jp)
Sayuri Hirooka (ayuri_hirooka@ybb.ne.jp)
Kimitaka Akaike (demio0601@gmail.com)
Keisuke Kojima (keisukekojima2000@yahoo.co.jp)
Takayuki Jodai (jojojojojody@gmail.com)
Yasumiko Sakamoto (mikomiko727@gmail.com)
Hideharu Ideguchi (ideguchi_hide@yahoo.co.jp)
Shohei Hamada (unagicurry@yahoo.co.jp)
Chieko Yoshida (chieko-m@ceres.ocn.ne.jp)
Susumu Hirosako (hirosako@kumamoto-u.ac.jp)
Shinichiro Okamoto (sokamoto@kuh.kumamoto-u.ac.jp)
Hirotsugu Kohrogi (kohrogi@kumamoto-u.ac.jp)

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Author’s response to reviews:

Prof. Martina Vasakova
Managing Editor
BMC Pulmonary Medicine
Dear Prof. Martina Vasakova:

Thank you very much for reviewing our manuscript (PULM-D-17-00150) and giving us an opportunity to respond to the reviewers’ comments. We found these comments to be helpful and have revised the manuscript accordingly.

Regarding the HRCT findings, we evaluated the HRCT scores using a semi-quantitative method reported by Ichikado et al. [Am J Respir Crit Care Med 2002; 165:1551–6]. We observed no significant differences in the HRCT scores or in the extent of interstitial and alveolar patterns between the survivor and non-survivor groups in our study.

To the Methods section, we added the following text: “Scoring of HRCT Findings.

The HRCT findings were graded using a classification method reported by Ichikado et al.; this method uses the following 6-point scale: (1) normal attenuation, (2) ground-glass attenuation (GGA) without traction bronchiectasis, (3) airspace consolidation without traction bronchiectasis, (4) GGA with traction bronchiectasis, (5) airspace consolidation with traction bronchiectasis, or (6) honeycombing [30]. Each lung was divided into upper, middle, and lower areas to yield a total of six zones, each of which was evaluated separately. The upper lung zone was defined as the area above the level of the tracheal carina, the lower zone was defined as the area below the level of the inferior pulmonary vein, and the middle zone was defined as the area between the upper and lower zones. In each zone, the extent of involvement of each finding was assessed visually and estimated to the nearest 10% of parenchymal involvement. The overall percentage of involvement of one type of abnormal finding in all six lung zones was obtained by averaging the corresponding values from all zones. Each abnormality score was calculated by multiplying the extent of involvement by each grading score, and the total HRCT score was calculated by summing up all of the grading scores. Two observers reviewed the HRCT findings. If the observers' scores differed, a consensus was reached after discussion.” (page 9, line 6).

In the Results section, we presented the HRCT scores in the revised Tables 2 and 3 and added the following sentence: “The baseline APACHE II scores and HRCT scores did not differ between the groups.” (page 14, line 7).

We have attempted to address each of the comments raised by the reviewers, and hope that you will now find the manuscript to have been satisfactorily improved. We would be very grateful for the acceptance and publication of our revised manuscript in your journal.
Thank you for your consideration of the revised version.

Sincerely yours,

Hidenori Ichiyasu, M.D., Ph.D.
Department of Respiratory Medicine
Faculty of Life Sciences, Kumamoto University
1-1-1 Honjo, Chuo-ku, Kumamoto 1860-8556, Japan
Phone No: +81-96-373-5012
Fax No: +81-96-373-5328
E-mail Address: ichiyasu@kumamoto-u.ac.jp

Dr. Nathan Hambly (Reviewer 1):
We have found the comments made by Dr. Nathan Hambly to be very helpful during the revision of our manuscript. We have accordingly attempted to address the questions raised by the reviewer.

Major comments
Background
1. Could the authors describe in more detail the histologic pattern of RPIP? Is this similar to DAD or is this term specific to CADM? Please provide supporting references.

(Response)
Thank you for these important questions.
The term RPIP is not specific to CADM and is not a clinicopathological term, but rather a medical term based on the clinical course of interstitial pneumonia.
We added the term “RPIPs” to the inclusion criteria and changed the following text in the Methods section to: “The inclusion criteria for RPIPs were as follows: (1) unexplained worsening of dyspnea within 1 month; (2) evidence of hypoxemia as defined by a P/F ratio < 300 mm Hg; (3) HRCT findings of newly developed ground-glass opacities and/or consolidations; (4) no evidence of pulmonary infection in bronchoalveolar lavage and sputum culture and negative results in blood tests for other potentially infectious pathogens; and (5) exclusion of left-heart failure, pulmonary embolism, pneumothorax, and other possible causes of acute respiratory failure” (page 8, line 9).

2. Need to clarify the strength of literature supporting the use of PMX-DHP in other forms of RPIP such as IPF. I believe that the majority of the data is from retrospective uncontrolled cohorts? What was the value seen in these trials from a clinical perspective?

(Response)

Thank you for these important comments.

In a larger, retrospective multi-center study of PMX-DHP in patients with RPIPs, including acute exacerbation of IPF, Abe et al. reported a favorable outcome; however, this study did not include a control group without PMX-DHP [Intern Med 2012; 51:1487–91]. Recently, the results of several retrospective studies (including ours) conducted to comparatively analyze PMX-DHP and control groups revealed significantly improved outcomes with PMX-DHP therapy [BMC Pulm Med 2015; 15:e15, Ther Adv Respir Dis 2017; 11:261–75].

We have added this text to the Background section as follows: “In a larger, retrospective multi-center study of PMX-DHP in patients with RPIPs, including acute exacerbation of IPF, Abe et al. reported a favorable outcome; however, this study did not include a control group without PMX-DHP [15]. Recently, the results of several retrospective studies including ours conducted to comparatively analyze PMX-DHP and control groups revealed significantly improved outcomes with PMX-DHP therapy [16, 17].” (page 6, line 12).

3. I think it needs to be stated that this is an investigational technique rather than a standard of care. Could the authors provide more detail regarding the mechanism by which PMX-DHP is thought to have benefit in RPIP?

(Response)

Thank you for this important comment and suggestion.

The mechanism by which PMX-DHP therapy affects RPIPs has not been fully elucidated. However, several reports have discussed the mechanism of the PMX-DHP. Abe et al. [Blood
Purif. 2010;29:321–6, Blood Purif. 2011;32:310–6] showed that PMX-DHP treatment eliminated activated neutrophils and humoral factors, including matrix metalloproteinase-9 and HMGB-1, which are relevant to an improved P/F ratio, from the blood circulation in patients with acute exacerbations of IPF. Furthermore, Oishi et al. reported significantly decreased serum levels of cytokines, including IL-9, IL-17, PDGF, and prominently, IL-12 and VEGF, after PMX-DHP therapy. Additionally, improved pulmonary oxygenation after PMX-DHP was found to correlate with the quantity of VEGF eluted from PMX-fiber cartridges.

Therefore, we have added the following text to the Discussion section: “The mechanism by which PMX-DHP therapy affects RPIPs has not been fully elucidated. However, several reports have discussed the mechanism of the PMX-DHP. Abe et al. [18, 19] showed that PMX-DHP treatment eliminated activated neutrophils and humoral factors, including matrix metalloproteinase-9 and HMGB-1, which are relevant to an improved P/F ratio, from the blood circulation in patients with acute exacerbations of IPF. Furthermore, Oishi et al. reported prominent decreases in the serum levels of vascular endothelial growth factor (VEGF) and IL-12 after PMX-DHP therapy, and observed a correlation between improved oxygenation after PMX-DHP therapy and the quantity of VEGF eluted from PMX-fiber cartridges [35].” (page 18, line 12).

Methods

1. At present there is no control arm where patients did not receive PMX-DHP. Is it standard of care in your institution to treat all patients with RPIP in such a fashion. It would be valuable to have a control group of CADM related RPIP without

(Response)

Thank you for your comments.

CADM-associated RPIPs are resistant to combination therapy with high-dose corticosteroids and immunosuppressive agents. Therefore, we have treated all patients with CADM-associated RPIPs with PMX-DHP therapy since 2008. Additionally, we could not perform a comparison to patients without hemoperfusion because the conventional therapy administered up to 2007 might have differed from the present conventional therapy used in this study in terms of the choice of immunosuppressant. In the future, a larger prospective cohort study should be conducted to evaluate the efficacy of PMX-DHP for CADM-associated RPIPs.
Conclusion

1. I think the major conclusion of this article should be that the RPIP in anti-MDA-5 CADM is associated with very poor prognosis. Feel the scope of the article should be directed at this rather than the PMX-DHP non-effect.

(Response)

Thank you for these important comments.

Per your suggestion, we have changed the relevant sentences in the Abstract and Conclusion sections.

In the Abstract section:

“Conclusion: CADM-associated RPIPs with anti-MDA-5 antibody is associated with a very poor prognosis. A higher P/F ratio and SP-D level, lower LDH and ferritin levels, higher platelet counts, and anti-MDA-5 antibody negativity are important prognostic markers in patients with CADM-associated RPIPs treated with PMX-DHP.” (page 4, line 4).

In the Conclusion section:

“CADM-associated RPIPs with anti-MDA-5 antibody is associated with a very poor prognosis. In CADM-associated RPIPs treated with PMX-DHP, higher P/F ratio, platelet counts in peripheral blood samples, and serum SP-D levels, lower serum LDH and ferritin levels before PMX-DHP, positivity for anti-ARS antibodies, and negativity for anti-MDA-5 antibody indicate a favorable prognosis. Further studies are needed to establish the effect of PMX-DHP therapy and develop better therapeutic management for patients with CADM-associated RPIPs.” (page 23, line 5)

Minor:

Background

1. Need reference for the statement that CADM patients frequently develop RPIP

(Response)

Thank you for this comment.

This reference is cited in the Background section as follows: “As reported extensively in Asia, patients with CADM often develop RPIPs with acute respiratory failure [2]” (page 5, line 9).

2. Anti-tRNA synthetase antibodies are commonly associated with fibrotic ILD. Why was a similar association with acute respiratory failure or acute exacerbation not observed?

(Response)

Thank you for this comment.

Interstitial lung disease with anti-ARS antibody positivity is usually associated with a chronic disease course. However, Hozumi et al. reported a significantly lower frequency of acute/subacute ILD onset with anti-ARS-antibody positivity vs. anti-MDA-5 antibody, although 42.3% of the patients with anti-ARS-antibody-associated interstitial pneumonia exhibited acute/subacute ILD onset [Respiratory Medicine 2016;121:91–99]. Therefore, we studied both RPIPs with anti-ARS antibodies and with anti-MDA-5 antibodies. Referral bias may have caused our study group to comprise patients with more severe or complicated disease; thus, the results of our study might not be generally applicable to patients in other settings.

Methods

1. Describe in more detail the protocol used for PMX-DHP. The number of sessions, frequency, etc.

(Response)

Thank you for this comment.

We have modified the following sentences to provide further detail: “Direct hemoperfusion was performed at a flow rate of 80–100 mL/min for 4 hours per day. PMX-DHP therapy was performed once daily on two successive days. Nafamostat mesylate (Torii Pharmaceutical Co. Ltd., Tokyo, Japan) was used as an anticoagulant” (page 10, line 13).

Results

1. Should describe whether patients were treated with lung protective strategy, were antibiotics empirically prescribed as per the recommendation for AE-IPF?

(Response)
Thank you for this comment.

We have added the following sentences to the Methods section: “Conventional treatments, including the systemic administration of high-dose corticosteroids, empirical antibiotics, and/or immunosuppressive agents such as cyclophosphamide, tacrolimus, and cyclosporine, were initiated before PMX-DHP therapy” (page 11, line 1) and “The treatment of patients requiring ventilator management was based on a lung-protective strategy, according to previous reports [31, 32].” (page 11, line 8).

2. Numerical difference in LDH levels does not appear to be clinically relevant between survivors and non-survivors in comparison to ferritin. Do the authors agree?

(Response)

Thank you for this comment.

We have reviewed the data and confirm its accuracy.

3. Why is it relevant that serum LDH correlate to serum ferritin? Serum ferritin is not a validated biomarker for ILD flares. Should be correlated to clinical outcomes.

(Response)

Thank you for this comment.

Although serum ferritin is not a validated biomarker for other ILD flares, Gono et al. reported a close correlation between increased serum ferritin levels and acute interstitial lung disease associated with dermatomyositis [Rheumatology 2010;49:1354–60]. In that study, the serum ferritin levels were significantly higher in patients with DM with acute/subacute interstitial pneumonia than in those with DM but without acute/subacute interstitial pneumonia. The serum LDH level reflects the ILD activity. Therefore, we examined the relationship between serum LDH and ferritin levels in this study.

4. Need to introduce what HMBG-1 is earlier in the manuscript

(Response)

Thank you for the kind comments.
Per your recommendation, we have added the following sentences to the Background section: “Abe et al. [18, 19] demonstrated that PMX-DHP treatment eliminated activated neutrophils and humoral factors, including matrix metalloproteinase-9 (MMP-9) and high-mobility group box protein 1 (HMGB-1), from the blood circulation of patients with acute exacerbations of IPF. These factors contribute to acute lung inflammation by inducing the accumulation of neutrophils and production of proinflammatory cytokines in the lung [20], both of which are relevant to an improvement of the P/F ratio.” (page 7, line 1).

The following sentences were deleted from the Discussion: “Abe et al. [27, 28] showed that PMX-DHP treatment in patients with acute exacerbations of IPF eliminates from the circulating blood activated neutrophils and humoral factors including matrix metalloproteinase-9 and HMGB-1, which are relevant to improvement of P/F ratio.” (original manuscript: page 16, line 4) and “HMGB-1 contributes to acute lung inflammation by inducing the accumulation of neutrophils and production of proinflammatory cytokines in the lung [36]” (original manuscript: page 19, line 5).

5. I am a little confused as to the relevance of comparing non-validated biomarkers in LDH, ferritin, and platelet count to the anti-MDA-5 status. What is the value of making this comparison given its clinical relevance and the very small sample size used.

(Response)

As noted, the importance of the relationships among these clinical parameters was unclear in patients with CADM-associated PRIPs, given the small sample size. However, as described above, serum LDH and ferritin levels in CADM have gradually garnered attention as biomarkers of disease activity. Therefore, we compared the levels of these biomarkers with other clinical findings. We have provided a description of the limitations of our study in the Discussion.

Discussion

1. Results suggest that anti-MDA-5 it is not only an important prognostic marker but an important pre-disposing biomarker. Are patients with CADM more predisposed to being MDA-5 positive

(Response)

Thank you for this comment.

According to a previous report, anti-MDA-5 antibody can be detected in patients with DM (11%–26%) or CADM (50%–73%) [Curr Rheumatol Rep 2012;14:264–74]. Anti-MDA-5
antibody has been closely associated with DM-associated acute progressive ILD and with a poor prognosis [Rheumatology 2010;49:433–40].

2. Far to much text in the conclusion regarding the potential value of non-specific biomarkers

(Response)

Thank you for this comment.

Per your suggestion, we deleted the following sentences from the Discussion section:

“Cluster of differentiation (CD) 163-positive activated macrophages are involved in muscle and lung inflammation in patients with PM/DM, in whom the number of macrophages and serum levels of soluble CD163 are significantly higher than in healthy individuals [30, 31]” (original manuscript: page 17, line 9).

“However, there were no significant differences in serum SP-D levels between the anti-MDA-5 positive and negative groups. Serum SP-D levels are correlated with the extent of alveolitis, but not with the progression of fibrosis [34]” (original manuscript: page 18, line 14).

“Elevated HMGB-1 levels are evident in patients with sepsis [37], acute lung injury [38], and human autoimmune diseases such as systemic lupus erythematosus [39] and rheumatoid arthritis [40]” (original manuscript: page 19, line 9).

Again, thank you very much for your comments and suggestions.

Dr. Chung-Tat Lun (Reviewer 2):

We are grateful to Dr. Chung-Tat Lun for providing critical comments and useful suggestions that have helped us to improve our paper. As indicated in the following responses, we have taken all of these comments and suggestions into account in the revised version.

Limitation:

1. Small sample/retrospective.

It is difficult to recruit patients with rare condition for a prospective study, but the small sample size of only 14 patients would definitely be prone to bias and type 2 error.

(Response)
Thank you for this comment.

As you mentioned, this study might be affected by various biases and type 2 errors because of the small sample size and retrospective design. Therefore, in the Discussion we described the limitations as follows: “Some negative or positive associations in the statistical analyses may have been due to the inadequate power afforded by the small sample size.” (page 22, line 5)

2. There was no predefined protocol for the choice of immunosuppressant and no data about the dosage of pulse steroid therapy, although there is no significant difference between survivor and non-survivor with regards to the choice of immunosuppressant.

(Response)

Thank you for this important comment.

All patients received steroid pulse therapy (methylprednisolone 1000 mg/day for three consecutive days), followed by tapering doses of prednisolone. Immunosuppressive agents were selected according to the attending physician’s judgment.

We have added the following sentences to the Methods section: “Conventional treatments, including the systemic administration of high-dose corticosteroids, empirical antibiotics, and/or immunosuppressive agents such as cyclophosphamide, tacrolimus, and cyclosporine, were initiated before PMX-DHP therapy. All patients received steroid pulse therapy (methylprednisolone 1000 mg/day for three consecutive days), followed by tapering doses of prednisolone with or without a cyclophosphamide pulse (500 mg/m2 every 3–4 weeks) or cyclosporine (2–3 mg/kg/day followed by adjustment to trough levels of 100–150 ng/ml), or both. Tacrolimus (0.075 mg/kg/day followed by adjustment to trough levels of 5–10 ng/ml), instead of cyclosporine, was also added to the regimen.” (page 10, line 16).

3. The primary outcome is to identify the risk factors for mortality in patients with hemoperfusion. The risk factors include presence of anti-MDA5 antibodies, low PF ratio, low platelet, elevated LDH, low SP-D and higher ferritin. Recently, there has been increasing evidence that the presence of anti-MDA5 antibodies is associated with poor outcome and presence of anti-MDA5 antibodies is associated with high ferritin and low SP-D. [satoshi I. Interstitial lung disease in clinically amopathic dermatomyositis with and without anti-MDA-5 antibody: to lump or split. BMC pulm Med 2015; 15: 159] On the other hand, it is not possible to perform multivariate analysis in such small sample size, to assess if ferritin/ SP-D level are confounders.
The low PF ratio in non-survivor may suggest they are suffering from more severe disease. I have some interest to know if the severity scores, e.g. APACHE, were similar between survivors and non-survivors.

The platelet counts were lower in the non-survivors, thought the median platelet were within normal range. I am curious what is the proportion of the patients diagnosed with thrombocytopenia in each group and the cause e.g. DIC identified in thrombocytopenic patients. You may check if any deranged clotting profile or low fibrinogen.

(Response)

Thank you for these important comments.

Although non-survivors exhibited significantly lower P/F ratios, there were no significant differences in APACHE II scores between the survivors and non-survivors. We added the result of APACHE II score in revised Table 2 and Table 3. Furthermore, we added the following sentence to the Results section: “The baseline APACHE II scores and HRCT scores did not differ between the groups.” (page 14, line 7).

The platelet counts were also lower among non-survivors, although only one patient in this group exhibited accompanying DIC. Three non-survivors had accompanying thrombocytosis. None of the survivors exhibited either accompanying condition.

We added the following sentence to the Results section: “The platelet counts were lower among non-survivors, and only one subject in this group exhibited accompanying disseminated intravascular coagulation (DIC), while three patients exhibited accompanying thrombocytosis. None of the survivors exhibited accompanying DIC and/or thrombocytosis (data not shown).” in the Results section. (page 14, line 13).

4. Hemoperfusion were well known to lead to decrease in platelet, white cell, HMGB-1 in septic patients. However the findings may not be translated to a better clinical outcome. In the study, the white cell and platelet counts decreased together with HMGB-1 level but the PF ratio did not improve. On contrary, a case series by the same authors showed improvement in PF ratio and good clinical outcome with hemoperfusion, and I am curious whether if any of the three cases had anti-MDA-5 antibodie. [Ichiyasu H. Favorable outcome with hemoperfusion of polymyxin B-immobilised fiber column for rapidly progressive interstitial pneumonia associated with clinically amyopathic dermatomyositis: report of three cases. Mod Rheumatol 2014; 24 (2): 361–5]

(Response)

Thank you for these comments.
The three cases from our previous study were resistant to high-dose corticosteroid and immunosuppressive agent therapy and required additional PMX-DHP treatment, although all survived [Mod Rheumatol 2014;24:361–5]. Per our later examination, these three cases were not anti-MDA-5 antibody positive. Two of the three patients expressed anti-ARS-antibodies, whereas the third lacked any antibodies. In our study, patients who expressed anti-MDA-5 antibodies had a very poor prognosis.

5. The effect of hemoperfusion versus conventional therapy is not well-investigated. The authors were experienced with 14 dermatomyositis patients treated with hemoperfusion. Maybe the authors can compare the survival of patients with and without hemoperfusion.

(Response)

Thank you for these comments.

CADM-associated RPIPs are resistant to combination therapy with high-dose corticosteroids and immunosuppressive agents. Therefore, we have treated all patients with CADM-associated RPIPs with PMX-DHP therapy since 2008. Additionally, we could not perform a comparison to patients without hemoperfusion because the conventional therapy administered up to 2007 might have differed from the present conventional therapy used in this study in terms of the choice of immunosuppressant. In the future, a larger prospective cohort study should be conducted to evaluate the efficacy of PMX-DHP for CADM-associated RPIPs.

Again, thank you very much for your comments and suggestions.

Dr. Ademola Fawibe (Reviewer 3):

We are grateful to Dr. Ademola Fawibe for providing critical comments and useful suggestions that have helped us to improve our paper.

Tables and Figures: the authors should indicate significant p values with asterisk in order to make it easy for the readers to quickly interpret and understand variables with significant p values.

(Response)

Thank you for this important comment.

We have added the suggested asterisks to the revised Tables and Figures.

Again, thank you very much.