Author’s response to reviews

Title: Acute kidney injury induced by thrombotic microangiopathy in a patient with hemophagocytic lymphohistiocytosis

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Author’s response to reviews: see over
November 10th, 2015

Dear Editor-in-Chief, BMC Nephrology

Thank you very much for evaluating our manuscript. We are submitting a revised version, which incorporates suggestions made by the Reviewers. An item-by-item response to the Reviewers’ suggestions has been made. We also highlighted the changes made to the manuscript by using bolded text. We hope that the suggestions of the Reviewers are adequately addressed in the revised manuscript.

Manuscript Title: **Acute kidney injury induced by thrombotic microangiopathy in a patient with hemophagocytic lymphohistiocytosis.**

Best regards,

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Response to Reviewers

Reviewer: Xinping Tian

1. The author has presented us a rare but interesting case. Both HLH and TMA are not so common in clinic but happened to occur in a single patient. The diagnosis of these two conditions could be confirmed by clinical manifestations and the ancillary tests. The report of this case will be helpful for clinicians to think about TMA and HLH when a patient has the clinical pictures of acute kidney insufficiency and pancytopenia. However, if the authors could provide the NK cell count and blood soluble CD25 level would make the evidence for the diagnosis solid.

Answer) Thank you for your comment. We agree with you. Regarding the Reviewer’s comment, we have added reads for soluble interleukin-2 receptor levels and NK cell activity. The revised paragraph is as follows:

Page 5

The patient’s soluble interleukin-2 receptor level in serum was 5.2% (normal range 5-30%) and natural killer (NK) cells activity was decreased (NK cells activity 3.7%; normal range 6-29%).

Page 6

Finally, the patient was diagnosed with HLH based on fever, progressive pancytopenia, hyperferritinemia, splenomegaly, decreased NK cells activity and hemophagocytosis in BM and negative results on viral and autoimmune marker studies.
2. The author mentioned that the major cause of AKI in HLH patients was ATN. However, according to the report from Lancet, the most common cause of AKI in HLH patients was collapsing glomerulonephropathy. So please re-check the literature and discuss the divergence if there is any.

**Answer** Thank you for your comment. As for the Reviewer’s comment, the report from Lancet was reviewed. The literature reports 24 cases with renal involvement. There have been 18 out of 24 cases presenting with renal histopathology. We reviewed 18 cases. In 13 cases (72.2 %), this was in combination with tubular necrosis lesions. According to the report from Nature Review of Nephrology, AKI in HLH results from inflammatory or ischemic lesions of the renal tubules. The most frequent presentation of renal damage in HLH is acute tubular necrosis. According to a report from Kidney International, the only patient without impairment of renal function exhibited normal tubular histologic features. All the others (10/11, 90.9%) exhibited the same tubulointerstitial histologic pattern including tubular necrosis. Thus, we think that the most frequent presentation of renal damage in HLH is ATN.

References


Karras A: **What nephrologists need to know about hemophagocytic syndrome.** *Nat Rev Nephrol* 2009, **5**:329-336.

Reviewer: Jin-Shuen Chen

General comments:

1. Under the condition of thrombocytopenia, if this kind of patient needs to receive any invasive procedures, such as renal biopsy, the risk of bleeding should be considered. So, the authors need to provide the data of PT/PTT and whether any methods of bleeding prevention were used on the patient. That will help readers follow their strategies easily.

**Answer** Thank you for your comment. As for Reviewer’s comment, we provided the data on PT/PTT which was within normal limits but the patient showed thrombocytopenia. We used prophylactic transfusion before kidney biopsy. The revised paragraph is as follows:

Page 6

PT times (PT INR, 1.13) and aPTT times (aPTT, 23.3 seconds) were within normal limits. But complete blood count showed thrombocytopenia. We used prophylactic transfusion of platelets in preparation for a kidney biopsy that could cause bleeding.

2. Cytotoxic therapy for the patient is very important information, so the actual dose of drugs should be shown on either Table 2 or in context.

**Answer** Thank you for your comment. In addressing the Reviewer’s comment, we have included the actual dose of the drugs in context. The revised paragraph is as follows:

Page 6
At this time, we planned 6 cycles of cytotoxic therapy comprised of dexamethasone, etoposide at 100 mg/m² and cyclosporine at 200 mg/day for the treatment of HLH based on the HLH-2004 protocol [7]. Dexamethasone was started at 10 mg/m² for 2 weeks, and then reduced to 50% of initial dose every 2 weeks. As etoposide is cleared by both renal and hepatic routes, dose adjustment of etoposide based on renal function is recommended for the HLH-2004 protocol. We started at etoposide 100 mg/m² with dose reductions of 25% based on the patient’s renal function.

3. For the issue of acute renal failure, the picture of the tubular region should be included in Figure 3A.

Answer) Thank you for your comment. For Reviewer’s comment, we have added Figure 3B which is included the tubular region. The revised paragraph is as follows:

Page 6

Tubules revealed focal moderate atrophy and loss with interstitial fibrosis (Figure 3B).

Page 16

Figure Legends

Figure 3. Kidney biopsy findings. A. Light microscopic findings show capillary lumens filled with fragmented red blood cells and platelet aggregates (H&E stain, ×400). B. Light microscopic findings show tubules reveal focal moderate atrophy and loss with interstitial fibrosis (H&E stain, ×200). C. Electron microscopy findings show focal effacement of podocyte foot processes. There are no electron-dense deposits (Original magnification, ×5000).
4. Why does the ferritin level increase with treatment?

Answer) Thank you for your comment. Ferritin levels at 35 days decreased compared to 25 days with treatment. After the 4th cycle of etoposide treatment, the patient suffered neutropenic fever due to vancomycin-resistant enterococci infection, which was successfully treated with linezolid and recombinant human granulocyte colony stimulating factor. Serum ferritin has been widely accepted as an acute-phase reactant and is nonspecifically elevated in a wide variety of inflammatory states including infection. Thus, we thought the cause of elevation of ferritin levels at 55 days was neutropenic fever due to the vancomycin-resistant
enterococci infection. Ferritin levels at 185 days decreased compare to 55 days. We included the ferritin levels at 185 days (Figure 1).

The revised figure 1 are follows:

![Graph showing body temperature and related treatments]

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<th>ALT (IU/L)</th>
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5. In Table 1, the authors summarized and analyzed the similar cases; this table should be included in the Discussion.

**Answer** We appreciate your comment. And for the Reviewer's comment, the revised paragraph is as follows:

Page 9
Clinical features in renal TMA in HLH patients are summarized in Table 1. AKI was present in 5/5 cases and oliguria in 2/5 cases. Proteinuria and microscopic hematuria was present in 5/5 and 4/5 cases, respectively. Our case showed generalized edema, proteinuria and hematuria compatible with nephritic syndrome. Nephritic syndrome associated with renal TMA in HLH is more frequent than nephrotic syndrome. Dialysis therapy was required for two patients. Four patients recovered and death occurred in one case.

Minor comments:

1. The article is readable with only a few minor diction errors. e.g. P8, para2, line16, “TMA in HLA”, not HLA, is HLH P15, Figure 3, H&E stein, not stein, is stain.

Answer) Thank you for your comment. From Reviewer’s comment, the revised paragraph is as follows:

Page 9

Nevertheless, **TMA in HLH** has been rarely reported maybe because diagnostic biopsy has been rarely done in HLH patients.

Page 16

Figure 3. Kidney biopsy finding. A. Light microscopic findings show capillary lumens filled with fragmented RBCs and platelet aggregates (H&E stain, ×400).

2. Use of abbreviations: when using abbreviations, you should give the complete phrase at the first use. And some abbreviations should be presented clearly in the text. For example, CRP,
LDH, HCV, TMA, AKI... should be corrected. Check all abbreviations and correct similar problems.

Answer) Thank you for your comment. We have checked the abbreviations and corrected similar problems. The revised abbreviations are as follows:

Page 10

Abbreviations

AFB: acid-fast bacillus; AKI: acute kidney injury; aPTT: activated partial thromboplastin times; ATN: acute tubular necrosis; BM: bone marrow; CT: computerized tomography; HD: hemodialysis; HLH: hemophagocytic lymphohistiocytosis; HUS: hemolytic uremic syndrome; MAHA: microangiopathic hemolytic anemia; NK: natural killer; PT: prothrombin times; TMA: thrombotic microangiopathy; TNF: tumor necrosis factor; TTP: thrombotic thrombocytopenic purpura.

3. Nephrotic syndrome associated with hemophagocytic lymphohistiocytosis has been well discussed in previous literatures. Please well discuss if this patient has nephrotic syndrome or nephritic syndrome.

Answer) Thank you for your comment. According to the report from Kidney International, nephrotic syndrome was associated with HLH in 63.6% of cases. Renal TMA in HLH has rarely been reported. There have been two of eleven cases (18.2%) of HLH showing renal TMA. The clinical features in renal TMA in HLH patients are summarized in Table 1. Proteinuria and microscopic hematuria was present in 4/5 cases. Our case showed generalized edema, proteinuria and hematuria compatible with nephritic syndrome. We considered the
pathogenesis of renal TMA in HLH patients and postulate that these inflammatory processes may cause severe vascular endothelial cell injury and affect the filtration capabilities of the glomerulus. Therefore, we thought that nephritic syndrome associated with renal TMA in HLH is more frequent than nephrotic syndrome. For Reviewer’s comment, the revised paragraph is as follows:

Page 9

Clinical features in renal TMA in HLH patients are summarized in Table 1. AKI was present in 5/5 cases and oliguria in 2/5 cases. Proteinuria and microscopic hematuria was present in 5/5 and 4/5 cases, respectively. Our case showed generalized edema, proteinuria and hematuria compatible with nephritic syndrome. Nephritic syndrome associated with renal TMA in HLH is more frequent than nephrotic syndrome. Dialysis therapy was required for two patients. Four patients recovered and death occurred in one case.

Reference


4. Figure 1 did not show triglyceride and total cholesterol.

Answer) Thank you for your comment. We provide the data of triglyceride and total cholesterol. The revised Figure 1 is as follows:
5. In this patient, authors said hepatitis C antigen was positive but AST and ALT levels were normal, which suggested that HCV was in an inactive state. Do you check any anti-HCV antibody or RNA in this patient?

**Answer** Thank you for your comment. We checked anti-Hepatitis C virus (HCV) antibody. Anti-HCV antibody was positive and HCV real time polymerase chain reaction was 8327 IU/ml. The revised paragraph is as follows:

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Page 5
Anti-Hepatitis C virus antibody was positive but alanine transaminase and aspartate transaminase levels were normal, which suggested that Hepatitis C virus was in an inactive state.

6. Some reference forms were not correct. Please recheck

**Answer**) Thank you for your comment. From Reviewer’s comment, we corrected the reference forms.

7. The language needs some improvement. Please find a native English speaker to revise the language for you.

**Answer**) Thank you for your comment. A native English speaker has subsequently edited our manuscript.
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Acute kidney injury induced by thrombotic microangiopathy in a patient with hemophagocytic lymphohistiocytosis

Manuscript Authors:
Myoung Nam Bae

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Yours truly,

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