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【 ORIGINAL ARTICLE 】

First Nationwide Survey of 199 Patients with Amyloid A Amyloidosis in Japan

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Abstract:

Objective To clarify the underlying diseases, clinical manifestations, and treatment strategies for Amyloid A (AA) amyloidosis (AAA) in Japanese patients.

Methods We conducted a survey on Japanese patients with AAA treated between January 1, 2012, and December 31, 2014.

Results A total of 199 patients with AAA were included in the present study. The underlying diseases of AAA were rheumatoid arthritis (60.3%), uncharacterized inflammatory disorders (11.1%), neoplasms (7.0%), other rheumatic diseases (6.5%), inflammatory bowel diseases (4.5%), chronic infection (4.5%), Castleman's disease (4.0%), and autoinflammatory diseases (2.0%). The clinical manifestations at the diagnosis of AAA were moderate to severe renal dysfunction (46.2%), moderate to severe proteinuria (30.7%), intractable diarrhea (32.2%), melena (4.5%), paralytic ileus (3.5%), heart failure (11.6%), cardiac conduction disturbances (10.1%), arrhythmia (5.5%), and hypothyroidism (11.6%). Diagnostic biopsies were performed most frequently in the gastrointestinal tract (66.3%), followed by the kidneys (22.1%), heart (5.5%), abdominal fat (4.0%), and others (3.0%). Biologics were used to treat 97 patients with AAA (48.7%). Tocilizumab (TCZ) was administered to 66 patients, with 95.5% showing good responses. Anti-TNF agents were administered to 27 patients, with 74.1% showing good responses. The treatment effects of TCZ were significantly superior to those of anti-TNF agents ($p<0.007$).

Conclusion The most common underlying diseases of AAA were rheumatic diseases. Uncharacterized inflammatory disorders and neoplasms were also frequently observed in patients with AAA. Renal and gastrointestinal manifestations were common and important for the diagnosis of AAA, with cardiac manifestations also being of significance. Biologics, particularly TCZ, were effective therapeutic modalities.

Key words: epidemiology, AA amyloidosis, Japanese patients, clinical manifestation, treatment modality, tocilizumab

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Introduction

Systemic amyloidosis is a condition in which insoluble fibrillar aggregates accumulate in the extracellular spaces of organs (1). Amyloid A (AA) amyloidosis, resulting from AA protein deposition in the extracellular matrices of various target organs, may lead to multiple organ dysfunction. The level of serum amyloid A (SAA), the precursor of the AA protein, was found to be elevated in livers stimulated by inflammation-associated cytokines, such as interleukin-6 (IL-6), tumor necrosis factor (TNF), and IL-1 (2). Prolonged elevations in SAA levels are a major risk factor for the development of AA amyloidosis in chronic inflammatory diseases (3). The prognosis of patients with advanced-stage AA amyloidosis is typically poor (4).

The underlying diseases and clinical features of AA amyloidosis vary among countries and eras (5-7). In Japan, rheumatic diseases, including rheumatoid arthritis (RA), are thought to be the main underling diseases of AA amyloido-

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Table 1. Underlying Diseases in 199 Patients with AA Amyloidosis.

Underlying diseases	Number of patients (%)
Rheumatoid arthritis	120 (60.3)
Uncharacterized inflammatory disorders	22 (11.1)
Neoplasm	14 (7.0)
renal cell carcinoma 3, lung cancer 2, gastric cancer 2, malignant lymphoma 1, MALT lymphoma 1, esophageal cancer 1, multiple myeloma 1, hair follicular cancer 1, left atrial myxoma 1	
Other rheumatic diseases	13 (6.5)
Adult onset still's disease 3, mixed connective tissue disease 2, carry-over juvenile idiopathic arthritis 1, intestinal Beçhet's disease 1, systemic lupus erythematosus 1, Sjögren's syndrome 1, ankylosing spondylitis 1, polymyalgia rheumatic 1, SAPHO syndrome 1, malignant rheumatoid arthritis 1	
Inflammatory bowel disease	9 (4.5)
Crohn's disease 8, ulcerative colitis 1	
Chronic infection	9 (4.5)
non-tuberculous mycobacteriosis 4, tuberculosis 2, pyogenic spondylitis 1, postoperative refractory infection of hip joint 1	
Castleman's disease	8 (4.0)
Autoinflammatory diseases	4 (2.0)
FMF 3, a suspicious case of FMF 1	

MALT: Mucosa Associated Lymphoid Tissue, SAPHO: Synovitis-acne-pustulosis hyperostosis-osteomyelitis, FMF: familial Mediterranean fever

sis, and chronic infections, such as tuberculosis, were previously considered to be the principal cause of this disease. However, a nationwide survey on AA amyloidosis in Japan has not yet been conducted. Therefore, the underlying diseases, clinical manifestations, and treatment strategies for AA amyloidosis need to be clarified in Japanese patients.

On behalf of the Amyloidosis Research Committee, Intractable Disease Division of the Japanese Ministry of Health and Welfare, we conducted a survey on this disease in Japan.

Materials and Methods

The Amyloidosis Research Committee, Intractable Disease Division, of the Japanese Ministry of Health and Welfare conducted a survey on AA amyloidosis patients treated between January 1, 2012, and December 31, 2014. A questionnaire was sent to 4,652 hospital departments, including those of Rheumatology, Nephrology, Gastroenterology, Cardiology, Neurology, Hematology, Neurosurgery, and Urology, which were randomly selected from all hospitals in Japan according to the number of hospital beds. The sampling fractions were as follows: 2.5% for general hospitals with 99 or fewer beds; 5% for 100 to 199 beds; 10% for 200 to 299 beds; 20% for 300 to 399 beds; 40% for 400 to 499 beds; 100% for 500 or more beds, university hospitals, and special hospitals that patients with amyloidosis were very likely to visit, irrespective of the number of beds. Responses were obtained from 2,321 departments (49.9%). A total of 369 cases were identified by the primary survey, and 199 cases of AA amyloidosis were collected during the second survey. The contents of the second survey questionnaire were as follows: age, gender, family history, medical history, underlying diseases, histological evidence for the diagnosis, manifestation at the diagnosis, therapeutic modality and response, laboratory data [C-reactive protein (CRP), SAA, creatinine, albumin, urinary protein], and echocardiographic findings.

The Institutional Review Boards of Kumamoto University, Kumamoto (No: 1001) and Dohgo Spa Hospital, Ehime (No: H27-003) approved the present study.

Statistical analyses

A categorical data analysis was performed using the chi-squared test.

Results

A total of 199 AA amyloidosis patients were included in the present study (49 men and 150 women; median age, 65 years, range: 22-90). The male-to-female ratio was approximately 1:3.

Underlying diseases of AA amyloidosis

The underlying diseases of AA amyloidosis were rheumatoid arthritis (120 patients, 60.3%), uncharacterized inflammatory disorders (22, 11.1%), neoplasms (14, 7.0%), other rheumatic diseases (13, 6.5%), inflammatory bowel diseases (9, 4.5%), chronic infections (9, 4.5%), Castleman's disease (8, 4.0%), and autoinflammatory diseases (4, 2.0%) (Table 1). Detailed diseases and the numbers of patients in these groups are listed in Table 1.

Initial symptoms

The clinical manifestations at the diagnosis of the 199 patients are summarized in Table 2. Proteinuria was detected in 102 patients (51.3%) and was moderate (≥0.5 g/day or 0.5 g/gCr) to severe (≥3.5 g/day or 3.5 g/gCr) in 61 (31.7%). Renal failure was noted in 152 patients (76.4%) and was moderate [estimated glomerular filtration rate (eGFR): 30 to <60 mL/min/1.73 m^2] to severe (eGFR: <30 mL/min/1.73 m^2) in 92 (46.2%). Fifteen patients (7.5%)

Table 2. Clinical Manifestations at Diagnosis of the 199 Patients with AA Amyloidosis.

Clinical manifestations	Number of patients (%)
Proteinuria	102 (51.3)
Moderate to severe case	61 (31.7)
Renal failure	152 (76.4)
Moderate to severe case	92 (46.2)
Dialysis	15 (7.5)
Serious gastrointestinal (GI) symptoms	79 (39.7)
Intractable diarrhea	64 (32.2)
GI bleeding	9 (4.5)
Paralytic ileus	6 (3.0)
Cardiac damage	
Cardiac failure	23 (11.6)
NYHA grade 3 or 4 case	10 (5.0)
Conduction disturbance	7 (3.5)
Atrial fibrillation	7 (3.5)
Ventricular tachycardia	4 (2.0)
Hypothyroidism	23 (11.6)

Proteinuria; moderate: not less 0.5 g/day or 0.5 g/gCr, severe: not less 3.5 g/day or 3.5 g/gCr.
Renal failure; moderate: eGFR-blow 60 to 30, severe: eGFR-below 30.
NYHA: New York Heart Association

were receiving dialysis.

The following severe gastrointestinal (GI) tract symptoms were detected in 79 patients (39.7%): intractable diarrhea, 64 (32.2%): GI bleeding, 9 (4.5%): and paralytic ileus, 6 (3.0%). Regarding cardiac damage, conduction disturbances were observed in 20 (10.1%) of the 199 patients. First-degree atrioventricular (AV) block was noted in 4 patients, while second- and third-degree complete AV blocks were detected in 3 (Wenckebach type 2: 2, complete: 1), and cardiac arrhythmia was observed in 11 (atrial fibrillation: 7, ventricular tachycardia: 4). Cardiac failure was detected in 23 (11.6%) of the 199 patients. Of these, 10 patients had cardiac failure of worse than grade 3 according to the New York Heart Association (NYHA) classification. Cardiac damage was the first symptom of AA amyloidosis in nine patients (renal and GI symptoms were negative at the time of the diagnosis of AA amyloidosis). Hypothyroidism was noted in 23 (11.6%) of the 199 patients.

The diagnosis (Table 3)

GI tract biopsies were performed on 137 patients (upper GI in 98, lower GI in 22, and both in 19). Amyloid deposition was detected in 114 out of 137 patients (83.2%). The vascular deposit pattern was noted in 17 patients (14.9%), and the vascular with stromal deposition pattern was noted in 97 (85.1%). Examinations of upper GI tract biopsies revealed higher positive rates with a biopsy of the stomach and duodenum (91.3%) than with that of the stomach only (62.1%; χ^2=10.1, p=0.0015).

Table 3. Biopsy Site for Diagnosis.

Site	Cases	Positive cases, N (%)
Gastrointestinal (GI) tract	137	114 (83.2%)
Upper GI tract	98	80 (81.6%)
Stomach	29	18 (62.1%)
Duodenum+Stomach	69	63 (91.3%)
Lower GI	22	18 (81.8%)
Upper GI+Lower GI	19	16 (84.2%)
Kidneys	61	59 (96.7%)
Heart	13	13 (100%)
Abdominal fat	8	4 (50.0%)
Others	18	10 (55.6%)

Comparison of sensitivity between stomach versus stomach+duodenum: χ^2=10.1, p=0.0015

Renal biopsies were conducted on 61 patients, and amyloid deposits were detected in 59 (96.7%). The vascular deposit pattern was observed in 9 patients (15.3%), while the vascular with extravascular tissue deposition pattern was noted in 50 (84.7%). Cardiac biopsies were performed on 13 patients, and amyloid deposits were detected in all patients. All biopsy-positive cases showed the vascular with extravascular tissue deposition pattern (100%). Abdominal fat biopsies were performed on 8 patients, and amyloid deposits were detected in 4 (50%). Other biopsy sites included the skin (5 patients), lymph nodes (2 patients), lungs (2 patients), bone marrow (2 patients), tongue (1 patient), bladder (1 patient), liver (1 patient), ear (1 patient), ureter (1 patient), pelvis (1 patient), and salivary gland (1 patient).

Immunohistochemical staining of AA was performed to diagnose AA amyloidosis in 118 patients (59.3%), while a proteomic analysis based on liquid chromatography/tandem mass spectrometry (LC-MS/MS) of tissue samples obtained via laser microdissection was conducted for 8 patients.

Laboratory findings (Table 4)

The median values of CRP (mg/dL), SAA (μg/mL), and creatinine (mg/dL) increased to 1.14 [interquartile range (IQR): 0.21-4.60], 59.9 (IQR: 30.7-212.9), and 1.1 (IQR: 0.7-2.3), respectively, in patients with AA amyloidosis. The median value of albumin (mg/dL) decreased to 3.1 (IQR: 2.2-3.7). The median 24-h urinary protein (g/day) value was 2.0 (IQR: 0.7-4.7) in 60 of the 199 patients. The median brain natriuretic peptide (BNP) (pg/mL) value was 304.0 (IQR: 121.2-693.2) in 60 patients. Ultrasound cardiography showed granular sparkling in 11 of 83 patients (13.3%). The median value of E/E' was 12.3 (IQR: 9.9-17.1).

Treatment

Biologics were administered to 97 patients (48.7%) to

Table 4. Laboratory Findings in AA Amyloidosis.

Clinical test items	Cases	Values (median, IQR)
CRP (mg/dL)	199	1.14 (0.21-4.60)
SAA (μg/mL)	199	59.9 (30.7-212.9)
Creatinine (mg/dL)	199	1.1 (0.7-2.3)
Albumin (g/dL)	199	3.1(2.2-3.7)
24-hour urinary protein (g/day)	63	2.0 (0.7-4.7)
BNP (pg/mL)	60	304.0 (121.2-693.2)
UCG: E/E'	63	12.3 (9.9-17.1)
UCG: Granular sparkling	83	13.3% (11/83 cases)

CRP: C-reactive protein, SAA: serum amyloid A, BNP: brain natriuretic peptide, UCG: ultrasonic cardiogram

Table 5. Biologic Treatment and Efficacy in AA Amyloidosis.

Biologics	Patient's number (effective cases, %)
Anti-interleukin 6 receptor antibody	
Tocilizumab	66 (63, 95.5%)
TNF inhibitors	27 (20, 74.1%)
Etarnercept	15
Infliximab	8
Adalimumab	2
Golimumab	2
Selective T cell costimulation modulator	
Abatacept	4 (3, 75%)

Comparison of efficacy between anti-interleukin 6 receptor antibody versus TNF inhibitors: χ^2=7.3, p=0.007

treat AA amyloidosis (Table 5). Tocilizumab (TCZ, an anti-IL-6 receptor antibody) was administered to 66 patients, and good responses were obtained in 63 (95.5%). The underlying diseases of patients administered TCZ were RA (51 patients), Castleman's disease (6 patients), other rheumatic diseases (4 patients), renal cell carcinoma (2 patients), uncharacterized inflammatory disorders (2 patients), and Familial Mediterranean fever (FMF) (1 patient). Good responses were obtained in all patients except for those with RA. Anti-TNF inhibitors (etanercept: 15, infliximab: 8, adalimumab: 2, golimumab: 2) were administered to 27 patients, and good responses were noted in 20 (74.1%). The underlying diseases of patients administered TNF inhibitors were RA (21 patients), other rheumatic diseases (3 patients), and Crohn's disease (3 patients). The efficacy of TCZ was significantly superior to that of TNF inhibitors (χ^2=7.273, p=0.007). Abatacept (ABT, a selective T cell co-stimulation modulator) was administered to 4 patients (RA), and good responses were observed in 3 (75%). Immunosuppressant or disease-modifying anti-rheumatic drugs (IS/DMARDs) were administered to 63 patients (31.7%) to treat AA amyloidosis. The agents were methotrexate (MTX) (29 patients), tacrolimus (13 patients), salazosulfapyridine (8 patients), bucillamine (3 patients), mizoribine (3 patients), azathioprine (2 patients), cyclosporine (2 patients), cyclophosphamide (1 patient), and auranofin (1 patient). Good responses to IS/DMARDs were observed in 39 out of 63 patients (61.9%). Corticosteroids (CSs) were administered to 102 patients (51.3%) to treat AA amyloidosis. Good responses to CSs were noted in 68 patients (66.7%). Colchicine was administered to 3 patients, all of whom achieved good responses.

Some patients received organ-supportive therapies, including dialysis (15 patients with end-stage renal failure), the administration of tolvaptan and angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers (5 with heart failure), octreotide (2 with intractable diarrhea), and renal transplantation (1 with end-stage renal failure).

Discussion

This is the first nationwide survey of AA amyloidosis in Japan and included 199 patients. Although the present study was based on a retrospective survey, the results obtained will help clarify the status of AA amyloidosis in Japan by providing information on the diseases underlying AA amyloidosis, the demographic features of patients with this disease, and treatment strategies.

The most common (two-thirds) underlying diseases of AA amyloidosis were rheumatic diseases [RA (120 patients, 60.3%) and other rheumatic diseases (13 patients, 6.5%)]. Uncharacterized inflammatory disorders were observed in 22 patients (11.1%), and neoplasms were observed in 14 (7.0%). These were characteristic results of this survey. Furthermore, inflammatory bowel diseases, chronic infections, and Castleman's disease (4-4.5%) were identified as important underlying diseases of AA amyloidosis. An increase in the incidence of uncharacterized inflammatory disorders and a decrease in that of idiopathic juvenile arthritis were recently reported in an English-language single-nation referral center survey of the underlying diseases of AA amyloidosis (7).

In Europe, FMF is a major underlying disease of AA amyloidosis. A nationwide survey of FMF in Japan revealed that the estimated number of Japanese FMF patients was approximately 300. Many of these patients were effectively treated using colchicine, and the incidence of AA amyloidosis was only 3.7% (8).

The diagnostic symptom of proteinuria was detected in 102 patients (51.3%) and was moderate to severe in 61 (31.7%). Moderate to severe renal failure was observed in 92 patients (46.2%), and 15 (7.5%) were receiving dialysis. Severe GI disorders were noted in 79 patients (39.7%). The present results confirmed that renal and GI damage were the two important symptoms of AA amyloidosis. Cardiac disorders [conduction disturbances or arrhythmia: 18 patients (9.0%) and cardiac failure: 23 (11.6%)] were also detected. Cardiac symptoms were important for the diagnosis of AA amyloidosis because they were the first symptom observed in nine patients with AA amyloidosis in this survey.

GI biopsies were the most frequently conducted biopsies for the diagnosis of AA amyloidosis. The upper GI tract was

a more frequent biopsy site than the lower GI tract. Examinations of upper GI tract biopsies revealed higher positive rates with a biopsy of the stomach and duodenum (91.3%) than with a biopsy of the stomach only (62.1%; χ^2=10.1, p= 0.0015). This result demonstrated the high sensitivity of the duodenum for amyloid biopsies and was consistent with the findings obtained in our upper GI biopsy study (9). The kidney was also a highly sensitive biopsy site for AA amyloidosis (96.7%). In patients with advanced renal failure or difficulties with body position maintenance due to joint deformities (particularly those with advanced RA), GI biopsies were performed instead of renal biopsies. Cardiac biopsies were performed on 13 patients (6.5%) and were highly sensitive (100%). Abdominal fat biopsies were performed in only 8 patients and exhibited low sensitivity (50%). The immunohistochemical staining of AA was recommended because of its high specificity (10) and was performed to diagnose AA amyloidosis in approximately 60% of patients in this survey.

Laboratory data showed high values for inflammatory markers and renal dysfunction as expected. The median value of 24-h urinary protein was 2.0 g/day in the 60 patients tested. Ultrasound cardiography revealed cardiac diastolic dysfunction (high value for E/E') and granular sparkling (13% of the 83 patients examined).

Biologics were administered to 97 patients (48.7%) to treat AA amyloidosis. TCZ was administered to 66 patients, and good responses were obtained in 63 (95.5%). Good responses were observed in all patients, except for 3 with RA. Anti-TNF inhibitors were administered to 27 patients, and good responses were noted in 20 (74.1%). The efficacy of TCZ was significantly superior to that of TNF inhibitors. (χ^2 =7.273, p=0.007). ABT was administered to 4 patients (RA), and 3 obtained good responses (75%). TCZ was the most frequently administered biologic for the various underlying diseases, including RA, and was found to be the most effective. Regarding AA amyloidosis complicated by rheumatic diseases, we previously reported that TCZ showed superior SAA suppression, clinical efficacy, and survival rates to TNF inhibitors (11). IS/DMARDs were administered to 63 patients (31.7%) to treat AA amyloidosis, and good responses were observed in 39 patients (61.9%). Non-responders to IS/DMARDs were subsequently treated with biologics or an increased dose of CSs. CSs were administered to 102 patients (51.3%) to treat AA amyloidosis, and good responses were observed in 68 patients (66.7%). However, there were concerns regarding the long-term efficacy and safety of moderate-to-high doses of CSs.

Several limitations associated with the present study warrant mention. First, the immunohistochemical staining of AA is important for the diagnosis of accurate AA type; however, staining was not conducted in about 40% of cases. Second, judgment of the treatment efficacy was left to each principal doctor's consideration.

In summary, we outlined the current status of AA amyloidosis in Japan. Although this was a retrospective survey with some limitations, it is the first and largest nationwide survey conducted to date and provides the following important results: 1) The most common (two-thirds) underlying diseases of AA amyloidosis were rheumatic diseases. Uncharacterized inflammatory disorders and neoplasms were observed in 11.1% and 7.0% of patients, respectively. 2) Renal and GI manifestations were commonly observed and were important for the diagnosis of AA amyloidosis. Cardiac manifestations were also of significance. 3) Biologics, particularly TCZ, were effective therapeutic modalities.

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

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