Dear Editor,

Re: **84888**

Title: **Membranous nephropathy with systemic light-chain amyloidosis: remission after rituximab therapy, a case report and literature review**

Thank you very much for giving us an opportunity to revise our manuscript. We have revised the manuscript carefully according to the Editor's and Reviewers' comments and recommendations. Briefly speaking, the revisions included the following issues:

1. Regarding the clinical significance and impact of MN complicated with amyloidosis. MN combined with renal amyloidosis is rare, and whether it is two independent diseases or their pathogenesis has some connection is not clear. We have investigated it from both immune and inflammatory perspectives in the latest research, with details in the following paragraphs.
2. Regarding the relevant medical history or comorbidities. In middle-aged or elderly patients with nephrotic syndrome who do not respond to standard diuretic, antihypertensive, and urinary protein-lowering drugs, those with heart failure whose ejection fraction does not decrease, those with hypotension, macroglossia, and hepatomegaly, we would consider combined amyloidosis.
3. Regarding the treatment and adverse effects. Glucocorticoids, rituximab and chemotherapy were given after the renal biopsy. The patient had an abnormal liver function when receiving methylprednisolone at the beginning of treatment, which was then immediately discontinued and switched to prednisone, with no subsequent complaints or adverse effects. Neither intolerable adverse effects during rituximab treatment nor chemotherapy were observed.
4. Regarding the implications of this case. The presentation of our rare cases can increase clinicians' awareness about such conditions. Our case also provided a reference for the early diagnosis and treatment of such patients. Additionally, the pathogenesis of MN combined with renal amyloidosis is not fully understood and could be a point for subsequent scientific research.

5. Regarding the language of the manuscript. We have invited the native editor to revise the language of the manuscript. The language editorial certificate has been uploaded and it is hoped that the language will meet the publication requirements of your respected journal.

The response to each question or comment of reviewers was listed point-by-point in the attached pages. We hope the revised form is suitable for publication in your respected journal.

Yours sincerely,
Wenge Li

Answer to Reviewer 1

1. Can the authors provide more information about the clinical significance and impact of MN complicated with amyloidosis?

Answer: Thank you very much for your valuable suggestions, which make our manuscript more complete. MN combined with renal amyloidosis is rare, and whether it is two independent diseases or their pathogenesis has some connection is not clear from current studies. Kuroda et al[1] reported six cases of MN combined with rheumatoid arthritis-related amyloid A amyloidosis and suggested that the cause of MN in these patients may be related to the Disease-Modifying Antirheumatic Drugs. Veelken et al[2] suggested a possible association between rheumatoid arthritis and AL amyloidosis. On the one hand, persistent activation of the immune system by autoantigens was associated with an increased rate of cancerous transformation in B-lymphocytes[3], and on the other hand, precancerous clones were associated with inflammation. MN is an autoimmune disease. Recent research found that gene expression of inflammatory signalling pathways (like the IL-6 signalling pathway) was elevated in MN[4]. Inflammation may be another mechanism in the pathogenesis of MN[5]. However, whether MN is also associated with the development of AL amyloidosis is unknown. Khalighi et al[6] suggested that non-branching fibrils could disrupt the glomerular basement membrane, but there were few studies suggesting that MN may be caused by antigenic exposure following amyloid deposition. Therefore, the clinical significance of MN combined with amyloidosis needs to be explored by further studies.

Lines 278-294 (In the latest modified version): MN combined with renal amyloidosis is rare, and whether it is two independent diseases or their pathogenesis has some connection is not clear from current studies. Kuroda[1] reported six cases of MN combined with rheumatoid arthritis-related amyloid A amyloidosis and suggested that the cause of MN in these patients may be related to the Disease-Modifying Antirheumatic Drugs. Veelken[2] suggested a possible association between rheumatoid

arthritis and AL amyloidosis. On the one hand, persistent activation of the immune system by autoantigens was associated with an increased rate of cancerous transformation in B-lymphocytes[3], and on the other hand, precancerous clones were associated with inflammation. MN is an autoimmune disease. Recent research found that gene expression of inflammatory signalling pathways (like the IL-6 signalling pathway) was elevated in MN[4]. Inflammation may be another mechanism in the pathogenesis of MN[5]. However, whether MN is also associated with the development of AL amyloidosis is unknown. Khalighi[6] suggested that non-branching fibrils could disrupt the glomerular basement membrane, but there were few studies suggesting that MN may be caused by antigenic exposure following amyloid deposition. Therefore, the clinical significance of MN combined with amyloidosis needs to be explored by further studies.

2. Is there any relevant medical history or comorbidities that could be important in understanding the case?

Answer: Thank you for your advice. In middle-aged or elderly patients with nephrotic syndrome who do not respond to standard diuretic, antihypertensive, and urinary protein-lowering drugs, those with heart failure whose ejection fraction does not decrease, those with hypotension, macroglossia, and hepatomegaly, we would consider combined amyloidosis. In our case, a middle-aged male was admitted with nephrotic syndrome and found to be positive for serum PLA2R antibodies; hence, the patient was considered to have PLA2R-related MN. Although there were no obvious signs of involvement of other systems, such as macroglossia or heart failure, the patient was treated with supportive therapy with no effect. Furthermore, in view of the fact that it was his first time receiving a systematic consultation, he was screened with immunofixation electrophoresis and free light chain examination. Serum and urine immunofixation electrophoresis were positive for IgG-lambda-M protein, and serum free light chain-lambda was slightly higher. Finally, the patient was advised to undergo a renal biopsy and was diagnosed with MN combined AL renal amyloidosis.

Lines 225-236 (In the latest modified version): In middle-aged or elderly patients with nephrotic syndrome who do not respond to standard diuretic, antihypertensive, and urinary protein-lowering drugs, those with heart failure whose ejection fraction does not decrease, those with hypotension, macroglossia, and hepatomegaly, we would consider combined amyloidosis[7]. In our case, a middle-aged male was admitted with nephrotic syndrome and found to be positive for serum PLA2R antibodies; hence, the patient was considered to have PLA2R-related MN. Although there were no obvious signs of involvement of other systems, such as macroglossia or heart failure, the patient was treated with supportive therapy with no effect. Furthermore, in view of the fact that it was his first time receiving a systematic consultation, he was screened with immunofixation electrophoresis and FLC examination. Finally, we confirmed the diagnosis of amyloidosis with renal biopsy.

3. Was the renal biopsy performed before or after the patient started treatment?

Answer: Thank you for your suggestions, which make our manuscripts more accurate. Glucocorticoids, rituximab and chemotherapy were given after the renal biopsy. The patient was first admitted on December 24, 2020, and the renal biopsy was performed on December 30, 2020. Before the renal biopsy, he had only received supportive treatment. As soon as the diagnosis was established, a therapeutic program was created. From January 5, 2021, to February 9, 2022, the patient took 32 mg methylprednisolone once a day (then changed to 40 mg prednisone once a day due to abnormal liver function tests), with 1 g rituximab (three times successively) and support treatment; then, the patient's edema improved significantly, and prednisone was slowly and regularly reduced to 2.5 mg once daily for maintenance, without adverse effects or complications. Hematology advised continued monitoring of M-protein. At this stage, MN reached immunological remission but M-protein was slightly increased compared to the previous level. Thus, in the second stage, which was from February 9, 2022, to September 12, 2022, the patient was transferred to the hematology department for further treatment. He was advised to undergo autologous

hematopoietic stem cell transplantation but declined it for financial reasons. He eventually received CyBorD chemotherapy and completed 21 cycles of treatment without intolerable adverse effects or complications. During this period, he was treated with 2.5 mg prednisone as maintenance. Eventually, the patient was in complete remission with normal renal function.

Lines 128-129 (In the latest modified version): After assessing the risk of bleeding, renal biopsy was advised. The patient accepted and underwent a renal puncture biopsy on December 30, 2020.

Lines 156-169 (In the latest modified version): Before the renal biopsy, the patient had only received supportive treatment. As soon as the diagnosis was established, a therapeutic program was created. Treatment and follow-up were divided into two stages. From January 5, 2021, to February 9, 2022, the patient took 32 mg methylprednisolone once a day (then changed to 40 mg prednisone once a day due to abnormal liver function tests), with 1 g rituximab (three times successively) and support treatment. Notably, we examined the possibility of potential infections before proceeding with rituximab therapy to ensure safety. Then, the patient's edema improved significantly, and prednisone was slowly and regularly reduced to 2.5 mg once daily for maintenance, without adverse effects or complications. Later, the patient was advised to undergo autologous hematopoietic stem cell transplantation (ASCT) but declined it for financial reasons. He eventually received CyBorD chemotherapy and completed 21 cycles of treatment without intolerable adverse effects or complications. During this period, he was treated with 2.5 mg prednisone as maintenance.

4. Were there any complications or adverse effects associated with the treatment?

Answer: Thank you very much for your valuable suggestions, we have completed it in the revised manuscript. The patient had an abnormal liver function when receiving methylprednisolone orally at the beginning of treatment, which was then immediately

discontinued and switched to prednisone, with no subsequent complaints or adverse effects. He was thoroughly screened for possible infections prior to treatment with rituximab, which was confirmed to be safe before treatment, and no adverse effects occurred during treatment. In addition, chemotherapy was also administered with careful evaluation, and there were no intolerable adverse effects.

Lines 158-169 (In the latest modified version): From January 5, 2021, to February 9, 2022, the patient took 32 mg methylprednisolone once a day (then changed to 40 mg prednisone once a day due to abnormal liver function tests), with 1 g rituximab (three times successively) and support treatment. Notably, we examined the possibility of potential infections before proceeding with rituximab therapy to ensure safety. Then, the patient's edema improved significantly, and prednisone was slowly and regularly reduced to 2.5 mg once daily for maintenance, without adverse effects or complications. Later, the patient was advised to undergo autologous hematopoietic stem cell transplantation (ASCT) but declined it for financial reasons. He eventually received CyBorD chemotherapy and completed 21 cycles of treatment without intolerable adverse effects or complications. During this period, he was treated with 2.5 mg prednisone as maintenance.

5. What are the implications of this case for clinical practice or future research?

Answer: Thank you for your advice. We report a rear case of PLA2R-related MN combined with primary AL amyloidosis, with the kidney as the only involved organ. The presentation of rare cases can increase clinicians' awareness about such conditions. Renal amyloidosis combined with renal diseases such as MN is particularly easy to be missed and misdiagnosed. For middle-aged and old patients with nephrotic syndrome who consult the doctor for the first time, we suggest that protein electrophoresis, immunofixation electrophoresis, and free light chain tests can be completed routinely, with perfecting Congo red staining as far as possible in patients receiving renal biopsy,

to achieve early detection and early treatment. In this case, the combination of rituximab with glucocorticoids and CyBorD chemotherapy regimen showed good efficacy and provided a reference for the treatment of such patients. Additionally, the pathogenesis of MN combined with renal amyloidosis is not fully understood and could be a point for subsequent scientific research.

Lines 297-308 (In the latest modified version): We report a rear case of PLA2R-related MN combined with primary AL amyloidosis, with the kidney as the only involved organ. The presentation of rare cases can increase clinicians' awareness about such conditions. Renal amyloidosis combined with renal diseases such as MN is particularly easy to be missed and misdiagnosed. For middle-aged and old patients with nephrotic syndrome who consult the doctor for the first time, we suggest that protein electrophoresis, immunofixation electrophoresis, and FLC tests can be completed routinely, with perfecting Congo red staining as far as possible in patients receiving renal biopsy, to achieve early detection and early treatment. In this case, the combination of rituximab with glucocorticoids and CyBorD chemotherapy regimen showed good efficacy and provided a reference for the treatment of such patients. Additionally, the pathogenesis of MN combined with renal amyloidosis is not fully understood and could be a point for subsequent scientific research.

References

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