Author’s response to reviews

Title: Dasatinib-induced nephrotic syndrome in a patient with chronic myelogenous leukemia: a case report

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Author’s response to reviews:

Dear Editor:

We thank both referees for their careful reading our manuscript and giving useful comments. In response to the Referees' comments, we revised the manuscript BNEP-D-18-00230. We worked hard to incorporate your feedback, and hope that these revisions persuade you to accept our submission.

Sincerely,

Shoko Ochiai, MD

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Our responses to the referees' reports are as follows:

<Response for the first Referee>
Maike Büttner-Herold (Reviewer 1):

The authors describe an interesting case of a 40 year old patient developing nephrotic syndrome in the course of dasatinib therapy. Proteinuria greatly improved after switching therapy to another TKI and the authors argue that the renal side effects might be a consequence of VEGF inhibition. Additionally, fibrillary glomerular deposits were observed, which were interpreted as coincidental fibrillary glomerulonephritis. As the spectrum of TKI increases steadily it is very important to discover and understand the side effects induced by such drugs.

With regard to the coincidental fibrillary GN diagnosed by the authors, it would be very important to know whether Kongo-red staining was performed and negative to exclude amyloidosis. Moreover, it is not typical to find negative results of immunofluorescence, especially with regard to IgG and C3c, in fibrillary GN. It therefore would be helpful to stain the present case with an antibody specific for DNAJB9, which has been suggested as a specific marker of fibrillary GN (Nasr et al, Kidney Int rep. 2017 Aug 8; 3(1):56-64) to prove fibrillary GN.

Because the Congo red staining and immunofluorescent study for immunoglobulins and light chains were negative, we therefore considered that the present case wasn’t compatible with amyloidosis. Indeed, it is reasonable suggestion that Fibrillar glomerulopathy was as atypical as amyloidosis in the aspect of the immunofluorescent findings. According to your recommendation, we performed an additional immunehistochemical staining using anti-DNAJB9 antibody, which showed negative in the current case. The result suggested the possibility that the fibril we found in the EM study might not be derived from true Fibrillar glomerulopathy but
induced by TKI-associated endothelial injury. Therefore we revised the manuscript on the basis of above findings.

As the authors suggest that renal side effects induced by dasatinib might be a consequence of VEGF-inhibition, it would also be very interesting for us to know whether characteristic morphologic changes described by our group in the context of aVEGF-therapies were also detected in the present case (Pfister et al., Histopathology. 2018 Jul 17. Doi:10.1111/his.13716).

In the current case, the lesion which you pointed out characteristics in using aVEGF drug was absent except for endothelial cytoplasm expansion and double-contoured GBM. We thought that dasatinib developed nephrotic syndrome without TMA in current case. Further studies are necessary for understanding the characteristics in using dasatinib.

It should also be explained more clearly why the authors believe that fibrillary GN is coincidental and not associated with the therapy. So far it is very difficult to follow the reasons for this assumption.

We thought that it was incidental because we didn’t find any previous study regarding the relationship between Fibrillary glomerulopathy and TKI. Although the electron microscopic study showed fibrils in mesangial area suggesting those of Fibrillary glomerulopathy, considering with negative results of immunofluorescent study for immunoglobulins and immunehistochemical study for DNAJB9, it might not suggest true Fibrillary glomerulopathy. Moreover, it couldn’t be denied that the fibril was induced by unknown effect of Dasatinib. We revised the manuscript according to above description.

Page 4, line 17: do the authors really mean "myoblastic"?

We fixed this word to “leukemia-cell proliferation”.

Page 6, line 7: does "hepatitis" refer to viral hepatitis?

We thought that "hepatitis" mean viral hepatitis. So we modified “hepatitis” to “viral hepatitis”.

Page 6: As at a later time point it is stated that "hematuria almost disappeared" it would be helpful to include findings of urinalysis here.
We removed "almost" from this sentence because the patient's urinalysis revealed occult blood (-) after switching drug.

Page 7, line 11: why was it "possible nephrotic syndrome" and not nephrotic syndrome in the reported patients?
In accordance with your comment, we have changed this to "nephrotic syndrome".

Page 8, lines 15-17: can this sentence please be clarified.
We revised the sentence.

Page 9, line 6: maybe "document" should be replaced by "reports" and "dasanitib" is misspelled and should be "dasatinib".
We modified the word to what you suggested.

Page 15, line 6: in figure D also subepithelial fibrillary deposits are depicted.
Thank you for your suggestion. We revised figure legends as you suggested.

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Michiko Shimada, MD,PhD (Reviewer 2):
The authors presented a case of nephrotic syndrome caused by dasatinib. It is known that dasatinib often cause proteinuria. However, detailed mechanism remains unknown, and there are not many case reports with the findings of renal biopsy. Therefore, this case report would have a significant value for the readers.

The manuscript needs proofread for language corrections.

- Is there any relationship between endothelial damage and the deposition of the fibrils? Is it really just a coincidence?
We thought that it was incidental because no previous study regarding the relationship between FGN and TKI was available. However, considering with your and another reviewer’s concern, we performed additional immunostaining study using anti-DNABJ9 antibody, which was reported as a marker FGN, and it showed negative result in the current case. The finding suggests the possibility that the fibril we found in the EM study might not be derived from true FGN but induced by TKI-associated endothelial injury. Thus, we revised the manuscript on the basis of above description.

- p6 line4 platelet count of 1,320,000/μL→Is it correct?
  We corrected platelet count of 1,320,000/μL to 132,000/μL.

- Please indicate if there was any hematuria or abnormal urine casts.
  We added them at p6 line9.

- p8 line3 binds to the VEGF-2 receptor of cells→ Which kind of the cells do you mean here?
  We modified “cells” to “endothelial cells”.

- p8 line4 the cell's function→ cellular function?
  We modified the word to what you suggested.

- p8 line14 The electron microscopy study of our case showed diffuse effacement of the foot process that was due to massive proteinuria. Does it make sense?
  We revised the sentence.

- p8 line 15 endothelia → endothelial
  We corrected this misspell.
• Please use arrows or something to indicate swelling of the endothelial cells, and the fibrils in Fig1C and D.

• Please indicate original magnification in Fig1.

Thank you for your suggestion. We modified figure as you suggested.