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【 CASE REPORT 】

The Long-term Outcomes after VAD plus SCT Therapy in a Patient with AL Amyloidosis and Severe Factor X Deficiency

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Abstract:
A 55-year-old man was admitted to our institute to undergo evaluation for proteinuria (5.4 g/day) with lambda-type Bence-Jones protein (BJP). Primary amyloid light chain (AL) amyloidosis and acquired factor X deficiency were diagnosed. High-dose melphalan combined with autologous stem cell transplantation was performed. After three years, the patient's proteinuria normalized, he was negative for urinary BJP, and his factor X activity improved to 105%. Serial renal biopsy showed no progression of amyloid deposition at a biopsy after 5 years, but showed a slight increase in the amyloid deposition after 11 years. This therapy can improve the prognosis of AL amyloidosis; however, there are limitations to the strategy.

Key words: AL amyloidosis, factor X deficiency, vincristine, adriamycin, and dexamethasone (VAD), high-dose melphalan, autologous stem cell transplantation (SCT)

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Introduction

Amyloidosis is a disease in which fibrils composed of low molecular weight subunits of various serum proteins are deposited in the extracellular tissue. These fibrils predominantly show an antiparallel ß-pleated sheet conformation. Thus far, more than 30 proteins have been shown to form amyloid fibrils (1). The most frequent type of amyloidosis is amyloid light chain (AL) amyloidosis. AL amyloidosis is a clonal hematological disorder that arises when antibody-producing cells function incorrectly and produce abnormal protein fibers composed of immunoglobulin light chains which are deposited as amyloid fibrils. It is a systemic disorder that can present with various symptoms, including proteinuria, edema, unexplained heart failure, neuropathy, skin manifestations, and bleeding diathesis. Over the past decade, the median survival of patients with AL amyloidosis has nearly doubled (2). In 2004, the median survival time of patients with AL amyloidosis was only 2.2 years, and the estimated 4-year overall survival rate was 38% (3). The treatment of AL amyloidosis targets the clonal bone marrow plasma cells using the same methods that are employed for multiple myeloma (3). On 2004, as treatment for AL amyloidosis, induction chemotherapy with a combination of vincristine, adriamycin, and dexamethasone (VAD) prior to autologous stem cell transplantation with high-dose melphalan, autologous stem cell transplantation (HDM/SCT) was proposed by Perz et al. (4).

A close association has been reported between AL amyloidosis and factor X deficiency. Systemic AL amyloidosis patients with reduced factor X levels is associated with in-

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Table. Laboratory Tests before HDM/SCT and at 3, 5, 10, 11 and 13 Years after Transplantation.

	Before	after 3 years	after 5 years	after 10 years	after 11 years	after 13 years	normal range
Total protein, g/dL	5.4	6	6.8	7.2	7.1	6.4	6.9-8.4
Albumin, g/dL	2.5	3.5	3.8	3.9	3.9	4	3.9-5.2
UN, mg/dL	10	21	21	20	21	24	8-20
Creatinine, mg/dL	0.6	1	1	1	1.07	1.16	0.4-0.6
eGFR	107	60.5	59.9	58.5	52.8	49.1	>100
White blood cell /μL	6,500	5,900	5.4	4.2	6.1	6,600	3,400-9,200
Red blood cell, $\times10^4$/μL		3.2	3.25	3.41	3.55	3.3	400-566
Hb, g/dL	13.7	11.2	12	12.6	12.2	10.3	13.0-17.0
Platelet, $\times10^4$/μL	37.1	13.8	13.9	10.9	12.8	15.5	14.1-32.7
IgG, mg/dL	421	558	965		984	904	870-1,700
IgA, mg/dL	84		154		212	49.5	110-410
IgM, mg/dL	48		71		114	53	35-220
serum M protain	negative	negative	negative	negative	negative	positive	negative
APTT, second	51.3	30	25.3		29.5	33	27-40
PT, second	21	114	109.4		94.8	79.3	>70
INR	1.8		0.94		1.92	1.13	
factors X activity, %	12.9	105					70-130
urinary protain (g/day)	5.4	0.03	0.04	0.04	0.89	0.38	<0.1
urunary BJP	positive	negative	negative	negative	negative	positive	negative
LVIDd, mm	50	54	46		46		34-54
IVST, mm	15	9	9		11.4		6-10
PWT, mm	13	11	10		9.2		6-10
LVM, g	276	206	148		162		122-174

LVIDd: left ventricular internal diameter at end-diastole, IVST: interventricular septal thickness, PWT: posterior wall thickness, LVM: left ventricular mass, UN: urea nitrogen, eGFR: estimated glomerular filtration rate, APTT: activated partial thromboplastin time, PT: prothrombin time, PT-INR: prothrombin time-international normalized ratio, BJP: Bence-Jones protein

creased hemorrhagic morbidity (5).

In 2004, we initiated HDM/SCT therapy for a patient who had AL amyloidosis combined with severe factor X deficiency according to the report of Perz et al. (4). We herein report the patient's long-term outcome in the 10 years after this therapy was administered.

Case Report

In 2004, a 55-year-old Japanese man was admitted to our hospital to undergo evaluation for proteinuria and weight loss. On admission, he was 168.0 cm tall and weighed 58.0 kg (he had lost 5 kg of body weight during the previous 5 months). His blood pressure was 100/70 mmHg, his pulse rate was 90 beats/min, and his body temperature was 36.6℃. Laboratory tests revealed the following: serum albumin, 2.5 g/dL; total protein, 5.4 g/dL; urea nitrogen (UN), 10 mg/dL; serum creatinine (Cre), 0.60 mg/dL; and estimated glomerular filtration rate (eGFR), 107.0 mL/min/1.73 m^2) (Table). The patient's C-reactive protein (CRP) level was 0.1 mg/dL, and his serum amyloid A protein (SAA) was 2.0 μg/mL (normal: <13.0). Coagulation tests showed an activated partial thromboplastin time (APTT) of 51.3 seconds (normal range: 27.0-40.0 seconds), a prothrombin time of 21 seconds (normal range: >70 seconds), and a prothrombin time-international normalized ratio (PT-INR) of 1.77; however, clinical bleeding was not noted. Abnormal coagulation was observed because the patient's factor X activity was reduced to 15.2% (normal range: 70-130%); his other coagulation factors were normal. An immunological evaluation revealed the following: IgG, 421 mg/dL (normal range: 870-1,700 mg/dL); IgA, 83.6 mg/dL (normal range: 110-410 mg/dL); and IgM, 47.4 mg/dL (normal range: 35-220 mg/dL). The patient was negative for all autoantibodies, including antinuclear antibodies and anti-cardiolipin-β2 glycoprotein I complex antibodies. Immunofixation revealed that the patient was negative for serum M-protein. A urinalysis detected proteinuria of (5.36 g/day), while protein electrophoresis showed that albumin accounted for 57.5% and the patient was positive for lambda-type Bence-Jones protein (BJP).

Low voltage electrocardiography was performed. Echocardiography revealed generalized cardiac hypertrophy and the left ventricular mass was found to be 276 g by the formula of Devereux: $0.8[1.04(IVST+PWV+LVIDd)^3-LVIDd^3]+0.6$. IVST: interventricular septal thickness, PWV: posterior wall thickness, LVIDd: left ventricular internal diameter at end-diastole. These findings were consistent with cardiac amyloidosis. Renal biopsy was performed to evaluate the pathogenesis of nephrotic-range proteinuria.

Renal biopsy

A renal biopsy specimen was obtained, which contained 21 glomeruli, with 3 showing global sclerosis. Mesangial

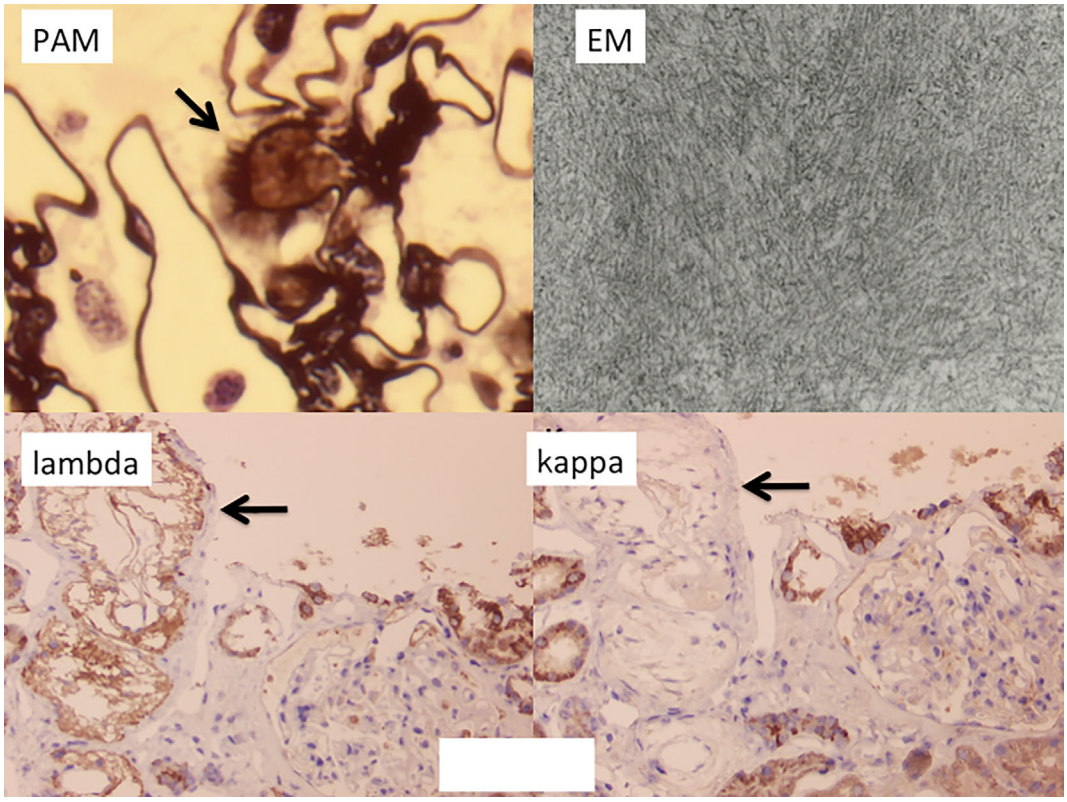

Figure 1. **PAM staining demonstrated spicule formation in the subepithelial region (arrow). Electron microscopy showed randomly arranged fibrils measuring 7 to 12 nm in diameter. An immunoenzyme analysis of an amorphous lesion (paraffin-embedded section) was positive for lambda light chain (arrow), but negative for kappa light chain (arrow).**

cell proliferation was not noted, but periodic acid-Schiff (PAS) staining showed partial expansion of the mesangial matrix. In addition, PAM staining detected subepithelial spicule formation (Fig. 1). The small arteries showed swelling due to the presence of an amorphous material. Congo red staining was positive for these lesions (Fig. 2). Immunofluorescence staining was negative for immunoglobulins (IgG, IgA, IgM), complement components (C3, C4, C1q), and light chains (kappa and lambda). An immunoenzyme analysis of an amorphous lesion (paraffin section) was positive for lambda light chain (Fig. 1), but negative for kappa light chain (Fig. 1), amyloid A, beta-2 microglobulin, and transthyretin. Electron microscopy showed randomly arranged fibrils that were 7-12 nm in diameter (Fig. 1). Tubulointerstitial fibrosis occupied approximately 10% of the total renal cortical area (Fig. 3).

To examine the extra-renal involvement of the amyloidosis, a biopsy of abdominal skin and adipose tissue, as well as an endoscopic biopsy of the colon, was performed. The colon specimen showed the same type of amyloid deposits in the small arteries and surrounding tissues of the submucosal layer (Fig. 4a). The same type of amyloid deposition was also confirmed in the abdominal skin and adipose tissue specimen. An examination of the bone marrow revealed 8.4% (<10%) monoclonal plasma cells, but the patient did not fit the criteria for multiple myeloma since no osteolytic lesions were detected by a full skeletal survey. Thus, the patient was diagnosed with primary lambda (λ) type systemic AL amyloidosis.

Clinical course

In 2004, autologous stem cell transplantation (SCT) was performed according to the published criteria and regimen (6, 7). Before SCT, the patient received 2 cycles of chemotherapy with VAD, as well as high-dose melphalan (HDM). In 2007, his proteinuria decreased to 0.03 g daily, he became negative for urinary BJP, his serum albumin level increased to 3.5 mg/dL, his APTT and PT were normalized, and his factor X activity improved to 105% (Table). In 2009, his left ventricular mass decreased to 148 g.

In 2015, Cre was 1.07 mg/dL, and proteinuria became positive at 0.89 g daily. In 2016, he complained of malaise, and his WBC count was found to be 58,600/μL with 94.5% myeloblasts (Fig. 5). Acute myeloid leukemia (AML) was diagnosed. He received conditioning with cytarabine (ara-C), BU/ fludarabine, and melphalan, followed by unrelated cord blood transplantation. At 12 months after transplantation (May 2017), his AML remains in complete remission. However, his urinary protein was 0.36 g/day and he became positive for urinary BJP.

The sequential renal biopsy findings

In 2007, a second renal biopsy was performed because of a decrease in the eGFR from 107 mL/min to 60.5 mL/min,

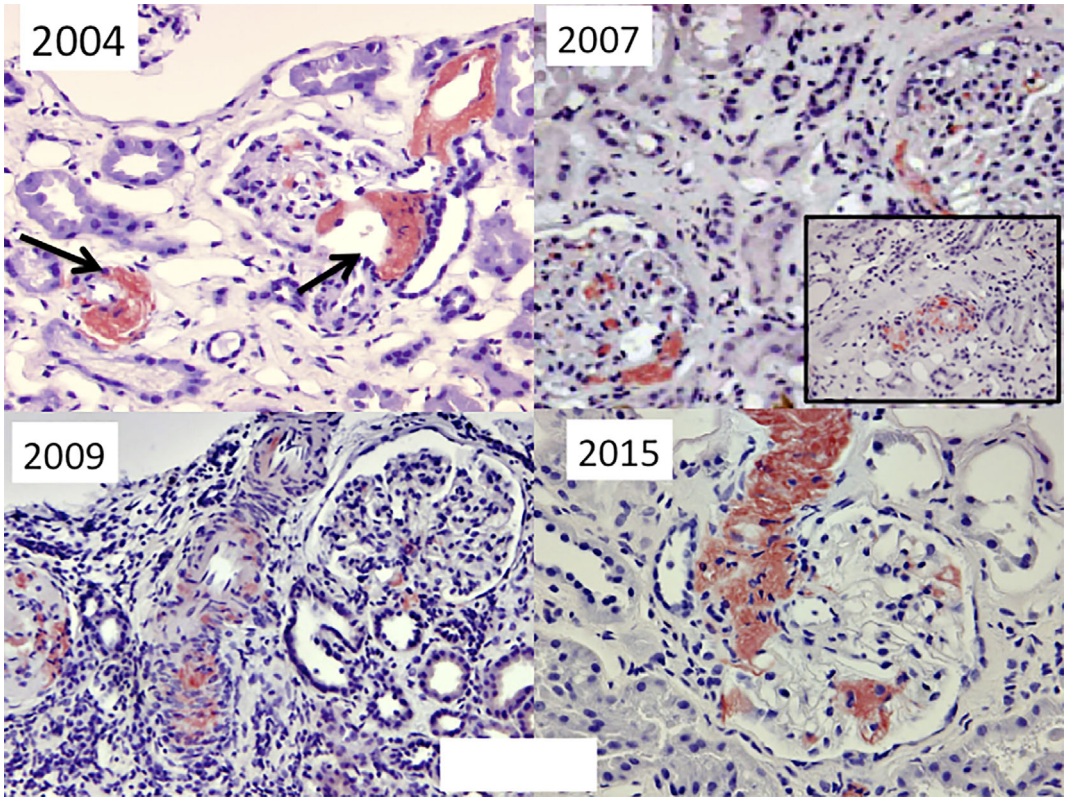

Figure 2. **In 2004, Congo red staining was positive in the mesangial region and small arteries (arrow). In 2007, there was a decrease in the deposition of amyloid in small arteries and non-sclerotic glomeruli. In 2009, the amyloid deposition in the small arteries and non-sclerotic glomeruli remained almost unchanged. In 2013, the amyloid deposition in the renal arterioles and interlobular arteries showed progression in comparison to the third biopsy specimen; however, the amyloid deposition in the non-sclerotic glomeruli remained unchanged.**

although his proteinuria had subsided. Light microscopy showed global sclerosis in 3 of 18 glomeruli. Amyloid deposition in small arteries and non-sclerotic glomeruli was less prominent than before (Fig. 2), but tubulointerstitial fibrosis had progressed to occupy approximately 50% of the total renal cortical area (Fig. 3), reflecting a decrease in the renal function.

In 2009, a third biopsy was performed to determine the timing of further treatment for amyloidosis. Light microscopy showed global sclerosis in 34/40 glomeruli. The deposition of amyloid in the small arteries and non-sclerotic glomeruli was similar to that shown by the second biopsy (Fig. 2). The tubulointerstitial findings were also similar to those of the second biopsy (Fig. 3).

In 2015, the fourth renal biopsy was performed after the patient's proteinuria increased to 0.89 g daily. Light microscopy displayed global sclerosis in 30 of 42 glomeruli. In comparison to the third biopsy, amyloid deposition in the renal arterioles and interlobular arteries showed progression, although amyloid deposition in non-sclerotic glomeruli was unchanged (Fig. 2). The tubulointerstitial findings were similar to those at the third biopsy (Fig. 3).

The sequential colon biopsy findings

In 2011, endoscopic biopsy of the colon was performed. A marked decrease in the amyloid deposits was confirmed; although amyloid deposition was only confirmed in a small part of the small arteries (Fig. 4b).

Discussion

The treatment of AL amyloidosis involves the targeting of clonal plasma cells in the bone marrow with the same regimens that are used for multiple myeloma. Cibeira et al. reported the outcomes of 421 consecutive patients with AL amyloidosis who were treated with HDM/SCT. The complete remission (CR) rate was 34% and the median event-free survival time (EFS) and overall survival time (OS) were 2.6 and 6.3 years, respectively. Among the patients who achieved a CR, the median EFS and OS were 8.3 and 13.2 years, respectively (8). Modern treatment options (including SCT, bortezomib, and lenalidomide) have improved the outcomes of low- and intermediate-risk patients, but the prognosis of patients with severe cardiac dysfunction remains poor (9). However, Hattori reported that the left ventricular mass (LVM) decreased from 320 g to 180 g after the administration of tocilizumab in a hemodialysis patient with AA Amyloidosis secondary to rheumatoid arthritis, and that this patient' cardiac disease improved (10). The LVM of our patient also improved from 276 g to 162 g, even at 11 years

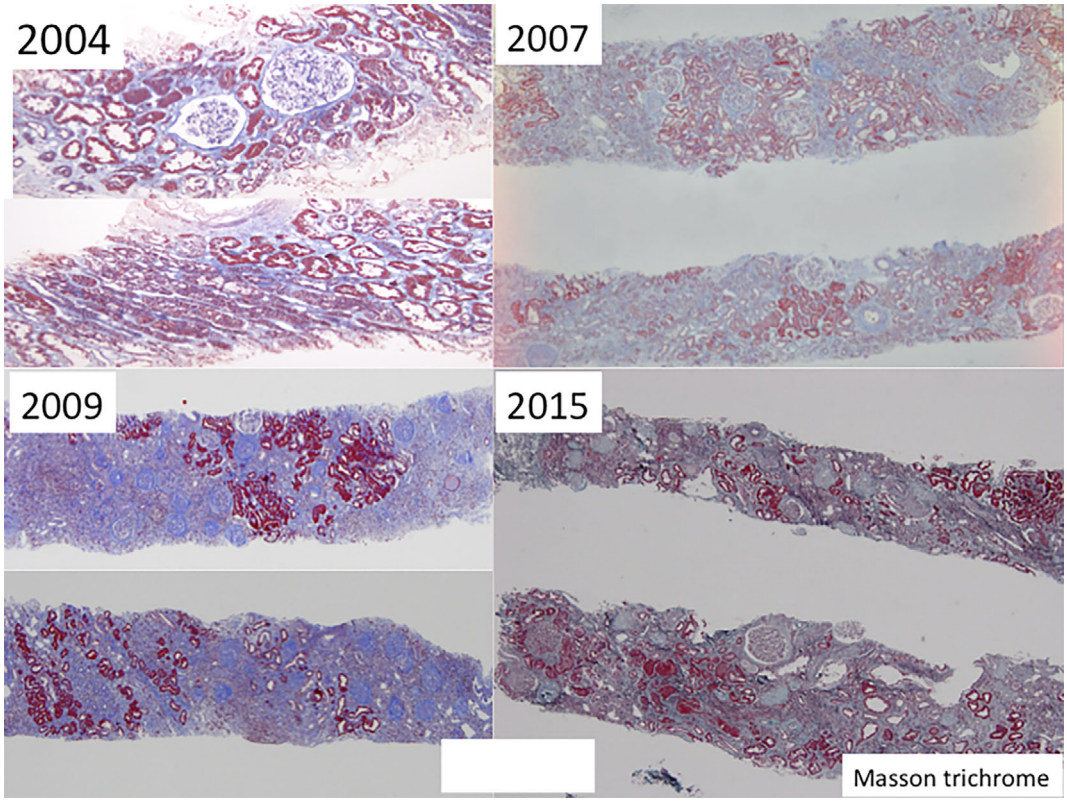

Figure 3. **In 2004, tubulointerstitial fibrosis occupied approximately 10% of the total renal cortical area. In 2007, tubulointerstitial fibrosis had progressed to approximately 50% of the total renal cortical area. In 2009, the tubulointerstitial findings were similar to those of the second biopsy. In 2015, the tubulointerstitial region showed no further changes. (Masson trichrome staining)**

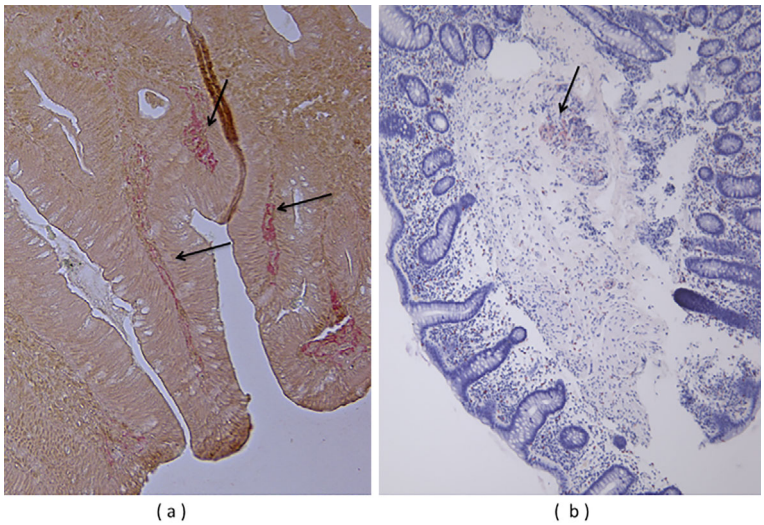

Figure 4. **(a): In 2004, the patient's colon showed the same type of amyloid deposits (arrows) in the small arteries and the surrounding tissues of the submucosal layer. (b) In 2011, a marked decrease in the amyloid deposits was confirmed; however, amyloid deposition was only confirmed in a small number of the small arteries (arrow).**

after HDM/SCT. HDM/SCT may improve cardiac complications in AL amyloidosis.

Acquired factor X deficiency (Stuart factor) is the most common coagulation factor deficiency that has been identified in patients with AL amyloidosis. It occurs because factor X is adsorbed into amyloid fibrils, which shortens the plasma half-life (11). Choufani et al. evaluated 368 patients with systemic AL amyloidosis. 32 patients (8.7%) had factor X levels below 50% of normal. Eighteen of these patients (56%) had bleeding complications, which were more frequent and severe in the 12 patients below 25% of normal (5). High-dose melphalan chemotherapy followed by

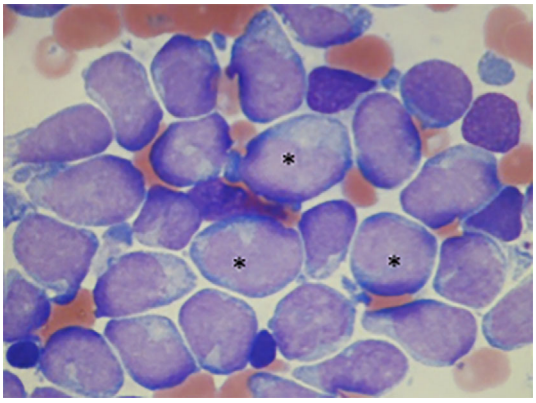

Figure 5. Bone marrow puncture shows the accumulation of myeloblasts (＊).

SCT can lead to the amelioration of amyloid-related factor X deficiency as well as amyloid (5). Accordingly, if the deposition of amyloid is reduced by HDM/SCT and the adsorption of factor X by amyloid fibrils is consequently decreased, the factor X levels can be normalized.

Recently, some novel agents for the treatment of plasma cell dyscrasias have been investigated. For example, immunomodulators such as thalidomide and lenalidomide have been used in combination with dexamethasone and the proteasome inhibitor bortezomib has also shown efficacy (12).

In conclusion, we have managed a patient with AL amyloidosis and severe factor X deficiency who has survived for more than 13 years. LVM of this patient improved from 276 g to 162 g, and remained improved, even at 11 years after HDM/SCT. It seems that a cardiac response was achieved, which may have contributed to long-term outcome of this patient. In addition, a marked decrease of amyloid deposits was confirmed in the colon, and the complete remission of proteinuria persisted for approximately 7 years. These findings may show that this treatment is effective, even in an AL amyloidosis patient with cardiac involvement, the prognosis of which has been considered poor. A significant decrease in the amyloid deposition in kidney tissue, which may show that the clearance of AL amyloid from renal tissue differs from that of the intestine, was not noted. Hematological relapse, including the reappearance of urinary BJP, as well as the new occurrence of leukemia may require the next therapeutic option.

The authors state that they have no Conflict of Interest (COI).

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