Author's response to reviews

Title: Renal thrombotic microangiopathy and podocytopathy associated with the use of carfilzomib in a patient with multiple myeloma

Authors:

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Author's response to reviews: see over
September 8, 2014

Dear Dr. Henderson:

Thank you very much for reviewing our manuscript MS: 6434658671262973 entitled “A case of renal thrombotic microangiopathy associated with the use of Carfilzomib following autologous stem cell transplantation for multiple myeloma” and for giving us the opportunity to respond to your Editorial Board in reference to the Decision letter-email submitted to us on June 9th, 2014. We have made an effort to address every concern raised by the referees. In our view, the changes made have significantly improved the quality of the manuscript and we certainly thank you and your panel of reviewers for the insightful comments. We hope that you find the revised version of improved quality and suitable for publication. An item-by-item rebuttal letter, a marked manuscript file and an unmarked manuscript file are included in this resubmission.

Sincerely,

Liliane Hobeika, MD
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RESPONSE TO THE REVIEWERS:

Editorial Comments:
This is an interesting case report describing a possible case of TMA associated with carfilzomib. In addition to the comments/suggestions of the reviewers, I would suggest the following:

1. A more complete description of the classic clinical, serological, and pathological features of TMA and those features that were and were not present in this case should be provided.

   Thank you for your comment. The requested information was added to the revised manuscript as below:

   The clinical features of TMA syndromes include microangiopathic hemolytic anemia, thrombocytopenia, and organ injury. The pathological features are vascular damage manifested by arteriolar and capillary thrombosis with characteristic abnormalities in the endothelium and vessel wall. Renal pathology in TMA is characterized by thickened capillary walls, occlusion of vascular lumens, fibrin deposition and endothelial separation with expansion of subendothelial zone. Our patient had TMA limited to the kidney with no systemic manifestations. Although his serum haptoglobin was slightly low, LDH and bilirubin were within normal range, and anemia and thrombocytopenia were chronic and preceded the clinical presentation (Table 1).

2. A table describing the patient's kidney function, paraprotein levels, and urinary protein during the course of his MM and treatment would be useful.

   Table 1 was edited to include UPEP monoclonal spike (M- spike) % and albumin %, proteinuria, serum M- spike and kidney function, at time of biopsy, 2 months before the biopsy and 2 months after the biopsy. The patient had serum free light chain ratio checked at the time of initiation of carfilzomib and demonstrated recurrence of multiple myeloma (around 6 weeks prior to kidney biopsy). Serum free light chain ratio was not checked thereafter. Instead, SPEP and UPEP were checked by the patient's hematologist.

3. The authors describe the initial clinical features of AKI, Htn, and proteinuria as occurring 6 weeks after chemotherapy, but then state that the worsening hypertension and proteinuria occurred a few weeks after receiving carfilzomib. The timing of this should be consistent throughout.

   We have clarified that the syndrome (proteinuria and HTN, no AKI) occurred 6 weeks after the initiation of carfilzomib in both portions of the manuscript.
Reviewer 1: Dr. Anthony Chang

Reviewer’s report:

_Hobeika and Velez describe a case of thrombotic microangiopathy (TMA) associated with carfilzomib after autologous stem cell transplantation. This report is confounded by several factors that are more likely to account for the finding of TMA._

Major Compulsory Revisions:
1. _More details about the kidney biopsy should be provided. Were there any thrombi in glomerular capillaries or arterioles by light microscopy? Could the possible arterial thrombus that was staining for fibrin, C1q, IgM and C3 be visualized on the H&E cryosections of the IF specimen?_  
   Thank you for your question. The missing details about the biopsy report were added to the revised manuscript. No thrombi were identified in the glomerular capillaries or arterioles. Immunofluorescence showed 4 out of 14 glomeruli globally sclerotic with one small artery that stained intensely for fibrin (Figure 2b), C1q, IgM and C3. The corresponding H&E stained cryosection showed thrombus in the small artery.

2. _Was there evidence of “myeloma” cast nephropathy or monoclonal immunoglobulin deposition disease? If these findings were absent, it should be stated._
   There was no evidence of myeloma cast nephropathy or monoclonal immunoglobulin deposition disease. The specimen was negative for linear deposition of IgG or kappa along the glomerular and tubular basement membranes. This information has been added to the revised manuscript.

3. _The pathologist should at least be acknowledged for providing figures 2a-c and probably should be included as a co-author._
   Excellent point and we regret the prior omission. We added Dr. Self, the pathologist at MUSC, as a co-author.

4. _In two of the largest kidney biopsy series by Chang A et al (Clin J Am Soc Nephrol 2007; 2: 1014-1023) and Troxell M et al (Mod Pathol 2008; 21:396-406), radiation and chemotherapy are both well known causes of TMA. Radiation therapy is not mentioned for this patient, but the chemotherapy (particularly cyclophosphamide) is the more likely cause of this patient’s TMA particularly the chronic features of duplication of the GBM (as seen in figure 2c). This needs to be acknowledged. Many of the patients in these 2 series had similar timeframes when their symptoms presented as the current case report. The timing of the administration of carfilzomib is more likely a coincidence._
   We thank the reviewer for raising this concern. We carefully reviewed the 2 publications by Chang et al and Troxell M et al. We have added both informative
articles to our citations. The study by Chang in CJASN reported a comprehensive examination of renal pathologies occurring in relationship with hematopoietic stem cell transplantation (HSCT). In their 20-patient series, 3 cases of TMA were reported, 2 allogeneic and 1 autologous HSCT. No description of a specific association with cyclophosphamide therapy was found in the manuscript. On the other hand, the 3 cases of TMA in that series correspond to the recognized association with allogeneic HSCT that has been mentioned in the Discussion of our paper. Similarly, in the paper by Troxell, 2 cases of TMA were reported in a 15-patient series of renal pathologies occurring after HSCT, none of which were reported to receive cyclophosphamide. In fact, we are not aware of a particular association of cyclophosphamide with renal TMA. We would be extremely grateful if the reviewer could provide us with a reference describing it. Coincidence is in the realm of possibilities. Consequently, we applied the Naranjo criteria to conduct a rigorous examination of the association between the clinical syndrome and the drug exposure, which was included in the opening paragraph of the Discussion. Therefore, we consider that coincidence is an unlikely explanation.

5. P.7, Conclusion – Therefore, the 3rd point that “no alternate explanation was plausible” is not accurate. The authors even acknowledge (at the end of page 9) that “the long-standing history of essential hypertension could be responsible of some of the histologic findings...,” but they dismiss this possibility by simply restating their hypothesis.
We have deleted the sentence (“no alternate explanation was plausible”) to eliminate the inconsistency and soften the tone of our statement, for a more balanced conclusion.

6. Discontinuation of carfilzomib is unlikely to reverse the mesangiolysis or duplication of the GBM, which are the features of TMA. The serum creatinine did not improve after discontinuation of carfilzomib.
The patient’s kidney function had been stable for the past 1.5 years, since his 2nd HSCT (baseline serum creatinine around 2 mg/dL). Post carfilzomib he developed poorly controlled hypertension and worsening proteinuria but his kidney function remained stable. We did not expect his kidney function to significantly improve after stopping the drug. However, a modest (19%) decrease from 2.1 to 1.7 mg/dL was indeed observed. Because the patient was not re-biopsied, we cannot comment of reversal of mesangiolysis or duplication of the GBM. However, discontinuation of carfilzomib led to partial improvement in proteinuria and blood pressure control.

7. The patient ultimately succumbed to myeloma and carfilzomib may have been his only hope to treat the myeloma. Was any other therapy given after discontinuation of carfilzomib? Was there a consideration to re-administer carfilzomib when there was only a modest improvement in proteinuria? The
authors should be careful, because this portion of the manuscript currently gives the impression that the myeloma was not adequately treated. This may have been a joint decision with the patient but that should be a bit more clear.

As suspected by the reviewer, the patient and the team of hematologist took a joint decision of not pursuing further treatment due to poor quality of life and dismal prognosis. Nevertheless, he was kept on thalidomide until the day of his death. We have added a sentence to describe this issue and eliminate the potential perception of inadequate therapy.

8. Were there serial quantitations of the paraprotein before and after discontinuation of carfilzomib? Was the improvement in proteinuria due to a decrease in the paraprotein?

Proteinuria improved after stopping carfilzomib but monoclonal spike (M-spike) on UPEP got worse due to worsening MM. Please refer to Table 1. The paraprotein did not improve after stopping carfilzomib, it got worse. Total amount of proteinuria improved from 2.6 g to 1 g per 24 hour.

9. It may be true that bortezomib and carfilzomib have anti-VEGF activity, but the exact contribution of this pathway is unclear and speculative. Citing reports of TMA in myeloma patients treated with bortezomib is not compelling, because these myeloma patients undergo many other therapies as previously mentioned that can cause TMA. The authors might consider reviewing the literature on kidney transplant patients that have been treated with bortezomib in the setting of antibody-mediated rejection.

We have reviewed the literature of bortezomib in the setting of antibody-mediated rejection and we were unable to identify cases of TMA. This has been acknowledged in the Discussion to recognize that the association requires further validation.

10. The authors are very focused on creating a compelling argument that carfilzomib caused their patient’s TMA. However, what lesson can be learned about treating future patients like this one? Would they treat this patient differently if confronted with the same situation in the future?

We have added a final statement suggesting that: “clinicians should be aware of the possibility of an association between exposure to carfilzomib and the development of a clinical syndrome of worsening proteinuria and uncontrolled HTN and pathological evidence of renal TMA. Discontinuation of the drug should be considered only after careful evaluation of risks and benefits of the chemotherapy and the prognosis of the existing malignancy.”

Minor Essential Revision:
1. page 4 – the authors state that “bortezomib has also been reported to be associated with TMA.” There is another mention in the conclusion, references #15 and 16 should be added each time this is mentioned.

Done.
Reviewer 2: Dr. Riyaj Kasekar

Reviewer's report:

• **Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)** –

  1. **Mention values in SI units in brackets alongside the conventional units e.g. \( \mu \text{mol/L} \) for serum creatinine.**
     Done.

  2. **Page 6 line 121 – replace ‘paleness’ with ‘pallor’**.
     Changed.

• **Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)**

  3. **Please use the subheading ‘Discussion’ between lines 164 and 165 on page 8.**
     We corrected the title.

  4. **The abrupt rise and subsequent improvement in the proteinuria is congruent with the patient’s blood pressure control and could be solely explained by that.**
     We recognized that the change in proteinuria could be solely explained by the exacerbated hypertension. However, this fact does not negate that the argument that carfilzomib was the likely triggering factor to incite the exacerbated hypertension that resulted in demonstration of glomerular pathology.

  5. **Page 7 line 143 – EM appearance of diffuse podocytopathy – the authors should attempt to explain this finding in the discussion as it is not typically seen in renal thrombotic microangiopathy to my understanding.**
     Foot process effacement is indeed a feature of TMA that tends to be forgotten. We the authors admit not knowing it a priori. We have added a paragraph to elaborate more on the podocyte findings and have added the term podocytopathy to the title of the paper to highlight this feature.

• **Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached).**
6. Page 7 line 150-151. Authors fail to mention whether the laboratory parameters like LDH, Haptaglobin and platelet count improved weeks after stopping the offending agent as that will make a stronger case for the association of the drug and the adverse event.

As we mentioned in the original manuscript, we believe the patient had TMA limited to the kidney, not a systemic TMA. LDH was normal at time of biopsy and remained normal thereafter (information added to Table 1), although haptoglobin was slightly low at time of biopsy. Patient had chronic thrombocytopenia and anemia, his hemoglobin and platelet count did not change since his 2nd HSCT.

Reviewer 3: Dr.Deepika Jain

Reviewer's report:

Minor Essential revisions:

1. Page 8, line 174 "with only ____ case reports in the literature":
The sentence needs restructuring.
The sentence has been restructured.

2. Page 6, Line 124: addition of reference range for serum C3 and C4 is recommended
Added.

Discretionary revisions:

1. Page 5, line 99. "the serum creatinine stabilized at a level of 1.4mg/dl" Is there any laboratory results on proteinuria, urine protein electropheresis? Even if negative, these results could be worthwhile mentioning in the case presentation.
All the available laboratory values have been included. Serum creatinine of 1.4 mg/dL was after the 1st HSCT. Patient had a 2nd HSCT after which he developed CKD secondary to ATN with a new baseline creatinine around 2 mg/dL. Thereafter, he always had mild proteinuria of less than 0.5 g/day as mentioned in the case presentation and no M-spike until MM relapse and initiation of carfilzomib.

2. Page 6, Line 137: "An ultrasound guided percutaneous kidney biopsy was performed." Addition of information on kidney size, texture on ultrasound can be informative.
We have added the requested description.

3. Page 7, Line 148/149: "Eight weeks later, proteinuria slightly improved to 1 gram on a 24 hour urine collection." If there is a repeat creatinine at the time of improvement of proteinuria, it could be added here.
Serum creatinine at that time was 1.7 mg/dL. This information was added to the manuscript.