

*Letter to the Editor*

## **Rituximab therapy in nephrotic syndrome due to AH amyloidosis**

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**Key words:** *AH amyloidosis, nephrotic syndrome, B-cell lymphoproliferative disorder, rituximab*

**Running title:** *Rituximab therapy in AH amyloidosis*

**Abbreviations:** *AH: AH amyloidosis, AL: AL amyloidosis, CD: cluster differentiation, FLCs: free light chains, WBC: white blood cell*

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**Abstract**

We report a patient with AH amyloidosis associated with lymphoplasmacytic leukemia who has remained in a stable state of nephrotic syndrome for 17 months since commencement of cyclic rituximab therapy aimed at suppression of pathogenetic  $\gamma$  heavy chains. Free light chains in serum and CD20-positive cells in peripheral blood were useful as hematological markers in the patient. Rituximab might be a potent therapeutic option for AH amyloidosis associated with a B-cell lymphoproliferative disorder.

Immunoglobulin light chains sometimes cause primary systemic AL amyloidosis (AL), which has a poor prognosis due to the involvement of multiple visceral organs. This disease is the most common form among the systemic amyloidoses, and intensive chemotherapies, such as high-dose melphalan with autologous peripheral blood stem cell transplantation, have recently been established as a standard treatment. AH amyloidosis (AH) is another disorder ascribed to immunoglobulin-derived fibrillary protein deposits. The precursor protein in AH is immunoglobulin heavy chains. Because this disease is much less frequent than AL [1-10], the treatment strategy has not yet been established. In this report we describe a patient with AH associated with a B-cell lymphoproliferative disorder. The patient showed progressive nephrotic syndrome, but urinary protein excretion and serum albumin have remained stable for 17 months since starting the cyclic rituximab therapy. We focus upon the therapeutic usefulness of rituximab in AH.

## Case Report

A 61-year-old woman with progressive nephrotic syndrome was diagnosed as having AH based on biochemical and molecular analyses of amyloid fibrils extracted from the biopsied renal tissue as reported previously [8]. Briefly, the amyloid deposits were not immunoreactive for either anti-A $\lambda$  or anti-A $\kappa$  antibodies, and the amino acid sequence of extracted fibrils was EVQLLESGGDLVQPGGSLR and DNSENTLYLQMNSLR, which showed homology to the human immunoglobulin  $\gamma$  heavy chain variable region. Bone marrow biopsy showed infiltration of CD20-positive lymphoplasmacytoid cells producing pathogenetic  $\gamma$ -heavy chains. The patient received supportive treatment, such as diuretics and anti-hypertensive drugs, without specific chemotherapy at her request. Leg edema and general fatigue gradually worsened in parallel with a decrease in serum albumin. When she was admitted to our hospital 3 years after diagnosis, routine laboratory tests showed severe hypoalbuminemia (albumin: 1.8 g/dL, normal 4.2-5.1 g/dL) with proteinuria (daily excretion in urine: 8.0 g/day), anemia (hemoglobin: 7.6 g/dL, normal 12-17 g/dL), a decrease in creatinine clearance (53.3 mL/min, normal 80-130 mL/min) and an increase in white blood cells (WBC, 19,770/ $\mu$ L, normal 4,000-8,000/ $\mu$ L) mainly comprising CD20-positive lymphoplasmacytoid cells (79%). Serum creatinine was elevated slightly (1.11 mg/dL, normal 0.4-0.8 mg/dL), and blood urea nitrogen (19 mg/dL, normal 9-22 mg/dL) and liver function were within normal limits. Immunofixation demonstrated IgG $\kappa$ -type M-protein in serum and  $\kappa$ -type Bence Jones protein in urine. Free light chains (FLCs) in serum were 108.0 mg/L in  $\kappa$  (normal 3.3-19.4 mg/L) and 28.7 mg/L in  $\lambda$  (normal 6.5-26.3 mg/L), and the  $\kappa/\lambda$  ratio was 3.76 (normal 0.26-1.65). Bone marrow aspirates showed 13% CD20-positive lymphoplasmacytoid cells. There were no abnormal findings in either the electrocardiogram or echocardiogram suggestive of amyloid heart disease. Brain natriuretic peptide was slightly increased (31.3 pg/mL, normal <20 pg/mL) possibly because of excess of body fluid. Computed tomography demonstrated mild hepatosplenomegaly but no obvious lymphadenopathy.

To eliminate the pathogenetic lymphoplasmacytoid cells, rituximab was given at a dose of 375 mg/m<sup>2</sup> once a week for 3 cycles after obtaining informed consent. Mild infusion reactions, such as transient nausea and back pain, occurred only at the first administration of rituximab, and the patient did not show any other serious adverse events. The number of WBC rapidly became normal in conjunction with disappearance of CD20-positive lymphoplasmacytoid cells in peripheral blood and bone marrow (Fig. 1A). Maintenance treatment with rituximab at a dose of 375 mg/m<sup>2</sup> has been repeated at an interval of approximately 6 months. There were no serious adverse events ascribable to rituximab. After

the second maintenance therapy FLCs in serum were 94.0 mg/L in  $\kappa$  and 53.9 mg/L in  $\lambda$ , and the  $\kappa/\lambda$  ratio was 1.74. CD20-positive lymphoplasmacytoid cells could not be detected in either peripheral blood or bone marrow. The patient has remained in good general condition with a slight increase in serum albumin despite the persistence of leg edema for 17 months since starting the rituximab therapy (Fig. 1B). M-protein in both serum and urine is still detectable on immunofixation, but WBC in peripheral blood has stayed within the normal range (Fig. 1A).

## Discussion

AH in the present patient manifested as progressive nephrotic syndrome, which was uncontrollable by supportive treatment alone. M-protein produced mainly from the bone marrow acts as the precursor protein of amyloid in AH, and frequently causes glomerular deposits leading to nephrotic syndrome and/or renal dysfunction. To avoid worsening of nephrotic syndrome in the present patient, therefore, reduction of the circulating M-protein using chemotherapy was considered to be necessary. As plasma cell dyscrasia is the most common pathology of the bone marrow in AH as seen in AL [1, 2, 5, 6], chemotherapy targeting it has been reported to show a favorable outcome [5]. In the present patient, however, lymphoplasmacytic leukemia is the underlying disorder of AH. Because lymphoplasmacytoid cells in both peripheral blood and bone marrow were positive for CD20, we decided to employ rituximab for treatment of the present patient. This drug is a humanized chimeric monoclonal antibody capable of killing CD20-positive cells by complement-mediated cytotoxicity, antibody-dependent cellular cytotoxicity, and possibly direct induction of apoptosis [12, 13]. Lymphoplasmacytic leukemia in the present patient belongs to low-grade malignancy of B cells, in which the therapeutic efficacy of rituximab has already been confirmed [14, 15]. Another advantage of this drug is the rarity of serious adverse events except for infection. Because of her bad general condition with severe edema and anemia, the present patient was considered to be unsuited to other cytotoxic agents causing severe adverse effects, such as gastrointestinal symptoms and myelosuppression.

After starting the rituximab therapy, WBC and CD20-positive cells in peripheral blood were quickly decreased in the present patient. M-protein remained positive, but the FLC analysis showed an obvious decrease in serum  $\kappa$ -chains and the  $\kappa/\lambda$  ratio. As M-protein in the present patient was IgG $\kappa$ , lymphoplasmacytoid cells expressing CD20 on the cell surface probably produced pathogenetic  $\gamma$ -heavy chains as well as  $\kappa$ -light chains. Free heavy chains in serum were still unquantifiable, and instead of them FLC and an intact form of IgG $\kappa$  may be useful as clinical markers indicating the disease activity of AH [11, 16]. No obvious change was seen in serum albumin and urinary protein excretion of the present patient after the rituximab therapy possibly because of irreversible impairment of the kidneys. Transient decreases in urinary protein excretion after administration of rituximab may be due to renal damage following rapid disruption of massive leukemic cells. Considering that the patient had shown a chronic progressive course before starting the rituximab therapy, the absence of significant deterioration in serum albumin and urinary protein excretion was thought to be rather important. The present patient has been able to continue the rituximab therapy at intervals of 6 months without serious adverse events.

## Acknowledgement

This work was supported by a grant from the Intractable Disease Division, the Ministry of Health and Welfare, Amyloidosis Research Committee in Japan.



## References

1. Eulitz M, Weiss DT, Solomon A. Immunoglobulin heavy-chain-associated amyloidosis. *Proc Natl Acad Sci USA* 1990; 87: 6542-6546.
2. Solomon A, Weiss DT, Murphy C. Primary amyloidosis associated with a novel heavy-chain fragment (AH amyloidosis). *Am J Hematol* 1994; 45: 171-176.
3. Tan SY, Murdoch IE, Sullivan TJ, Wright JE, Truong O, Hsuan JJ, Hawkins PN, Peppys MB. Primary localized orbital amyloidosis composed of the immunoglobulin gamma heavy chain CH3 domain. *Clin Sci (Lond)* 1994; 87: 487-491.
4. Mai HL, Sheikh-Homad D, Herrera GA, Gu X, Truong LD. Immunoglobulin heavy chain can be amyloidogenic: morphologic characterization including immunoelectron microscopy. *Am J Surg Pathol* 2003; 27: 541-545.
5. Copeland JN, Kouides PA, Grieff M, Nadasdy T. Metachronous development of nonamyloidogenic lambda light chain deposition disease and IgG heavy chain amyloidosis in the same patient. *Am J Surg Pathol* 2003; 27: 1477-1482.
6. Yazaki M, Fushimi T, Tokuda T, Kametani F, Yamamoto K, Matsuda M, Shimojo H, Hoshii Y, Higuchi K, Ikeda S. A patient with severe renal amyloidosis associated with an immunoglobulin gamma-heavy chain fragment. *Am J Kidney Dis* 2004; 43: e23-28.
7. Kaplan B, Martin BM, Boykov O, Gal R, Pras M, Shechtman I, Saute M, Kramer MR. Co-deposition of amyloidogenic immunoglobulin light and heavy chains in localized pulmonary amyloidosis. *Virchows Arch* 2005; 447: 756-761.
8. Gono T, Yazaki M, Fushimi T, Suzuki T, Uehara T, Sano K, Kametani F, Ito N, Matsushita M, Nakamura S, Hoshii Y, Matsuda M, Ikeda S. AH amyloidosis associated with lymphoplasmacytic lymphoma secreting a monoclonal  $\gamma$  heavy chain carrying an unusual truncated D segment. *Am J Kidney Dis* 2006; 47: 908-914.
9. Nasr SH, Colvin R, Markowitz GS. IgG1  $\gamma$  light and heavy chain renal amyloidosis. *Kidney Int* 2006; 70: 7.
10. Miyazaki D, Yazaki M, Gono T, Kametani F, Tsuchiya A, Matsuda M, Takenaka Y, Hoshii Y, Ikeda S. AH amyloidosis associated with an immunoglobulin heavy chain variable region (VH1) fragment: a case report. *Amyloid* 2008; 15: 125-128.
11. Matsuda M, Yamada T, Gono T, Shimojima Y, Ishii W, Fushimi T, Sakashita K, Koike K, Ikeda S. Serum levels of free light chain before and after chemotherapy in primary systemic AL amyloidosis. *Intern Med* 2005; 44: 428-433.
12. Demidem A, Lam T, Alas S, Hariharan K, Hanna N, Bonavida B. Chimeric anti-CD20 (IDEC-C2B8) monoclonal antibody sensitizes a B cell lymphoma cell line to cell killing by cytotoxic drugs. *Cancer Biother Radiopharm* 1997; 12: 177-186.
13. Reff ME, Carner K, Chambers KS, Chinn PC, Leonard JE, Raab R, Newman RA, Hanna N, Anderson DR. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. *Blood* 1994; 83: 435-445.
14. McLaughlin P, Grillo-López AJ, Link BK, Levy R, Czuczman MS, Williams ME, Heyman MR, Bence-Bruckler I, White CA, Cabanillas F, Jain V, Ho AD, Lister J, Wey K, Shen D, Dallaire BK. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. *J*

- Clin Oncol 1998; 16: 2825-2833.
15. van Oers MH, Klasa R, Marcus RE, Wolf M, Kimby E, Gascoyne RD, Jack A, Van't Veer M, Vranovsky A, Holte H, van Glabbeke M, Teodorovic I, Rozewicz C, Hagenbeek A. Rituximab maintenance improves clinical outcome of relapsed/resistant follicular non-Hodgkin lymphoma in patients both with and without rituximab during induction: results of a prospective randomized phase 3 intergroup trial. *Blood* 2006; 108: 3295-3301.
  16. Harding S, Drayson M, Hobbs J, Mead G, Bradwell A. Analysis of the involved IgG kappa/IgG lambda ratios may give a more sensitive measure of response to treatment in multiple myeloma. *Haematologica* 2008; 93 (s1): Abstr 662.

**Figure legends**

Figure 1: Clinical course of the patient. The  $\kappa/\lambda$  ratio and serum IgG decreased in conjunction with a rapid reduction in CD20-positive lymphoplasmacytoid cells in peripheral blood after starting the rituximab therapy (A). Serum albumin increased slightly, although creatinine clearance gradually decreased (B). ALB: albumin, TP: total protein, CCr: creatinine clearance



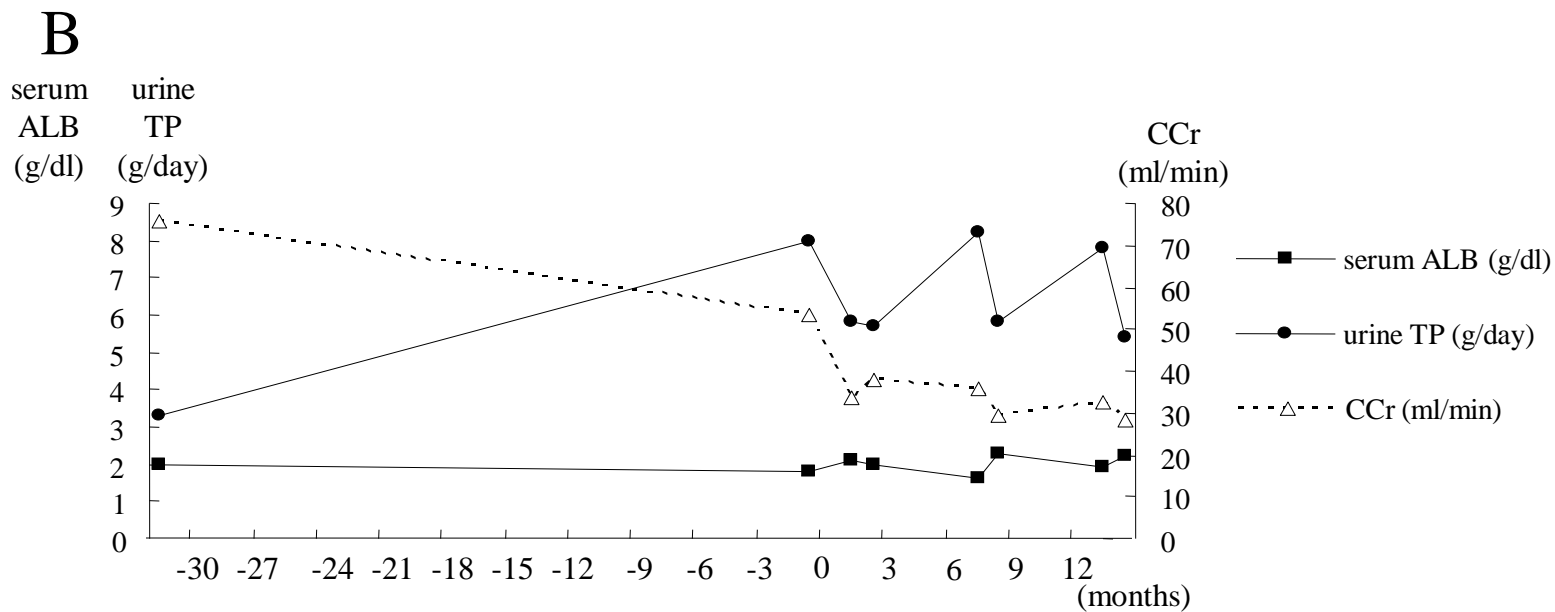
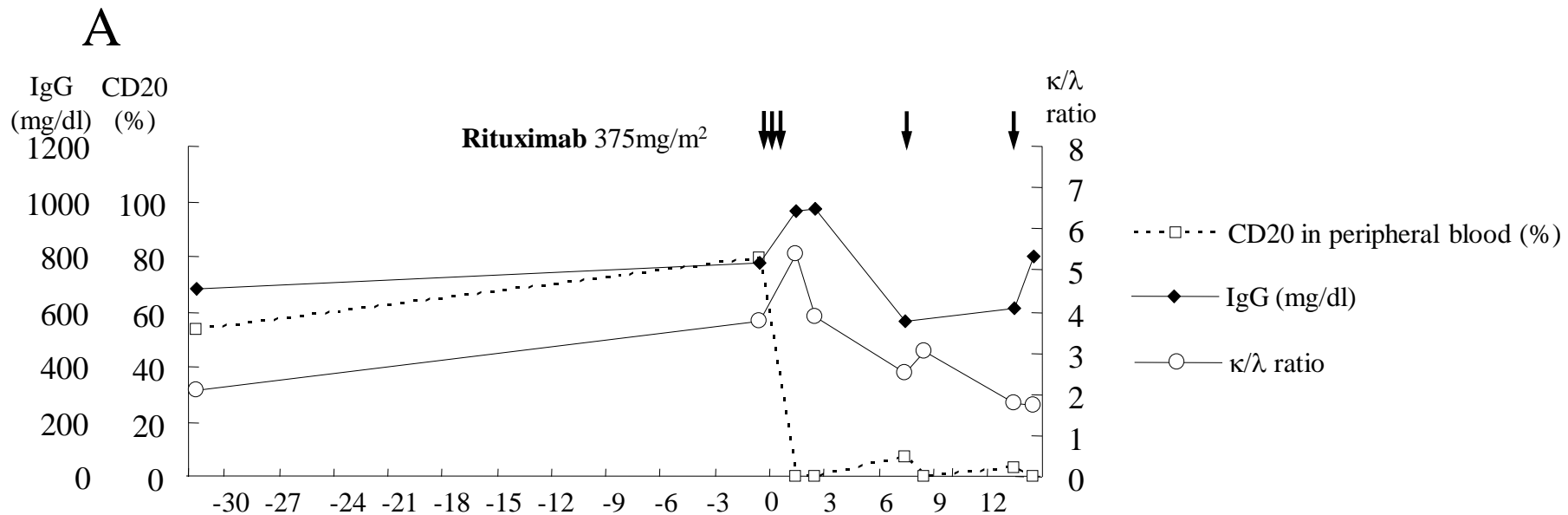


Figure 1