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 have revised the manuscript BNEP-D-18-00230. We have worked hard to incorporate your feedback and used Editage (www.editage.jp) for English language editing. We hope that these revisions persuade you to accept our submission.

Sincerely,

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Our responses to the referees' reports are as follows:

<Response for the first Referee>

Reviewer #1: Many issues have been addressed in your revision, however, the issue of fibrillary deposits remains very confusing.

Major comments:

As EM looks very suggestive of amyloidosis it would be very helpful to show Congo red staining as well as DNAJB9 immunohistochemistry in the figure. Has Congo red staining been performed on sufficiently thick sections? Was AA-amyloidosis excluded by immunohistochemistry?

The theory that fibrils are derived from TKI therapy is very speculative and it is difficult to understand why this is presumed. Has by chance a follow-up biopsy been performed, which could show an effect on fibrillary deposits after cessation of TKI therapy? Why should subepithelial fibrils be induced by endothelial damage? Was the distribution of fibrils a focal finding?

Thank you for your useful suggestion. We performed Congo red staining with thick sections (4 and 8 μm), as well as AA-amyloid immunohistochemical staining, but these sections were negative. In addition, we compared the electron microscopic photographs of the current case with that of other amyloidosis cases, which suggests that the diameter of the deposition is larger than that of the amyloid (10-20 nm vs 8-10 nm). We think that these findings are not in favor of a diagnosis of amyloidosis.

We thought that the deposition is consistent with that of FGN based on the EM findings, but the negative results of the IF and anti-DNAJB9 IHC staining did not support the diagnosis of FGN; moreover, the fibril distribution was relatively focal. Therefore, we initially hypothesized an
association between the deposit and dasatinib-induced endothelial injury; however, we could not state this definitively because we did not perform a follow-up biopsy and could not find any previous clinical and basic scientific studies to support this association. Further case accumulation with detailed EM and IF study is needed to establish an association.

Therefore, we added the following sentences

in the Case presentation section: page 5, lines 16~19 and page 6, line 1

~, but their distribution was relatively focal. AA amyloidosis was deemed unlikely because of negative Congo-red staining and immunohistochemistry (Fig 2-A). In addition, negative immunohistochemistry for DNAJB9 did not suggest the diagnosis of fibrillary glomerulonephritis (Fig 2-B). We thought that these renal histological changes were caused by dasatinib, but the cause and diagnosis of fibril were not evident.

in the Discussion section: page 8, lines 8-11 and page 9, lines 2-4

In addition, we found a glomerular deposit (called fibrils) in the EM study, which was negative for a Congo-red staining, suggesting non-amyloid deposit. At first, we suspected the fibril may be consistent with a diagnosis of fibrillary glomerulonephritis (FGN), because the diameter of the fibril seen in this case was larger than that seen in amyloidosis (φ10-20 nm vs φ8-12 nm).

We found the fibril coincidentally, after performing an electron microscopy study. Considering these results, we could not clarify the cause of fibril and the association between the fibril and dasatinib-induced renal damage.

In accordance with the reviewer’s suggestion, we have also added the figures (Congo red and anti-DNAJB9 IHC staining).

Minor comments:
Moreover, the authors report diffuse effacement of podocyte foot processes, which is difficult to appreciate in the figure. Maybe this could be depicted more clearly.

The manuscript would also benefit from improvement of language.

Thank you for your suggestion. We have revised Figure 1 as you suggested and have had the manuscript language checked by a native speaker.

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<Response for the second Referee>

Reviewer #2: I assume that the authors responded appropriately to the reviewers comments. I would suggest that the authors present immunofluorescent study, Congo-red and DNAJB9 staining even if they are negative. I believe this case report can be improved by English proofreading.

Thank you for your suggestion. We have included a new Figure 2 to further illustrate Congo red staining and DNAJB9 immunohistochemistry as you suggested.

· p5 line19

fibrils found in the EM study is fibrillary glomerulonephritis (FGN) or caused by TKI.

→ fibrils found in the EM study is fibrillary glomerulonephritis (FGN) caused by TKI.

We have removed this sentence (from page 5, line 19) and have revised the manuscript regarding the diagnosis of FGN and the association between the deposit and dasatinib-induced endothelial injury.

· p8 line18

which is specific for FGN [23], was not stain, too.

→ which is specific for FGN [23], was not positive.

We modified the word to what you suggested.

· p9 line6
To the best of our knowledge, current report is the first case of,..

After careful consideration, we have removed line 6 on page 9.