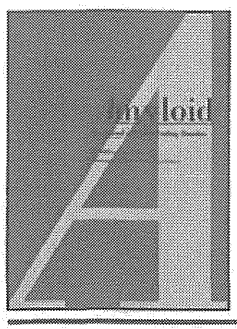


Taylor & Francis

Amyloid

The Journal of Protein Folding Disorders

ISSN: 1350-6129 (Print) 1744-2818 (Online) Journal homepage: http://www.tandfonline.com/loi/iamy20

Carpal tunnel syndrome: a common initial symptom of systemic wild-type ATTR (ATTRwt) amyloidosis

Michitaka Nakagawa, Yoshiki Sekijima, Masahide Yazaki, Kana Tojo, Tsuneaki Yoshinaga, Tadashi Doden, Jun Koyama, Shin Yanagisawa & Shu-Ichi Ikeda

To cite this article: Michitaka Nakagawa, Yoshiki Sekijima, Masahide Yazaki, Kana Tojo, Tsuneaki Yoshinaga, Tadashi Doden, Jun Koyama, Shin Yanagisawa & Shu-Ichi Ikeda (2016) Carpal tunnel syndrome: a common initial symptom of systemic wild-type ATTR (ATTRwt) amyloidosis, Amyloid, 23:1, 58-63, DOI: 10.3109/13506129.2015.1135792

To link to this article: http://dx.doi.org/10.3109/13506129.2015.1135792

Published online: 08 Feb 2016.

Amyloid
The Journal of Protein Folding Disorders

http://informahealthcare.com/amy
ISSN: 1350-6129 (print), 1744-2818 (electronic)

Amyloid, 2016; 23(1): 58–63
© 2016 Taylor & Francis. DOI: 10.3109/13506129.2015.1135792

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Carpal tunnel syndrome: a common initial symptom of systemic wild-type ATTR (ATTRwt) amyloidosis

Michitaka Nakagawa[1], Yoshiki Sekijima[1,2], Masahide Yazaki[1,2], Kana Tojo[1], Tsuneaki Yoshinaga[1], Tadashi Doden[1], Jun Koyama[3], Shin Yanagisawa[4], and Shu-Ichi Ikeda[1,2]

[1]*Department of Medicine (Neurology and Rheumatology),* [2]*Institute for Biomedical Sciences,* [3]*Department of Cardiovascular Medicine, and* [4]*Department of Radiology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Japan*

Abstract

Background: Systemic wild-type ATTR (ATTRwt) amyloidosis is a prevalent aging-related disorder. However, a limited number of systemic ATTRwt amyloidosis patients have been diagnosed antemortem, and therefore, the prevalence of ATTRwt is underestimated. Here, we investigated clinical findings of a series of systemic ATTRwt amyloidosis patients with antemortem diagnosis.

Methods: Thirty-one consecutive patients diagnosed with systemic ATTRwt amyloidosis at Shinshu University Hospital were included in this study. Systemic ATTRwt amyloidosis was diagnosed based on proven ATTR amyloid deposition in biopsy specimens and confirmation of wild-type TTR genotype.

Results: The systemic ATTRwt amyloidosis patients consisted of 24 men and seven women, and mean age of onset was 69.8 ± 9.0 years. The most common initial symptom was carpal tunnel syndrome (CTS, 17 patients), followed by heart failure symptoms (14 patients). The mean age at diagnosis was 74.5 ± 8.3 years and the duration of illness from onset to diagnosis was 5.4 ± 4.4 years. Cardiogenic embolism and renal dysfunction are also frequently seen during the course of the disease.

Conclusions: CTS is the most common initial symptom of systemic ATTRwt amyloidosis. Our results suggest the possibility of systemic ATTRwt amyloidosis diagnosis at an early stage by carefully examining patients with CTS.

Abbreviations ATTRwt amyloidosis, wild-type ATTR amyloidosis; BNP, brain natriuretic peptide; CTS, carpal tunnel syndrome; DT, E-wave deceleration time; E/A ratio, ratio of the early to late ventricular filling velocities; ECG, electrocardiogram; E/e' ratio, Ratio of early mitral inflow velocity to mitral annular early diastolic velocity; FS, fractional shortening; IVSThd, diastolic thickness of the interventricular septum; LVDd, left ventricular diastolic diameter; LVEF, left ventricular ejection fraction; PCR, polymerase chain reaction; siRNAs, small interfering RNAs; ^{99m}Tc-PYP, technetium-99m pyrophosphate; TTR, transthyretin

Keywords

Amyloid, carpal tunnel syndrome, senile systemic amyloidosis, transthyretin, wild-type ATTR (ATTRwt) amyloidosis

History

Received 27 September 2015
Revised 20 December 2015
Accepted 21 December 2015
Published online 5 February 2016

Introduction

Systemic wild-type ATTR (ATTRwt) amyloidosis (also called senile systemic amyloidosis) is an incurable sporadic disease caused by the extracellular deposition of amyloid fibrils composed of wild-type transthyretin (TTR) [1]. Systemic ATTRwt amyloidosis is a prevalent aging-related disorder, as 12–25% of people over the age of 80% and 37% of people over the age of 95 were shown to have ATTR deposition in postmortem studies [2–4]. However, a limited number of systemic ATTRwt amyloidosis patients have been diagnosed antemortem, probably due to reduced attention given to ATTRwt amyloidosis as no effective disease-modifying therapies are currently available for this disease. An alternative rationale or explanation for the low rate of antemortem diagnosis is that ATTR amyloid deposits found on autopsy do not contribute to the clinical syndromes that patients experience in a certain proportion of cases.

Recently, however, the clinical effects of TTR tetramer stabilizers, diflunisal [5] and tafamidis [6], on hereditary ATTR amyloidosis were demonstrated in randomized clinical trials, and tafamidis has been approved for the treatment of hereditary ATTR amyloidosis in several European countries, Japan, Argentina and Mexico. In addition, gene therapies with antisense oligonucleotides [7] and small interfering RNAs (siRNAs) [8] are promising strategies for the amelioration of ATTR amyloidosis and are currently in Phase-III

Address for correspondence: Yoshiki Sekijima, Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan. Tel: +81-263-37-2673. Fax: +81-263-37-3427. E-mail: sekijima@shinshu-u.ac.jp

clinical trials. Based on their mechanisms of action, these newly developed therapies are expected to be effective for not only hereditary ATTR amyloidosis but also ATTRwt amyloidosis. Importantly, both TTR tetramer stabilizers and gene silencers are expected to prevent amyloid deposition but do not address amyloid already present. Therefore, early diagnosis and therapy will be required in systemic ATTRwt amyloidosis.

Here, we present the clinical, radiological and pathological findings of a series of systemic ATTRwt amyloidosis patients with antemortem diagnosis and discuss indicators for the early diagnosis of this disease. We reported, for the first time, the high prevalence of carpal tunnel syndrome (CTS) in systemic ATTRwt and showed that CTS is the most common initial symptom of the disease. Our findings suggest the possibility of systemic ATTRwt amyloidosis diagnosis at an early stage by careful examination of patients with CTS.

Materials

Patients

Thirty-one consecutive patients diagnosed with systemic ATTRwt amyloidosis and evaluated at Shinshu University between February 2008 and August 2015 were included in this study. Systemic ATTRwt amyloidosis was diagnosed based on proven ATTR amyloid deposition in biopsy specimens and confirmation of wild-type TTR genotype (i.e. all patients were diagnosed as systemic ATTRwt by Congo red/immunohistochemical analysis and DNA analysis). Patients with localized tenosynovium ATTRwt amyloidosis were excluded from the study.

Methods

Analysis of clinical findings

We performed diagnostic interviews with patients and their family members very carefully at the first medical examination and followed up at least once a year. Additional telephone interviews with patients and their families and inquiries to other hospitals that the patients had consulted were performed in cases in which medical information was insufficient. All patients were evaluated by neurologists, and no ATTRwt patients had obvious cognitive dysfunction. Initial symptoms were defined as symptoms considered to be related to ATTRwt amyloidosis. We regarded cardiac symptoms, cardiogenic embolism, CTS and peripheral neuropathy as ATTRwt-related symptoms based on previous reports. All symptoms related to other diseases were excluded from ATTRwt-related symptoms. Age at onset was defined as the age when ATTRwt-related symptoms first developed. Patient history, laboratory, radiological and pathological findings were retrospectively obtained from medical records.

Congo red and immunohistochemical analysis

Biopsy tissues specimens were analyzed by phenol Congo red and immunohistochemical staining. Immunohistochemical analysis was performed as follows. After deparaffinization, sections were treated with peroxidase-blocking solution (Dako, Glostrup, Denmark) to inhibit endogenous peroxidase activity and with protein block, serum free (Dako, Carpinteria, CA) to inhibit nonspecific binding. Anti-λ (118–134), anti-κ (116–133) [9], anti-AA [10] and anti-TTR (115–124) [11] antibodies were applied to the sections as primary antibodies for 30 minutes at room temperature. The sections were then incubated with EnVision + (Dako) as the secondary antibody for 30 min at room temperature. Immunoreactivity was visualized with DAB + (3,3-diaminobenzidine, Dako).

DNA analysis for detecting TTR mutations

Direct DNA sequencing of the entire TTR gene was performed to detect TTR gene mutations. DNA was extracted from peripheral leukocytes of the patients according to the standard protocol after obtaining written informed consent. All four exons of the TTR gene were amplified by polymerase chain reaction (PCR) and the PCR-amplified DNAs were analyzed by direct sequencing.

Technetium-99m pyrophosphate (^{99m}Tc-PYP) myocardial scintigraphy

Myocardial scintigraphy with ^{99m}Tc-PYP was performed using a triple-head gamma camera (PRISM IRIX; Shimadzu, Kyoto, Japan) equipped with low-energy, high-resolution (LEHR) collimators. Patients received 15–20 mCi of ^{99m}Tc-PYP intravenously, and planar images (anterior, lateral and left anterior oblique views) with the heart centered in the field of view were obtained approximately 3.5 h after injection. The parameters of acquisition were as follows: matrix = 512 × 512, zoom factor = 1.422, acquisition time = 240 s. In addition, single-photon emission computed tomography (SPECT) images were obtained with the following acquisition parameters: matrix = 64 × 64, zoom factor = 1.488. The Butterworth filter was used with a cutoff of 0.24 cycle/pixel and order of 8.00. Results of ^{99m}Tc-PYP myocardial scintigraphy (i.e. positive or negative) were assessed by radiologists.

Statistical analysis

Statistical comparisons were performed between the cardiac symptom onset group and carpal tunnel onset group. Demographic data of both groups were assessed using the χ^2 test for binary outcomes. For continuous variables, we used the Mann–Whitney *U*-test (when data were not normally distributed), Student's *t*-test (when data had a normal distribution with equal variance) or Welch's *t*-test (when data had a normal distribution with unequal variance). All statistical analyses were conducted using SPSS version 18.0J software (SPSS Inc., Chicago, IL). In all analyses, $p<0.05$ was taken to indicate statistical significance.

Results

Demographic characteristics and initial symptoms of ATTRwt amyloidosis patients

The clinical features of systemic ATTRwt amyloidosis patients are summarized in Supplementary Table 1. The systemic ATTRwt amyloidosis patients consisted of 24 men and seven women (male–female ratio, 3.4:1), and the age of

Table 1. Comparison between carpal tunnel onset patients and cardiac onset patients.

	Carpal tunnel onset group	Cardiac onset group	p
Number of individuals	17	14	
Sex (male:female)	14:3	10:4	0.47*
Age at onset (mean ± SD)	66.5 ± 8.6	73.9 ± 7.9	0.01**
Age at diagnosis (mean ± SD)	73.5 ± 8.7	75.8 ± 7.8	0.58***
The duration of illness from onset to diagnosis (mean ± SD)	6.9 ± 4.2	1.9 ± 2.7	<0.0001***

*p Values were obtained using χ^2 test.
**Student's t-test.
***Mann–Whitney U-test.

onset ranged from 44 to 97 years (mean ± SD, 69.8 ± 9.0). The female ratio in our series was higher than those in previous reports (male–female ratio, 25 – 50:1) [12–14]. In addition, the average age of onset in women (79.0 ± 8.5 years old) was significantly older than that in men (67.1 ± 7.2 years old, $p = 0.001$) in our series.

The most common initial symptom was CTS ($n = 17$, 55%), followed by heart failure symptoms ($n = 14$, 45%). The age at diagnosis ranged from 47 to 97 years (mean ± SD, 74.5 ± 8.3) and the duration of illness from onset to diagnosis was 0–16 years (mean ± SD, 5.4 ± 4.4). The duration of illness from first cardiac symptoms to diagnosis was 2.1 ± 2.8 years.

Confirmation of ATTR amyloid deposition by tissue biopsy and myocardial scintigraphy

ATTR amyloid deposition was confirmed by endomyocardial biopsy in 17 patients (55%), gastroduodenal mucosa biopsy in 16 patients (52%), surgical skin biopsy including the deep subcutaneous fat pad in 10 patients (32%) and tenosynovial tissues obtained at carpal tunnel release surgery in six patients (19%). ^{99m}Tc-PYP myocardial scintigraphy was performed in 24 patients and abnormal uptake was observed in 23 patients. The patient that did not have abnormal uptake was a 47-year-old male (patient 20 in Supplementary Table 1) diagnosed based on tenosynovial tissues obtained at carpal tunnel release surgery and gastroduodenal mucosa biopsy. Detailed clinical findings of this patient were described previously [15]. Two patients had abnormal uptake in ^{99m}Tc-PYP myocardial scintigraphy, although they did not have cardiac symptoms or signs.

Cardiac manifestations

At the time of diagnosis, 28 patients (90%) showed cardiac manifestations, including cardiac failure/dilatation ($n = 25$, 81%), bundle branch block/intraventricular conduction delay ($n = 15$, 48%), atrial fibrillation ($n = 13$, 42%), atrioventricular block ($n = 9$, 29%), sick sinus syndrome ($n = 4$, 13%) and ventricular tachycardia/fibrillation ($n = 3$, 10%). Three patients received a permanent pacemaker for complete atrioventricular block and one patient received an implantable cardioverter defibrillator. Plasma level of brain natriuretic peptide (BNP) was 284.6 ± 186.4 pg/mL (normal, ≤20.0).

Twenty-seven patients underwent echocardiography. The diastolic thickness of the interventricular septum (IVSThd) ranged from 9.8 to 24.1 mm (mean ± SD, 19.0 ± 4.5; normal, ≤12.0), and hypertrophy was observed in 24 patients (89%). The left ventricular diastolic diameter (LVDd) ranged from 14.4 to 53.9 mm (mean ± SD, 42.2 ± 7.8; normal, ≤54.0). Left ventricular ejection fraction (LVEF) and fractional shortening (FS) were 43.8%±17.2% (normal, ≥55) and 24.2%±8.5% (normal, ≥28), respectively, and decreased systolic function was observed in 18 patients (67%). Ratio of the early to late ventricular filling velocities (E/A ratio) and E-wave deceleration time (DT) were 1.80 ± 1.04 and 178.6 ± 49.4, respectively. Ratio of early mitral inflow velocity to mitral annular early diastolic velocity (E/e′ ratio) was evaluated in 26 patients and diastolic dysfunction (E/e′≥15) was observed in 23 patients (89%; mean ± SD, 34.7 ± 24.3).

Noncardiac manifestations

CTS was present in 21 patients (68%), seven of whom had a history of carpal tunnel release surgery. Eight patients developed cardiogenic embolism (cerebral infarction, $n = 5$, transient ischemic attack, $n = 1$, renal infarction, $n = 1$ and acute subclavian artery occlusion, $n = 1$) during the course of the illness. Four of eight patients (50%) with cardiogenic embolism had atrial fibrillation. Mild-to-moderate renal dysfunction (decreased glomerular filtration rate) was observed in 14 patients (45%), although no patients developed end-stage kidney disease (EKD) or nephrotic syndrome. Spinal canal stenosis was observed in four patients (13%) and peripheral polyneuropathy was observer in two patients (6%). Symptoms and signs of autonomic dysfunction, macroglossia, or purpura were absent in all patients. Clinical, radiological and pathological findings of a representative patient (patient 13 in Supplementary Table 1) is shown in Figure 1.

Comparison between the carpal tunnel onset group and cardiac onset group

There were no statistically significant differences in sex ratio and age at diagnosis between the carpal tunnel onset group and cardiac onset group. However, the average age of onset in the carpal tunnel onset group (66.5 ± 8.6 years) was much younger than that in the cardiac onset group (73.9 ± 7.9 years, $p = 0.01$, Table 1). In addition, the duration of illness from onset to diagnosis was longer (6.9 ± 4.2 years) in the carpal tunnel onset group than the cardiac onset group (1.9 ± 2.7 years, $p < 0.0001$, Table 1). In the carpal tunnel onset group, CTS preceded cardiac symptoms by 1–14 years (6.1 ± 4.6 years).

Discussion

Although systemic ATTRwt amyloidosis is usually associated with cardiac disease, TTR deposition is not limited to the heart and is found in systemic organs, including the aorta, lung, gastrointestinal tract, liver, kidney and connective tissues [2,16–21]. Especially, recent studies showed that ATTRwt amyloid deposition in joints and ligaments is very common [18–22], and CTS could be an initial symptom of systemic ATTRwt amyloidosis in some patients [15,23–25].

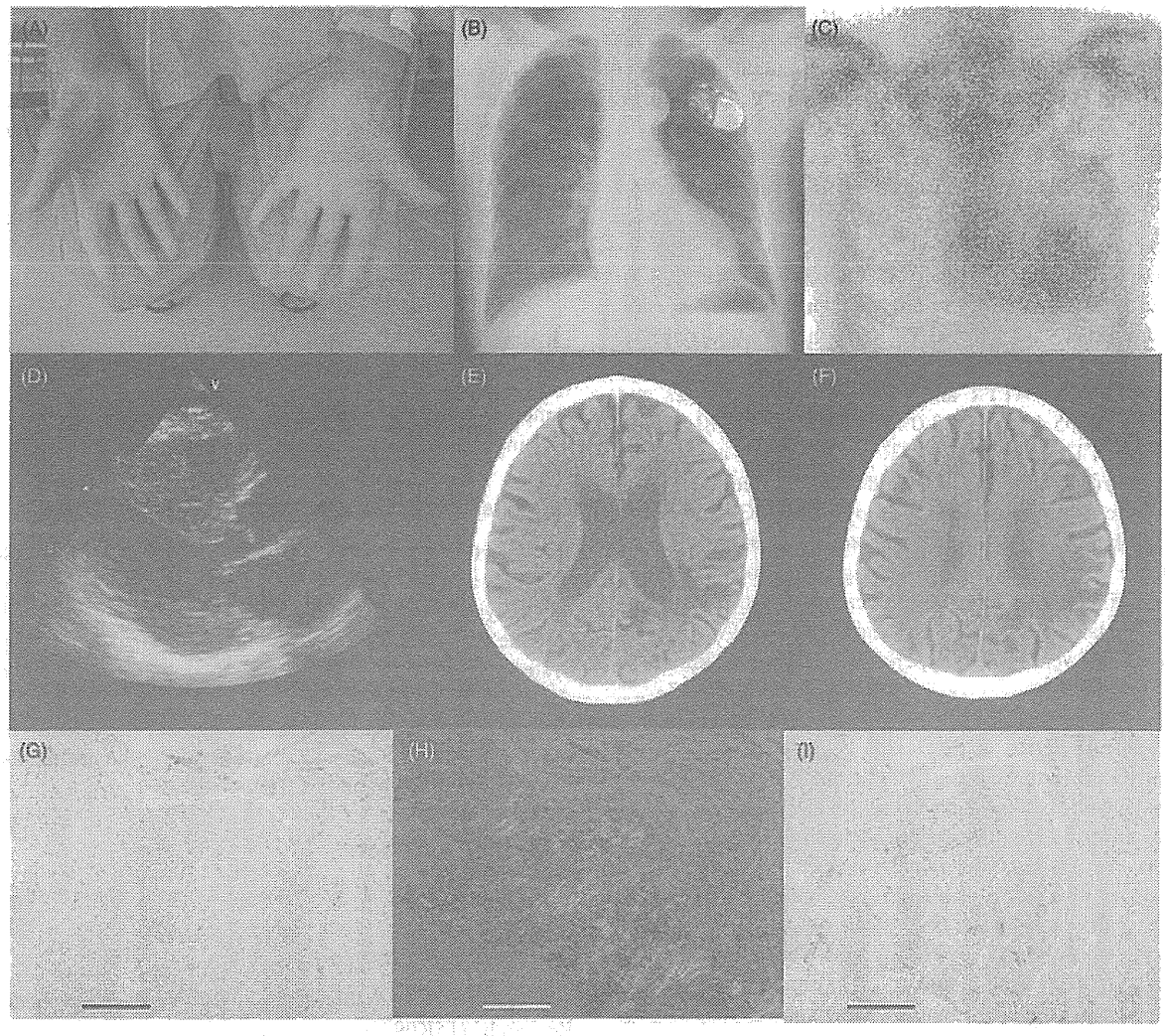


Figure 1. Clinical, radiological and pathological findings of a representative ATTRwt amyloidosis patient starting with carpal tunnel syndrome followed by cerebral embolism, finally showing cardiac amyloidosis. (A) Atrophy of hand muscles, especially in the right thenar muscle. (B) Chest X-ray showing enlarged cardiac shadow and implantation of a pacemaker. (C) Positive technetium-99m pyrophosphate myocardial scintigraphy. (D) Echocardiography showing marked symmetrical thickening of ventricular wall and interventricular septum (thickness of left ventricular posterior wall: 18.7 mm; thickness of interventricular septum: 21.4 mm). (E, F) Brain CT showing an area of low density in the left parietooccipital lobe. (G, H) Myocardial biopsy specimen demonstrating deposits of congophilic materials (G) and apple green birefringence of the deposited materials under polarized view (H). Amyloid deposits were positively immunolabeled with an anti-TTR antibody (I).

However, only one small epidemiological study [13] regarding this issue has been published to date. Rapezzi et al. [13] reported that two of 15 (13%) systemic ATTRwt patients developed CTS. However, the prevalence of CTS may be underestimated as not all patients were evaluated by neurologists. Here, we showed, for the first time, that CTS is the most common initial symptom in systemic ATTRwt amyloidosis by careful neurological examinations. Importantly, CTS preceded cardiac symptoms by 6.1 ± 4.6 years in the carpal tunnel onset group. We have also previously demonstrated that wild-type ATTR deposition is commonly seen in elderly patients with idiopathic CTS [18], suggesting that these patients with localized tenosynovium ATTRwt amyloidosis have the potential to develop systemic ATTRwt amyloidosis in the future. In addition to CTS, several studies [19,21,22] demonstrated that ATTR amyloid deposits were frequently found in the ligamentum flavum of the lumbar spine. Interestingly, spinal canal stenosis was observed in four systemic ATTRwt amyloidosis patients in our series (lumbar spinal canal stenosis, $n = 2$; cervical spinal canal stenosis, $n = 2$), although pathological examination of the ligamentum flavum was not performed.

It is clear that systemic ATTRwt amyloidosis is a prevalent aging-related disorder [2–4]; however, this disorder is rarely recognized before death, and even when diagnosed antemortem, most patients are already in the advanced stage of the disease. At present, there are no effective disease-modifying therapies for ATTRwt amyloidosis, and mean survival period from the onset of congestive heart failure symptoms is 75 months [12]. However, newly developed therapeutic strategies for hereditary ATTR amyloidosis (i.e. TTR tetramer stabilizers and gene therapies) are expected to be effective for ATTRwt amyloidosis. Therefore, early diagnosis of systemic ATTRwt amyloidosis will become critical in the near future. Our results suggest that early-stage systemic ATTRwt amyloidosis may be diagnosed by careful examination of patients

with CTS. It is important to perform pathological investigation (i.e. Congo red staining) of the tenosynovial tissues at carpal tunnel release surgery, especially in patients older than 50 years old [18]. Immunohistochemical analysis should be performed if tenosynovial tissues are positive for amyloid. In previous reports [18,19,26,27], the frequencies of ATTR amyloid deposition in specimens obtained at the time of carpal tunnel release were variable (6.7 – 34%). These discrepancies are likely due to differences in study populations and sensitivity of anti-TTR antibodies. Recently, we analyzed 100 idiopathic CTS patients (average age 67.3 years) and showed that wild-type ATTR deposition on tenosynovial tissues was commonly observed in elderly men (prevalence rates of wild-type ATTR amyloid deposition in males and females were 65.4% and 23.0%, respectively) [18]. When staining for TTR is positive, TTR gene analysis and ^{99m}Tc-PYP myocardial scintigraphy should be performed in addition to routine chest X-ray, electrocardiogram (ECG) and echocardiography, as we detected asymptomatic amyloid deposition in the heart by this type of scintigraphy. Considering the high sensitivity of ^{99m}Tc-PYP myocardial scintigraphy, this test could also be used as a screening test for ATTRwt in patients with ventricular hypertrophy and arrhythmia of unknown etiology.

Another important finding in the present antemortem diagnosed case series is that cardiogenic embolism occurs frequently during the course of systemic ATTRwt amyloidosis. In general, cardiogenic embolism is closely associated with atrial fibrillation, and the prevalence of atrial fibrillation (42%) was very high in our ATTRwt series, as indicated in a recent paper by Longhi et al. [28]. Intriguingly, half of the patients with cardiogenic embolism did not show atrial fibrillation on routine 12-lead ECG and Holter ECG, although patients may have low-frequency paroxysmal atrial fibrillation. Our results indicated that ATTRwt cardiac amyloidosis itself may be a risk factor for cardiogenic embolism and atrial thrombi may be present even during sinus rhythm, as shown in AL amyloidosis [29,30]. Atrial contraction velocities from echo Doppler may be useful to stratify patients regarding the risk of thromboembolism.

In addition, we showed that mild to moderate renal dysfunction is common in patients with systemic ATTRwt amyloidosis. It has been reported that the kidneys in ATTRwt amyloidosis are typically free of amyloid and renal dysfunction is rare [4,31]; however, Westermark et al. reported that seven of 33 patients were uremic in their postmortem series [16]. Renal dysfunction in our patients was milder compared with the previous report [16], probably because we diagnosed patients antemortem. Renal dysfunction in ATTRwt amyloidosis is likely attributable to amyloid deposition in the renal medullary papillae [16]. Other possibilities accounting for renal dysfunction in ATTRwt patients include age related changes and renal dysfunction attributable to poor cardiac output. Additional investigations are required to elucidate the precise pathophysiological mechanism of renal dysfunction in ATTRwt amyloidosis.

Finally, confirmation of the presence of ATTR amyloid deposition by tissue biopsy is necessary for accurate diagnosis of systemic ATTRwt amyloidosis. In most diagnosed cases, tissue samples were obtained by endomyocardial biopsy; however, this is rarely performed due to its high degree of invasiveness, lack of technical expertise and limited availability. Instead, we showed that abnormal uptake of ^{99m}Tc in myocardial scintigraphy is highly suggestive of ATTR cardiac amyloidosis, although histopathological confirmation is necessary. We showed that gastroduodenal mucosa biopsy and surgical skin biopsy including the deep subcutaneous fat pad [32,33], and tenosynovial tissues obtained at carpal tunnel release surgery are useful alternative histopathological tool for diagnosis of ATTRwt amyloidosis.

Limitations

Our study is limited by its retrospective design, the small sample size and single center nature of the sample potentially limiting generalizability.

Conclusions

We reported a series of patients with antemortem diagnosis of ATTRwt amyloidosis, showing that CTS is the most common initial symptom of the disease. Our detailed clinical findings provide novel insights to increase our understanding of systemic ATTRwt amyloidosis. It is likely that systemic ATTRwt amyloidosis is markedly underdiagnosed at present. With the development of new drugs for treatment, major efforts should be made to increase physician awareness of ATTRwt amyloidosis and CTS.

Acknowledgements

The authors thank Ms. E. Nomura for her technical support.

Declaration of interest

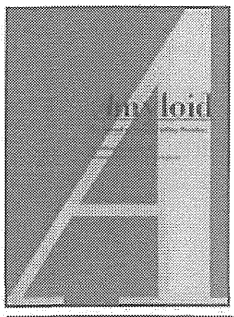
This study was supported by a Grant-in-aid for Scientific Research (15K09336 to YS), a grant from Amyloidosis Research Committee, the Ministry of Health, Labor and Welfare, Japan, and a Group Research Grant for the Pathogenesis and Therapy for Intractable Neuropathy in Japan.

References

1. Westermark P, Sletten K, Johansson B, Cornwell III GG. Fibril in senile systemic amyloidosis is derived from normal transthyretin. Proc Natl Acad Sci USA 1990;87:2843–5.
2. Cornwell III GG, Murdoch WL, Kyle RA, Westermark P, Pitkanen P. Frequency and distribution of senile cardiovascular amyloid. A clinicopathologic correlation. Am J Med 1983;75:618–23.
3. Tanskanen M, Kiuru-Enari S, Tienari P, Polvikoski T, Verkkoniemi A, Rastas S, Sulkava R, et al. Senile systemic amyloidosis, cerebral amyloid angiopathy, and dementia in a very old finnish population. Amyloid 2006;13:164–9.
4. Ueda M, Horibata Y, Shono M, Misumi Y, Oshima T, Su Y, Tasaki M, et al. Clinicopathological features of senile systemic amyloidosis: an ante- and postmortem study. Mod Pathol 2011;24:1533–44.
5. Berk JL, Suhr OB, Obici L, Sekijima Y, Zeldenrust SR, Yamashita T, Heneghan MA, et al. Repurposing diflunisal for familial amyloid polyneuropathy: a randomized clinical trial. JAMA 2013;310:2658–67.
6. Coelho T, Maia LF, Martins da Silva A, Waddington Cruz M, Plante-Bordeneuve V, Lozeron P, Suhr OB, et al. Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial. Neurology 2012;79:785–92.

7. Ackermann EJ, Guo S, Booten S, Alvarado L, Benson M, Hughes S, Monia BP. Clinical development of an antisense therapy for the treatment of transthyretin-associated polyneuropathy. Amyloid 2012;19:43–4.
8. Coelho T, Adams D, Silva A, Lozeron P, Hawkins PN, Mant T, Perez J, et al. Safety and efficacy of rnai therapy for transthyretin amyloidosis. N Engl J Med 2013;369:819–29.
9. Hoshii Y, Setoguchi M, Iwata T, Ueda J, Cui D, Kawano H, Gondo T, et al. Useful polyclonal antibodies against synthetic peptides corresponding to immunoglobulin light chain constant region for immunohistochemical detection of immunoglobulin light chain amyloidosis. Pathol Int 2001;51:264–70.
10. Imada N. Pathological study on amyloidosis. Isolation and purification of amyloid fibril protein and biochemical and immunological analysis. Yamaguchi Med J 1981;30:149–62.
11. Gustavsson A, Engstrom U, Westermark P. Mechanisms of transthyretin amyloidogenesis. Antigenic mapping of transthyretin purified from plasma and amyloid fibrils and within in situ tissue localizations. Am J Pathol 1994;144:1301–11.
12. Ng B, Connors LH, Davidoff R, Skinner M, Falk RH. Senile systemic amyloidosis presenting with heart failure: a comparison with light chain-associated amyloidosis. Arch Intern Med 2005; 165:1425–9.
13. Rapezzi C, Merlini G, Quarta CC, Riva L, Longhi S, Leone O, Salvi F, et al. Systemic cardiac amyloidoses: disease profiles and clinical courses of the 3 main types. Circulation 2009;120:1203–12.
14. Ruberg FL, Berk JL. Transthyretin (TTR) cardiac amyloidosis. Circulation 2012;126:1286–300.
15. Kodaira M, Sekijima Y, Tojo K, Tsuchiya A, Yazaki M, Ikeda S, Hoshii Y, et al. Non-senile wild-type transthyretin systemic amyloidosis presenting as bilateral carpal tunnel syndrome. J Peripher Nerv Syst 2008;13:148–50.
16. Westermark P, Bergstrom J, Solomon A, Murphy C, Sletten K. Transthyretin-derived senile systemic amyloidosis: clinicopathologic and structural considerations. Amyloid 2003;10:48–54.
17. Pitkanen P, Westermark P, Cornwell III GG. Senile systemic amyloidosis. Am J Pathol 1984;117:391–9.
18. Sekijima Y, Uchiyama S, Tojo K, Sano K, Shimizu Y, Imaeda T, Hoshii Y, et al. High prevalence of wild-type transthyretin deposition in patients with idiopathic carpal tunnel syndrome: a common cause of carpal tunnel syndrome in the elderly. Hum Pathol 2011;42:1785–91.
19. Sueyoshi T, Ueda M, Jono H, Irie H, Sei A, Ide J, Ando Y, et al. Wild-type transthyretin-derived amyloidosis in various ligaments and tendons. Hum Pathol 2011;42:1259–64.
20. Takanashi T, Matsuda M, Yazaki M, Yamazaki H, Nawata M, Katagiri Y, Ikeda S. Synovial deposition of wild-type transthyretin-derived amyloid in knee joint osteoarthritis patients. Amyloid 2013;20:151–5.
21. Yanagisawa A, Ueda M, Sueyoshi T, Okada T, Fujimoto T, Ogi Y, Kitagawa K, et al. Amyloid deposits derived from transthyretin in the ligamentum flavum as related to lumbar spinal canal stenosis. Mod Pathol 2015;28:201–7.
22. Westermark P, Westermark GT, Suhr OB, Berg S. Transthyretin-derived amyloidosis: probably a common cause of lumbar spinal stenosis. Ups J Med Sci 2014;119:223–8.
23. Kyle RA, Gertz MA, Linke RP. Amyloid localized to tenosynovium at carpal tunnel release. Immunohistochemical identification of amyloid type. Am J Clin Pathol 1992;97:250–3.
24. Takei Y, Hattori T, Gono T, Tokuda T, Saitoh S, Hoshii Y, Ikeda S. Senile systemic amyloidosis presenting as bilateral carpal tunnel syndrome. Amyloid 2002;9:252–5.
25. Takei Y, Hattori T, Tokuda T, Matsuda M, Saitoh S, Hoshii Y, Ikeda S. Senile systemic amyloidosis starting as bilateral carpal and left ulnar tunnel syndrome. Intern Med 2003;42:1050–1.
26. Stein K, Storkel S, Linke RP, Goebel HH. Chemical heterogeneity of amyloid in the carpal tunnel syndrome. Virchows Arch a Pathol Anat Histopathol 1987;412:37–45.
27. Nakamichi KI, Tachibana S. Amyloid deposition in the synovium and ligament in idiopathic carpal tunnel syndrome. Muscle Nerve 1996;19:1349–51.
28. Longhi S, Quarta CC, Milandri A, Lorenzini M, Gagliardi C, Manuzzi L, Bacchi-Reggiani ML, et al. Atrial fibrillation in amyloidotic cardiomyopathy: prevalence, incidence, risk factors and prognostic role. Amyloid 2015;22:147–55.
29. Dubrey S, Pollak A, Skinner M, Falk RH. Atrial thrombi occurring during sinus rhythm in cardiac amyloidosis: evidence for atrial electromechanical dissociation. Br Heart J 1995;74:541–4.
30. Santarone M, Corrado G, Tagliagambe LM, Manzillo GF, Tadeo G, Spata M, Longhi M. Atrial thrombosis in cardiac amyloidosis: diagnostic contribution of transesophageal echocardiography. J Am Soc Echocardiogr 1999;12:533–6.
31. Kyle RA, Spittell PC, Gertz MA, Li CY, Edwards WD, Olson LJ, Thibodeau SN. The premortem recognition of systemic senile amyloidosis with cardiac involvement. Am J Med 1996;101: 395–400.
32. Ikeda S, Sekijima Y, Tojo K, Koyama J. Diagnostic value of abdominal wall fat pad biopsy in senile systemic amyloidosis. Amyloid 2011;18:211–15.
33. Takashio S, Izumiya Y, Jinnin M, Yamamuro M, Kojima S, Ihn H, Ogawa H. Diagnostic and prognostic value of subcutaneous tissue biopsy in patients with cardiac amyloidosis. Am J Cardiol 2012; 110:1507–11.

Supplementary material available online



Taylor & Francis

Amyloid

The Journal of Protein Folding Disorders

ISSN: 1350-6129 (Print) 1744-2818 (Online) Journal homepage: http://www.tandfonline.com/loi/iamy20

The first pure form of Ostertag-type amyloidosis in Japan: a sporadic case of hereditary fibrinogen Aα-chain amyloidosis associated with a novel frameshift variant

Masahide Yazaki, Tsuneaki Yoshinaga, Yoshiki Sekijima, Saori Nishio, Yuji Kanizawa, Fuyuki Kametani, Kana Miyashita, Naomi Hachiya, Keiichi Higuchi & Shu-ichi Ikeda

To cite this article: Masahide Yazaki, Tsuneaki Yoshinaga, Yoshiki Sekijima, Saori Nishio, Yuji Kanizawa, Fuyuki Kametani, Kana Miyashita, Naomi Hachiya, Keiichi Higuchi & Shu-ichi Ikeda (2015) The first pure form of Ostertag-type amyloidosis in Japan: a sporadic case of hereditary fibrinogen Aα-chain amyloidosis associated with a novel frameshift variant, Amyloid, 22:2, 142-144, DOI: 10.3109/13506129.2015.1037389

To link to this article: http://dx.doi.org/10.3109/13506129.2015.1037389

Published online: 27 May 2015.

Amyloid
The Journal of Protein Folding Disorders

http://informahealthcare.com/amy
ISSN: 1350-6129 (print), 1744-2818 (electronic)

Amyloid, 2015; 22(2): 142–144
 DOI: 10.3109/13506129.2015.1037389

informa
healthcare

LETTER TO THE EDITOR

The first pure form of Ostertag-type amyloidosis in Japan: a sporadic case of hereditary fibrinogen Aα-chain amyloidosis associated with a novel frameshift variant

Masahide Yazaki[1,2], Tsuneaki Yoshinaga[2], Yoshiki Sekijima[1,2], Saori Nishio[3], Yuji Kanizawa[4], Fuyuki Kametani[5], Kana Miyashita[6], Naomi Hachiya[6], Kelichi Higuchi[7,1], and Shu-ichi Ikeda[2,1]

[1]Department of Biological Sciences for Intractable Neurological Disorders, Institute for Biomedical Sciences, Shinshu University, Matsumoto, Japan, [2]Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Matsumoto, Japan, [3]Division of Rheumatology, Endocrinology and Nephrology, Hokkaido University Graduate School of Medicine, Sapporo, Japan, [4]Department of Hematology, Oji General Hospital, Tomakomai, Japan, [5]Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, [6]Department of Neurophysiology, Tokyo Medical University, Tokyo, Japan, and [7]Department of Aging Biology, Institute of Pathogenesis and Disease Prevention, Shinshu University Graduate School of Medicine, Matsumoto, Japan

The various forms of hereditary systemic amyloidosis without peripheral neuropathy have often been referred to as ''Ostertag-type amyloidosis'' [1,2]. The majority of cases have been so far reported in Western countries and this type of amyloidosis is rare in Asia. Here, we report a sporadic case of rapidly progressive nephropathy due to severe renal amyloidosis associated with a novel frameshift mutation in the *fibrinogen Aα-chain* gene.

The patient is a 40-year-old Japanese woman presenting with nephropathy. At age 32, proteinuria was detected on a routine health checkup. She was diagnosed with nephrotic syndrome (serum total protein, 4.5 g/dL, urinary protein, 5.44 g/day). Renal biopsy demonstrated that massive amyloid deposits mainly in the glomeruli (Figure 1A and B). A diagnosis of primary AL amyloidosis was initially made, although there was no evidence of monoclonal gammopathy on immunofixation of serum or urine. Despite combined chemotherapy, the patient showed progressively deterioration of renal function and reached end-stage renal disease 18 months after detection of proteinuria and hemodialysis was begun at age 33. At age 40, she was referred to the Hokkaido University Hospital for further examination. There was no family history suggestive of renal amyloidosis. On physical examination, there were no signs of neuropathy, autonomic dysfunction, cardiomyopathy or gastroenteropathy. To identify the type of renal amyloidosis, we re-investigated a formalin-fixed paraffin embedded renal tissue sample obtained at age 32. We performed laser microdissection of the glomerular amyloid followed by liquid chromatography tandem mass spectrometry-based proteomics analysis [3,4]. In this proteomics study, several tryptic peptides were obtained, corresponding to the carboxyl terminal region of fibrinogen Aα-chain (data not shown). In addition, two tryptic peptides, LSLGAQNLASSQIQR and NPVLITLG, were detected and these corresponded to previously described sequences in the carboxyl terminal region of the variant fibrinogen (position 525–547) induced by a frameshift mutation (c.4904delG) [5]). After obtaining informed consent from the patient, direct DNA sequence analysis of exon 5 of the *fibrinogen Aα-chain* gene was carried out [6], revealing a heterozygous frameshift mutation (c.4899_4902delAGTG), a novel one in fibrinogen-related amyloidosis (Figure 1E). This deletion mutation is predicted to cause an abnormal amino acid sequence from position 523–546 (RLSLGAQNLASSQIQRNPVLITLG), which was identical to our proteomics analysis results. To confirm our results, immunohistochemical analysis was carried out using anti-human fibrinogen antiserum (Dako Agilent Technologies Inc., Denmark) and positive staining was observed mainly in the patient's glomeruli (Figure 1C and D). Thus, she was diagnosed with fibrinogen-related amyloidosis (AFib), associated with a novel 4-bp deletion mutation in the *fibrinogen Aα-chain* gene.

The first case of AFib amyloidosis was described in 1993 by Benson et al. [6], and to date, nine amyloidogenic mutations of *fibrinogen Aα-chain* gene have been described [5–10]. In Western countries, AFib amyloidosis is the leading cause of hereditary non-neuropathic systemic amyloidosis [11]. However, AFib amyloidosis is quite rare in Asia, and only a few cases have been reported in Korea and China [9,10]. Due to variable penetrance, a family history of renal disease or amyloidosis is frequently absent in AFib amyloidosis [10], and therefore, sporadic cases can frequently be misdiagnosed as primary AL amyloidosis [12].

Address for correspondence: Masahide Yazaki, MD and Yoshiki Sekijima, MD, Department of Biological Sciences for Intractable Neurological Disorders, Institute for Biomedical Sciences, Shinshu University, Matsumoto, Japan. Tel: +81-263-37-2673, Fax:+81-263-37-3427. E-mail: mayazaki@shinshu-u.ac.jp (M.Y.), sekijima@shinshu-u.ac.jp (Y.S.)

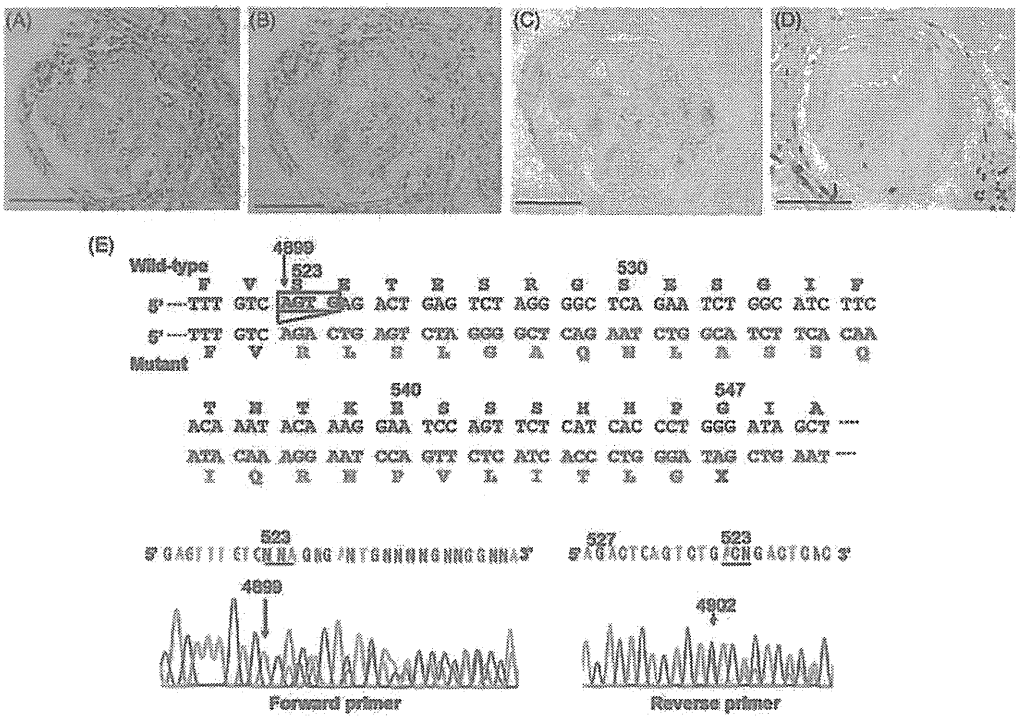


Figure 1. Renal biopsy findings of this patient (A,B,C) and DNA sequencing of exon 5 of *fibrinogen Aα-chain* gene (E) (A) Congo red staining. (B) Congo red staining under polarized light. (C and D) Immunohistochemical staining with anti-human fibrinogen antibody. Renal biopsy at age 32 shows heavy amyloid deposits mainly in the glomeruli with almost complete obliteration of the normal glomerular architecture (A). The deposited amyloid showed typical green birefringence under polarized light (B) and positive staining was observed on immunohistochemistry (C). We also performed immunohistochemistry on glomerular amyloid in an AH amyloidosis patient as a negative control, showing no positive staining (D). Bars = 50 μm (E) DNA sequence analysis of exon 5 of *fibrinogen Aα-chain* gene demonstrated a heterozygous c.4899_4902delAGTG mutation. This novel 4-bp deletion mutation gives a frameshift starting at codon 523 and premature termination at codon 547.

In Japan, there have been no previous reports of Ostertag-type amyloidosis. However, we recently identified two Japanese patients (a mother and her daughter) with gelsolin-related amyloidosis (AGel) associated with the most common amyloidogenic mutation (G654A) of the *gelsolin* gene [13]. Interestingly, both patients mainly presented with severe nephropathy despite heterozygosity of the most prevalent mutation, and the characteristic facial appearance, as seen in patients with Finnish-type familial amyloid polyneuropathy, was not remarkable in these two patients [13]. Thus, only three patients with hereditary renal amyloidosis have been found in Japan to date, including the patient described here. The clinical pictures and renal histopathology of the AGel patients were quite similar to those of Ostertag-type amyloidosis [13], but strictly speaking, their amyloidosis was not a non-neuropathic type because they had very mild cranial and peripheral neuropathy [13]. Of note, all of the AGel patients and our patient had been misdiagnosed with other types of amyloidosis and the time from initial presentation to correct diagnosis of amyloidosis was 8 years (our patient), 10 years [13] and 21 years [13]. A previous study by Stangou et al. [11] showed that median time from the initial presentation to diagnosis of AFib amyloidosis was only 23 months, and unfortunately, this delay is significantly remarkable in Japan, compared with Western countries where Ostertag-type amyloidosis is more prevalent.

Finally, even in Japan, patients with Ostertag-type amyloidosis may certainly be present, but the majority may have been misdiagnosed with other types of systemic amyloidosis.

Declaration of interest

The authors report no conflict of interest. This study was supported by a Grant-in-Aid for Scientific Research in Japan (25461275 to MY, 26670152 to K.H., N.H., M.Y.) and a grant from the Hokuto Foundation for Bioscience.

References

1. Ostertag B. Familäre Amyloid-Erkrankung. Z Menschl Vererbungs Konstit Lehre 1950;30:105–15.
2. Benson MD. Ostertag revisited: the inherited systemic amyloidosis without neuropathy. Amyloid 2005;12:75–87.
3. Sethi S, Theis JD, Vrana JA, Fervenza FC, Sethi A, Qian Q, Quint P, et al. Laser microdissection and proteomic analysis of amyloidosis, cryoglobulinemic GN, fibrillary GN, and immunotoctoid glomerulopathy. Clin J Am Soc Nephrol 2013;8:915–21.
4. Kametani F, Haga S. Accumulation of carboxy-terminal fragments of APP increases phosphodiesterase 8B. Neurobiol Aging 2015;36:634–7.
5. Uemichi T, Liepnieks JJ, Yamada T, Gertz MA, Bang N, Benson MD. A frame shift mutation in the Fibrinogen Aα chain gene in a kindred with renal amyloidosis. Blood 1996;87:4197–203.
6. Benson MD, Liepnieks J, Uemichi T, Wheeler G, Correa R. Hereditary renal amyloidosis associated with a mutant fibrinogen α-chain. Nat Genet 1993;3:252–5.
7. Hamidi Asl L, Liepnieks JJ, Uemichi T, Rebibou JM, Justrabo E, Droz D, Mousson C, et al. Renal amyloidosis with a frame shift mutation in fibrinogen aa-chain gene producing a novel amyloid protein. Blood 1997;90:4799–805.

8. Uemichi T, Liepnieks JJ, Benson MD. Hereditary renal amyloidosis with a novel variant fibrinogen. J Clin Invest 1994;93:731–6.
9. Kang HG, Bybee A, Ha IS, Park MS, Gilbertson JA, Cheong HI, Choi Y, et al. Hereditary amyloidosis in early childhood associated with a novel insertion-deletion (indel) in the fibrinogen Aα chain gene. Kidney Int 2005;68:1994–6.
10. Gillmore JD, Lachmann HJ, Rowczenio D, Gilbertson JA, Zeng CH, Liu ZH, Li LS, et al. Diagnosis, pathogenesis, treatment, and prognosis of hereditary fibrinogen Aα-chain amyloidosis. J Am Soc Nephrol 2009;20:444–51.
11. Stangou AJ, Banner NR, Hendry BM, Rela M, Portmann B, Wendon J, Monaghan M, et al. Hereditary fibrinogen Aα-chain amyloidosis: phenotypic characterization of a systemic disease and the role of liver transplantation. Blood 2010;115:2998–3007.
12. Lachmann HJ, Booth DR, Booth SE, Bybee A, Gilbertson JA, Gillmore JD, Pepys MB, et al. Misdiagnosis of hereditary amyloidosis as AL (primary) amyloidosis. N Engl J Med 2002;346:1786–91.
13. Yamanaka S, Miyazaki Y, Kasai K, Ikeda S, Kiuri-Enari S, Hosoya T. Hereditary renal amyloidosis caused by a heterozygous G654A *gelsolin* mutation: a report two cases. Clin Kidney J 2013;6:189–93.

Nephrol Dial Transplant (2015) 0: 1–8
doi: 10.1093/ndt/gfv276

ndt
Nephrology Dialysis Transplantation

Original Article

Significance of the decreased risk of dialysis-related amyloidosis now proven by results from Japanese nationwide surveys in 1998 and 2010

Junichi Hoshino[1], Kunihiro Yamagata[2], Shinichi Nishi[3], Shigeru Nakai[2], Ikuto Masakane[2], Kunitoshi Iseki[2] and Yoshiharu Tsubakihara[2]

[1]Nephrology Center, Toranomon Hospital, Tokyo, Japan, [2]Committee of Renal Data Registry, Japanese Society for Dialysis Therapy, Tokyo, Japan and [3]Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, Hyogo, Japan

Correspondence and offprint requests to: Kunihiro Yamagata; E-mail: k-yamaga@md.tsukuba.ac.jp

ABSTRACT

Background. Although dialysis technology greatly improved in recent years, it remained unclear whether those improvements helped decrease the incidence of dialysis-related amyloidosis (DRA). Accordingly, we retrospectively compared the incidence of first-time carpal tunnel surgery (CTS)—as proxy for DRA onset—in two cohorts of chronic hemodialysis patients, with the second cohort studied after dialysis methods (especially dialyzate quality control) had greatly improved.

Methods. We used the 1998 and 2010 Japan Renal Data Registries to compare crude risk of first-time CTS the following year. After adjusting for patient background and laboratory data, odds ratios (ORs) for CTS in the whole cohorts and the populations matched by propensity score (PS) for hemodialysis and hemodiafiltration were calculated at a 95% confidence interval.

Results. Of note, 2 02 726 patients were analyzed. In the 1998 cohort, 1.77% experienced first-time CTS compared with 1.30% of the 2010 cohort ($P < 0.001$); with 2010 as referent, the adjusted 1998 OR was 2.22 (1.68–2.95). Both crude risks and adjusted ORs were analyzed by dialysis vintage, age, pre-dialysis β2-microglobulin (β2m) and β2m clearance, risk of CTS trending 1.5–2.0 higher in 1998 than 2010. The reduction was most prominent in patients with longer dialysis vintage, patients who were younger, and those with lower pre-dialysis β2m levels. Similar results were obtained by PS-matched analysis. We also found that β2m clearance >80% may reduce risk of CTS.

Conclusions. The incidence of first-time CTS as proxy for DRA decreased significantly from 1998 to 2010. Several factors may have contributed to this decrease, including improved dialysis methods.

Keywords: amyloidosis, carpal tunnel surgery, dialysis technology, epidemiology, risks

INTRODUCTION

It was generally thought that amyloidosis associated with β2-microglobulin (β2m)—also known as dialysis-related amyloidosis (DRA)—might be an inevitable complication of long-term dialysis, especially for vintages >20 years [1, 2]. Carpal tunnel syndrome (CTS) is the most common presenting feature of DRA [3]. Although not all CTS in dialysis patients are associated with β2m amyloidosis, previous reports have suggested that the presence of β2m amyloid deposition in patients with CTS ranged from 45 to 100% [4, 5]. Reports suggested that, although elevated serum β2m appeared to be a constant, other possible major risk factors for DRA included age, dialysis vintage, chronic inflammation, oxidant stress etc. [6–9]. Because age, dialysis vintage and genetics are naturally unchangeable, attempts to decrease the development of DRA have centered on reducing chronic inflammation and oxidant stress, both considered possibly treatable risk factors.

It was suggested that therapy with high-flux synthetic membranes and/or with hemodiafiltration (HDF) might delay the appearance of clinical signs of β2m amyloidosis [10]. In addition, one center reported that the incidence and severity of DRA decreased by ~80% between 1988 and 1996 [11], suggesting

that improvement in dialysis technology—in this case, dialysis membranes—might play an important role in preventing development of DRA. Unfortunately, the number of patients in this study was very limited, and the observations from the study had never been confirmed.

Over the last two decades, however, dialysis technology has changed dramatically; but the effect of those changes on the risk of DRA has never been persuasively demonstrated. So there is now a clear need to determine whether all those changes have indeed been improvements, and whether they have reduced the incidence of DRA. If so, that finding might help clinicians optimize dialysis treatment.

Accordingly, we designed a study, using nationwide annual surveys in Japan, to evaluate those changes, looking at two large-scale cohorts of patients who received maintenance dialysis in two decades, 12 years apart, to gauge the impact of the putatively improved dialysis technology in preventing first-time carpal tunnel surgery (CTS) as a proxy for the onset of DRA.

MATERIALS AND METHODS

Database creation

The data were obtained from the annual nationwide surveys of dialysis patients conducted by the Japanese Society for Dialysis Therapy (JSDT). The surveys were conducted by JSDT volunteers, with details described previously [12–15]. Briefly, data covered 1 85 322 patients dialyzed at 3095 facilities in the 1998 survey, 1 97 213 patients at 3220 facilities in the 1999 survey, 2 97 126 patients at 4152 facilities in the 2010 survey and 3 04 592 patients at 4205 facilities in the 2011 survey. The study population consisted of two cohorts: patients who had received maintenance dialysis for 12 months, December 1998 through December 1999 (1998 cohort), and those who had received maintenance dialysis from December 2010 through December 2011 (2010 cohort). We excluded patients who had been dialyzed fewer than three times a week or fewer than 2 h per treatment, those who had received peritoneal dialysis or hemodialysis (HD) using a β2m absorption column, those with a history of organ transplantation, those with a history of CTS when follow-up began, and any whose records were incomplete.

After exclusions 2 02 726 patients remained. Demographic data and details of medical history were collected, with information on age, sex, dialysis vintage, height, body weight post dialysis, causes of renal failure, modality of dialysis (HD or HDF). Patients with no history of CTS at the beginning of the observation period and with a CTS recorded by the end of that period were considered to have experienced a first serious onset of CT syndrome (because it was first-time CTS) during the period.

Laboratory measures

Blood samples were drawn and measured at each dialysis center, typically within 24 h, and the most recent values at the time of survey were collected. Most laboratory values were measured monthly—and at least quarterly—including serum urea nitrogen, creatinine, albumin, C-reactive protein (CRP) and hemoglobin before dialysis. In addition, pre/post-dialysis serum β2m, single-pooled *Kt/V* and normalized protein catabolic rate (nPCR) were measured.

Statistical methods

Data were summarized using proportions, with means (±SD) as appropriate. Categorical variables were analyzed with the χ^2 test, and continuous variables were compared using the *t* test or ANOVA as appropriate. Laboratory data were refined using these limits: height 120–200 cm; body weight 20–150 kg; serum urea nitrogen 10–250 mg/dL; creatinine 3–20 mg/dL; albumin 1.0–5.0 g/dL; CRP < 50 mg/dL; hemoglobin 5.0–20.0 g/dL; β2m 10.0–100.0 mg/L; *Kt/V* 0.5–4.0 and nPCR 0.3–2.0. Clearance of β2m was calculated by the decrease of serum β2m values during dialysis (β2m removal), divided by the pre-dialysis β2m value. Logistic regression was used to obtain the adjusted odds ratios (ORs) at 95% confidence interval (95% CI) for first-time CTS. In this analysis, the covariates for calculating the adjusted ORs of 2 survey years were age, dialysis vintage, pre-dialysis β2m and β2m clearance. Other adjusting factors used were sex, dialysis vintage, body mass index (BMI), primary kidney disease, dialysis modality, serum albumin, serum creatinine, hemoglobin, CRP, single-pool *Kt/V* and PCR at the beginning of observation. Analyses adjusting for age at dialysis start, instead of current age, were also performed to calculate ORs for first-time CTS. Covariates whose P-values were <0.2 were included in the multivariate regression analysis. In addition, to minimize selection bias for dialysis modality, propensity score (PS) matching was performed to balance patients' background characteristics, including dialysis vintage, age, sex, primary kidney diseases, BMI, serum urea nitrogen, serum albumin, CRP, hemoglobin, β2m clearance, *Kt/V* and nPCR. To estimate the PS, we used a logistic regression model for the use of HDF as a function of background characteristics as noted above. Each patient who received HDF was matched with a patient who received HD using the nearest-neighbor matching method on the logit scale. If two or more patients with HD met this criterion, we randomly selected one patient for matching. Differences in clinical characteristics between the matched pairs were assessed using the *t* test or analysis of variance (ANOVA) for continuous variables and χ^2 test for categorical variables as appropriate. Adjusted ORs and 95% CI for first-time CTS were also calculated by logistic regressions in the whole population and the PS-matched population. All analyses were carried out using Stata® SE version 12.1 (StataCorp, College Station, TX, USA).

RESULTS

Change of patient characteristics in the decade

After excluding patients based on the criteria cited above, 2 02 726 patients were included in this study. There were 36 489 in the 1998 cohort (18%) and 166 237 in the 2010 cohort. Overall, the cohorts were 38.0% female, mean age 65.1 ± 12.7, mean dialysis vintage 7.2 ± 6.2 years. Background characteristics of these patients are shown in Table 1. The proportion of patients who experienced first-time CTS was higher in the 1998 cohort than in the 2010 cohort (1.77 versus 1.30%, P < 0.001). Several

Table 1. Baseline characteristics of patients in 1998 and 2010 cohorts

Survey year	Total	1998 cohort		2010 cohort		P-value
		CTS (+)	CTS (−)	CTS (+)	CTS (−)	
Number of patients	(*n* = 2 02 726)	(*n* = 647)	(*n* = 35 842)	(*n* = 2,157)	(*n* = 1 64 080)	
Proportion of patients		1.77%		1.30%		<0.001
Sex (female)	38.0%	43.6%	40.5%	50.4%	37.3%	<0.001
		40.5%		*37.4%*		*<0.001*
Dialysis vintage (years)	7.2 ± 6.2	17.4 ± 6.0	7.1 ± 5.3	18.2 ± 9.5	7.0 ± 6.2	<0.001
		7.3 ± 5.4		*7.2 ± 6.4*		*<0.001*
Modality						
Hemodialysis (HD)	95.0%	85.9%	95.2%	86.3%	95.2%	<0.001
HDF	5.0%	14.1%	4.8%	13.7%	4.9%	
		5.0%		*5.0%*		*0.85*
Primary disease						
Glomerulonephritis	41.8%	77.9%	57.3%	62.0%	38.0%	<0.001
Diabetic nephropathy	34.5%	7.6%	24.0%	19.9%	37.2%	
Nephrosclerosis	8.0%	2.3%	5.6%	3.7%	8.6%	
ADPKD	3.7%	2.9%	3.8%	3.2%	3.7%	
Lupus nephritis	0.9%	0.6%	1.1%	1.2%	0.8%	
Others or unknown	11.1%	8.7%	8.2%	10.1%	11.8%	
Age (years)	65.1 ± 12.7	61.0 ± 10.1	60.6 ± 12.9	65.8 ± 9.5	66.1 ± 12.5	<0.001
		60.6 ± 12.9		*66.1 ± 12.4*		*<0.001*
Body mass index (kg/m^2)	21.3 ± 3.5	20.0 ± 2.7	20.6 ± 3.0	20.7 ± 3.3	21.5 ± 3.6	<0.001
		20.6 ± 3.0		*21.5 ± 3.6*		*<0.001*
Urea nitrogen (mg/dL)	66 ± 17	74 ± 17	73 ± 18	66 ± 16	64 ± 16	<0.001
		73.0 ± 18.1		*64.4 ± 15.8*		*<0.001*
Creatinine (mg/dL)	10.6 ± 2.9	10.9 ± 2.9	11.2 ± 3.0	10.5 ± 2.3	10.4 ± 2.9	<0.001
		11.2 ± 3.0		*10.4 ± 2.9*		*<0.001*
Albumin (g/dL)	3.73 ± 0.40	3.81 ± 0.36	3.89 ± 0.40	3.66 ± 0.36	3.70 ± 0.39	<0.001
		3.89 ± 0.40		*3.70 ± 0.39*		*<0.001*
C-reactive protein (mg/dL)	0.45 ± 1.32	0.40 ± 0.62	0.39 ± 0.67	0.54 ± 1.61	0.46 ± 1.40	<0.001
		0.39 ± 0.67		*0.46 ± 1.41*		*<0.001*
Hemoglobin (g/dL)	10.5 ± 1.2	10.3 ± 1.4	10.2 ± 1.3	10.5 ± 1.2	10.6 ± 1.2	<0.001
		10.2 ± 1.3		*10.6 ± 1.2*		*<0.001*
β2m (pre-dialysis) (mg/L)	27.9 ± 7.5	30.7 ± 8.1	32.3 ± 8.8	27.3 ± 6.5	26.9 ± 6.8	<0.001
		32.2 ± 8.8		*26.9 ± 6.8*		*<0.001*
β2m (post-dialysis) (mg/L)	12.5 ± 7.0	17.6 ± 8.9	20.0 ± 10.1	10.2 ± 4.6	10.9 ± 4.8	<0.001
		20.0 ± 10.1		*10.9 ± 4.8*		*<0.001*
β2m clearance (%)	57.7 ± 15.1	46.0 ± 20.7	43.4 ± 19.4	63.7 ± 11.1	60.3 ± 12.4	<0.001
		43.4 ± 19.4		*60.4 ± 12.4*		*<0.001*
Kt/V	1.40 ± 0.30	1.47 ± 0.29	1.39 ± 0.29	1.53 ± 0.30	1.40 ± 0.30	<0.001
		1.39 ± 0.29		*1.41 ± 0.30*		*<0.001*
Normalized PCR	0.89 ± 0.18	1.00 ± 0.18	0.97 ± 0.19	0.92 ± 0.18	0.87 ± 0.18	<0.001
		0.97 ± 0.19		*0.88 ± 0.18*		*<0.001*

The values in italic type in the CTS (+) column for each cohort show the mean values for all patients in the cohort, both with and without CTS. P-values were calculated by χ^2 test or ANOVA as appropriate.

CTS, carpal tunnel surgery; HD, hemodialysis; HDF, hemodiafiltration; ADPKD, autosomal dominant polycystic kidney disease; β2m, beta-2 microglobulin.

significant changes were observed among the patients in the 2010 cohort compared with patients in the 1998 cohort: an increase in mean dialysis vintage [17.4 ± 6.0 (1998)–18.2 ± 9.5 (2010) years]; an increase in the proportion of patients with diabetes; higher mean age; and better β2m dialysis clearance (all P-values were <0.001). At the same time, there were significant decreases from 1998 to 2010 in urea nitrogen, serum albumin, pre-dialysis β2m and post-dialysis β2m (P < 0.001). Similar trends were seen when comparing patients in the two cohorts who did not have CTS.

Distribution of the unadjusted incidence proportion of first-time CTS

Next, we compared the crude incidence proportion (risk) of first-time CTS for dialysis vintage, age, pre-dialysis β2m and β2m clearance. As shown in Figure 1 and Supplementary data, Table S1, the risk of CTS was higher among the 1998 cohort in almost all categories, with the risk curves similar between two cohorts.

When we categorized by dialysis vintage, the crude risk of CTS for the 1998 cohort versus the 2010 cohort with vintage 15–20, 20–25 and >25 years was, respectively, 7.9 (95% CI,6.8–8.9) for 1998 versus 3.4 (3.0–3.7) for 2010; 16.5 (14.3–18.7) versus 7.8 (7.1–8.5); and 26.5 (20.4–32.5) versus 16.6 (15.4–17.9). So even though the shapes of the two cohorts' graphs were similar, when dialysis vintage was >10 years, the crude risk of CTS was only about half as great for 2010 patients. Furthermore, the risk of CTS for 1998 cohort patients with dialysis vintage >10 years was similar to that of 2010 cohort patients 5 years later, implying that improvement in dialysis technology during that period prolonged the onset of need for CTS by roughly 5 years.

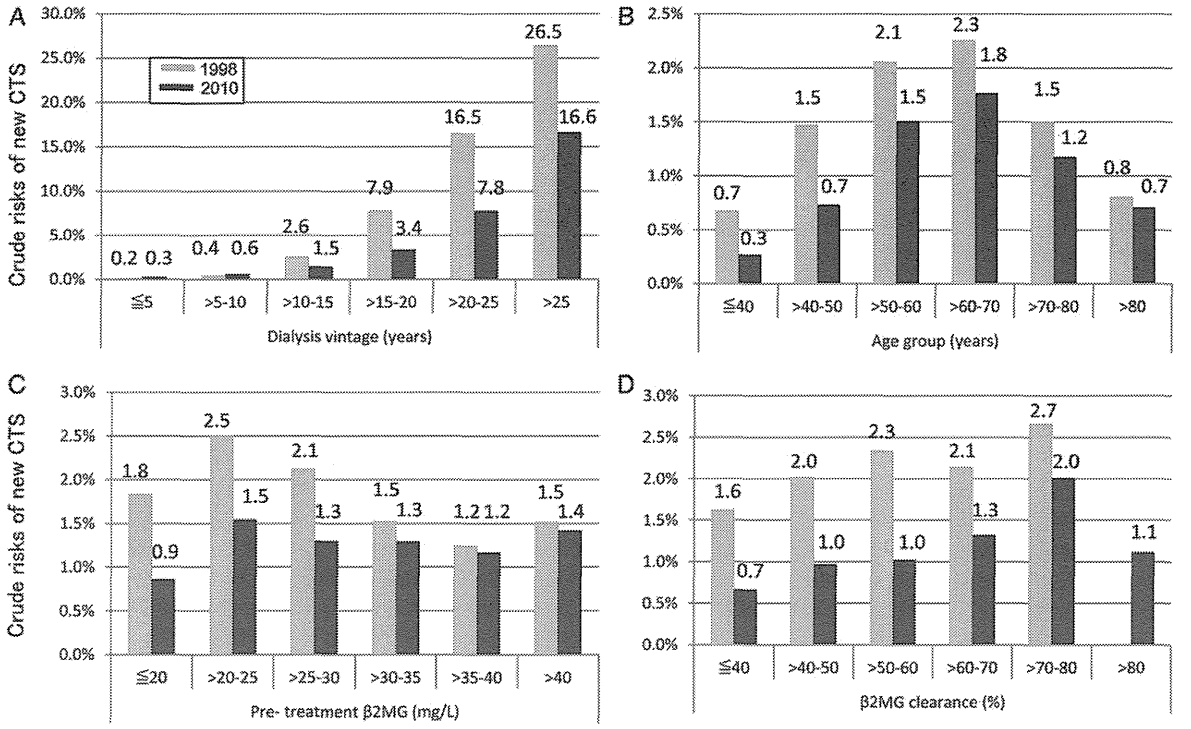


FIGURE 1: Crude risk of new CTS in 1998 and 2010 cohorts by dialysis vintage (A), age (B), pretreatment serum β2m (C) and β2m clearance during dialysis (D).

When we categorized by age group, the crude risk of CTS peaked in the 60–70 age group for both cohorts then tapered off until, >80, there was very little difference between cohorts, whereas among younger patients risk was ~1.5–2.0 times higher in the 1998 cohort.

When we categorized by pre-dialysis β2m, the crude risk of CTS for the 1998 cohort was 1.5–2.0 times higher than that for the 2010 cohort if pre-dialysis β2m was <30 mg/L, but similar with pre-dialysis β2m >30 mg/L.

When we categorized by β2m clearance, the crude risks of CTS in the 1998 cohort were similar in all categories—around 2.0%. As with vintage, although the graph shapes were similar, the risk was 1.5–2.0 times higher for the 1998 cohort.

Distribution of adjusted ORs for first-time CTS

Then we examined adjusted ORs for first-time CTS in the cohorts—and in the PS-matched population—depending on whether the dialysis modality was HD or HDF; comparison by dialysis modality of baseline characteristics of the two populations is shown in Supplementary data, Table S2. The crude risk of CTS was higher in patients with HDF than HD (3.83 versus 1.26%, $P < 0.001$), probably because high-risk patients were more likely to be prescribed HDF. As shown in Table 2 and Figure 2, the OR for first-time CTS in the 1998 cohort versus the 2010 cohort with dialysis vintage 15–20, 20–25 and >25 years (and using the 2010 dialysis vintage 10–15 as referent) was, respectively, 7.61 (4.78–12.09) versus 2.02 (1.44–2.84); 12.58 (7.36–21.50) versus 5.18 (3.68–7.28); and 26.45 (12.08–37.90) versus 11.20 (7.81–16.06). A similar trend was seen when PS-matched (Table 2 and Supplementary data, Figure S1). Every OR in groups with dialysis vintage ≥20 years was more than twice as great for 1998 as for 2010; and, again, the OR for first-time CTS in 1998 was similar to that for 2010 patients ~5–10 years later.

Next, we examined adjusted ORs for first-time CTS by age group. As shown in Table 2, the trend was similar in both cohorts to that for the crude risk analyses—mountain-shaped, peaking at age 60–70—although ORs in 2010 were less than half of those for the same age group in 1998. Results for the PS-matched populations were similar, although in the 1998 cohort, the OR for age group ≤40 was higher than for the 40–50 age group. In all, these results suggest that risk of CTS decreased by almost half in every age group between 1998 and 2010, with the decrease apparently larger for younger patients (Table 2 and Supplementary data, Figure S1).

Then we examined adjusted ORs for first-time CTS by pre-dialysis β2m level. In the 1998 cohort, the ORs decreased as pre-dialysis β2m increase, but these ORs were similar in the 2010 cohort. With PS matching, in the 1998 cohort the difference in OR between both lower and higher pre-dialysis β2m groups and the middle groups increased and the difference in OR between the 1998 and 2010 cohorts in the highest pre-dialysis β2m groups was getting smaller, although the overall trend was similar (Table 2 and Supplementary data, Figure S1).

Finally, we examined adjusted ORs for first-time CTS by β2m clearance. The ORs decreased as β2m clearance increased ≥60% in both the 1998 and 2010 cohort, and much decrease was seen when β2m clearance reached ≥80%. With PS matching, a similar trend was seen in the 2010 cohort. But in the PS-matched 1998 cohort, ORs were mountain-shaped, peaking in the middle range of β2m clearance; it remains unclear why that was true only for 1998, and not 2010. Because of this anomaly, we also examined

Table 2. Adjusted odds ratio for first-time CTS in source and propensity-matched population

	Source population						Propensity-matched population					
	1998			2010			1998			2010		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Overall	2.22	(1.68, 2.95)	<0.001	1.00			2.89	(1.70, 4.92)	*<0.001*	1.00		
Dialysis vintage (years)												
≤5	0.03	(0.01, 0.26)	*0.001*	0.16	(0.11, 0.24)	*<0.001*	n/a			0.06	(0.01, 0.29)	*<0.001*
≤10	0.24	(0.10, 0.61)	*0.00*	0.36	(0.25, 0.51)	*<0.001*	n/a			0.32	(0.13, 0.78)	*0.01*
≤15	2.81	(1.72, 4.61)	*<0.001*	1.00			1.53	(0.47, 5.01)	*0.49*	1.00		
≤20	7.61	(4.78, 12.09)	*<0.001*	2.02	(1.44, 2.84)	*<0.001*	6.68	(2.69, 16.57)	*<0.001*	1.31	(0.63, 2.73)	*0.46*
≤25	12.58	(7.36, 21.50)	*<0.001*	5.18	(3.68, 7.28)	*<0.001*	11.16	(4.17, 29.89)	*<0.001*	3.76	(1.88, 7.52)	*<0.001*
≤30	26.45	(12.08, 57.90)	*<0.001*	11.20	(7.81, 16.06)	*<0.001*	36.28	(10.82, 121.65)	*<0.001*	5.85	(2.80, 12.21)	*<0.001*
>30	n/a			17.56	(11.52, 26.77)	*<0.001*	n/a			13.09	(6.19, 27.71)	*<0.001*
Age group (years)												
≤40	0.85	(0.20, 3.62)	*0.83*	0.15	(0.02, 1.06)	*0.06*	3.46	(0.71, 16.75)	*0.12*	n/a		
≤50	1.19	(0.61, 2.33)	*0.61*	0.57	(0.34, 0.95)	*0.03*	1.84	(0.65, 5.18)	*0.25*	0.59	(0.25, 1.40)	*0.23*
≤60	2.41	(1.54, 3.76	*<0.001*	1.00			3.14	(1.41, 6.97)	*0.01*	1.00		
≤70	3.64	(2.34, 5.68)	*<0.001*	1.70	(1.29, 2.23)	*<0.001*	4.42	(1.75, 11.20)	*0.002*	1.94	(1.17, 3.20)	*0.01*
≤80	2.93	(1.55, 5.56)	*0.001*	1.45	(1.06, 1.99)	*0.02*	2.97	(0.60, 14.71)	*0.18*	1.34	(0.69, 2.61)	*0.39*
80<	2.14	(0.50, 9.19)	*0.31*	0.97	(0.58, 1.62)	*0.91*	n/a			0.60	(0.13, 2.73)	*0.51*
Pre-dialysis β2m (mg/L)												
≤20	3.77	(1.67, 8.49)	*0.001*	1.01	(0.64, 1.57)	*0.98*	4.25	(0.66, 27.21)	*0.13*	0.78	(0.29, 2.09)	*0.62*
>20–25	3.10	(1.87, 5.15)	*<0.001*	1.00			7.59	(2.92, 19.73)	*<0.001*	1.00		
>25–30	1.98	(1.26, 3.13)	*0.003*	0.86	(0.65, 1.15)	*0.31*	2.85	(1.18, 6.86)	*0.02*	1.06	(0.57, 1.97)	*0.85*
>30–35	1.70	(1.03, 2.79)	*0.04*	1.01	(0.71, 1.43)	*0.98*	2.28	(0.75, 7.00)	*0.15*	0.96	(0.43, 2.13)	*0.92*
>35–40	1.87	(1.03, 3.37)	*0.04*	1.08	(0.67, 1.74)	*0.75*	1.90	(0.49, 7.42)	*0.36*	1.05	(0.36, 3.04)	*0.94*
>40	1.52	(0.77, 2.97)	*0.22*	1.01	(0.55, 1.84)	*0.98*	1.17	(0.23, 5.92)	*0.85*	0.87	(0.25, 3.06)	*0.83*
β2m clearance (%)												
≤40	2.15	(1.31, 3.52)	*0.002*	0.94	(0.52, 1.70)	*0.85*	0.63	(0.06, 6.71)	*0.71*	2.18	(0.31, 15.27)	*0.43*
≤50	2.17	(1.16, 4.04)	*0.02*	1.00			1.77	(0.30, 10.45)	*0.53*	1.00		
≤60	2.56	(1.39, 4.73)	*0.003*	0.93	(0.61, 1.42)	*0.73*	3.92	(0.87, 17.58)	*0.07*	0.95	(0.23, 3.82)	*0.94*
≤70	1.59	(0.85, 3.00)	*0.15*	0.80	(0.54, 1.20)	*0.28*	1.96	(0.47, 8.19)	*0.35*	0.49	(0.13, 1.81)	*0.28*
≤80	1.46	(0.60, 3.53)	*0.41*	0.74	(0.48, 1.14)	*0.17*	1.47	(0.31, 6.94)	*0.63*	0.58	(0.16, 2.11)	*0.41*
>80	n/a			0.29	(0.11, 0.74)	*0.01*	n/a			0.32	(0.07, 1.48)	*0.14*
β2m removal (mg/l)												
q1 (min)	2.82	(1.86, 4.29)	*<0.001*	0.97	(0.55, 1.71)	*0.91*	2.24	(0.25, 20.04)	*0.47*	2.95	(0.45, 19.24)	*0.26*
q2	2.15	(1.24, 3.72)	*0.01*	1.24	(0.85, 1.80)	*0.26*	4.15	(0.99, 17.39)	*0.05*	2.51	(0.96, 6.54)	*0.06*
q3	2.49	(1.28, 4.82)	*0.01*	1.00			9.44	(2.93, 30.42)	*<0.001*	1.00		
q4	3.04	(1.71, 5.41)	*<0.001*	1.02	(0.73, 1.41)	*0.91*	5.22	(1.72, 15.90)	*0.004*	1.00	(0.45, 2.20)	*1.00*
q5	1.62	(0.74, 3.54)	*0.23*	0.93	(0.65, 1.33)	*0.71*	1.55	(0.37, 6.44)	*0.55*	0.94	(0.40, 2.25)	*0.90*
q6 (max)	1.81	(0.88, 3.75)	*0.11*	1.05	(0.71, 1.54)	*0.82*	2.99	(0.89, 10.05)	*0.08*	1.15	(0.45, 2.96)	*0.77*

β2m removal = (pre-dialysis β2m) − (post-dialysis β2m); β2m clearance = (β2m removal)/(pre-dialysis β2m).
OR, odds ratio; CI, confidence interval; β2m, beta-2 microglobulin.
These ORs were adjusted by dialysis vintage, age, sex, primary kidney disease, modality, body mass index, albumin, CRP, Kt/V, normalized PCR, and β2m clearance. In the analysis including pre-dialysis β2m, pre-dialysis of β2m and β2m removal were used instead of β2m clearance.

the association between β2m removal per dialysis (pre-dialysis minus post-dialysis β2m) and first-time CTS; and again, the adjusted ORs were similarly mountain-shaped in the 1998 cohort but not in the 2010 cohort (Table 2 and Supplementary data, Figure S1). One possible explanation is that less β2m clearance or removal may be associated with mortality, which may cause underestimation of ORs for the lower β2m clearance groups. Note that these results were very similar when we used age at dialysis start, instead of current age, to calculate adjusted ORs for first-time CTS (Supplementary data, Figure S2).

DISCUSSION

In this study, we examined nationwide survey data to determine whether risk of DRA decreased in the period between 1998 and 2010, and whether improvement of dialysis technologies, which had been dramatic in the decades of the 1990s and 2000s, did in fact help prevent first-time CTS, which can be considered a proxy for the onset of DRA. In Japan, biocompatible membranes became standard in the 1980s, use of high-flux membranes became widespread in 1990s, and reverse osmosis and endotoxin-cut filters came into use in the late 1990s [15]. Based on the JSDT surveys, the rate of reverse osmosis usage was already at 93.0% by 1999 although the proportion of facilities that attained an endotoxin concentration below 0.001 EU/mL—which is recommended by the JSDT ultrapure dialyzate quality-control standard—stood at only 13.4% in 1999 [16]. This, however, dramatically increased to 62.1% in 2010 [12]. These improvements suggested a distinct need for the investigation we made. Several studies had demonstrated a decrease in DRA associated with using synthetic high-flux membranes [10] and ultrapure dialyzates [17–19]. However, there was no large-scale data to confirm that what was thought to be

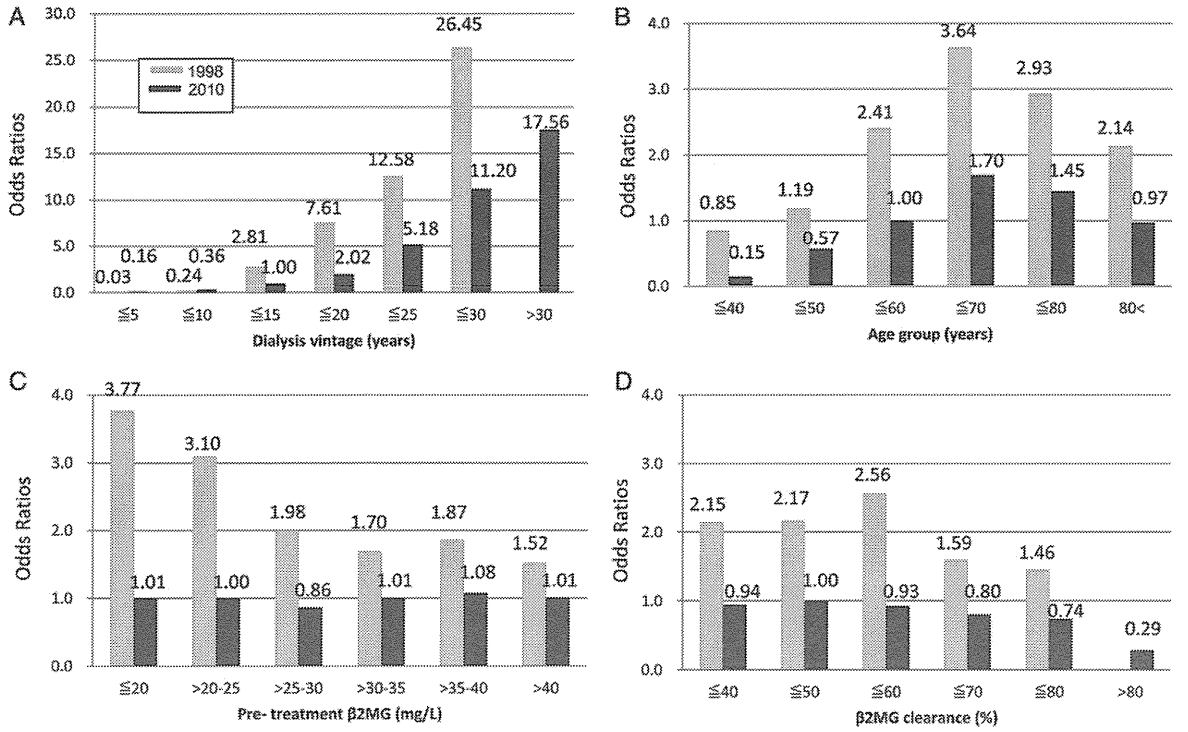


FIGURE 2: Adjusted OR of CTS in the whole two populations in 1998 and 2010, by dialysis vintage (A), age (B), pretreatment serum β2m (C) and β2m clearance (D). These ORs were adjusted by dialysis vintage, age, sex, primary kidney disease, modality, body mass index, albumin, CRP, *Kt/V*, normalized PCR and β2m clearance. In the analysis including pre-dialysis β2m, pre-dialysis of β2m and β2m removal were used instead of β2m clearance.

improvement in dialysis technologies did indeed help prevent DRA. Should more patients be given the resulting higher-quality dialysis? Our analyses show that the OR of the onset of DRA within the following year was significantly lower in the 2010 cohort than in the 1998 cohort after adjusting patients' background, laboratory values and dialysis methods in both the whole population and PS-matched population.

The main strengths of our study are that the analyses were of nationwide large-scale cohorts with long dialysis vintage, comparing two sequential surveys using the same method of data collection and same outcome measurement. Because DRA formation is a long process, large-scale cohorts with long dialysis vintage are essential for clarification of this kind of research question. In addition, our study compared two contrasting cohorts: the one in 1998, when the most dramatic improvements in dialysis technology were still developing, and the one in 2010, by which time ultrapure dialysis solutions and biocompatible membranes had become widely used. DRA had grown to a severe problem in the early 1980s when the number of patients surviving >10 years on dialysis grew. The response in the 1990s was the above-noted process of improving dialysis technology once the principal risk factors for DRA—dialysis vintage, age, biocompatibility of dialysis solution and membrane and advanced glycation end product—were clearly identified [1, 3, 17, 20].

There are several other new findings in this study. First, the trends of crude risk for first-time CTS surgery when analyzed by dialysis vintage, age, pre-dialysis β2m or β2m clearance were similar in the 1998 and 2010 cohort: in the 2010 cohort, when analyzed by dialysis vintage, that risk decreased by almost half from 1998. Second, the beneficial effect of improved dialysis technology may mainly affect patients with longer dialysis vintage, younger age, lower pre-β2m and less β2m clearance. Put another way, the crude risk of first-time CTS was similar in the two cohorts for patients with shorter dialysis vintage, much greater age, higher pre-β2m and higher β2m clearance. Finally, β2m clearance >80% may decrease risk of DRA which is consistent with our previous findings.

There are several limitations to this study. First, because our survey was questionnaire-based, information bias may exist. However, considering the large number of patients in this study, the proportion of bias should be very small. In addition, we used the same outcome for the surveys. Second, although this is a large-scale cohort study, the 1998 cohort comprised only 18% of the study population. That was because information about their history was missing for many 1998 patients —requiring their exclusion—which may cause some selection bias. Third, information about the dialysis membrane and dialysis solution used was not recorded, so we could not pinpoint with certainty the improvements in dialysis technology that decreased risk of DRA (though, of course, the chief role of those membranes and solutions was, at very least, strongly inferential since they were the focus of the improvement efforts). Finally, we did not have information about residual renal function, erythropoiesis stimulating agents, iron use or vitamin D use, which were considered as possible confounders. However, since the reported loss of renal function after dialysis start was

~2.0 mL/min/year [21] and the mean estimated glomerular filtration rate at dialysis initiation was 6.52 mL/min/1.73m^2 in 2007 throughout Japan [22], the impact of residual renal function on our cohort (mean dialysis vintage 7.2 ± 6.2 years) may be neglected.

Previously, we had found that dialysis vintage is a very strong risk factor for developing DRA, and that patients' age, lower serum albumin, and low β2m clearance—but not pre-dialysis β2m—are other risk factors [15]. We had also found that β2m clearance ≥80% is associated with lower risk of DRA, although it was unclear why that was so. This present study provides one possible answer. In this study, reduction in risk of first-time CTS between the two cohorts was greater for patients with longer dialysis vintage, younger age, lower pre-dialysis β2m and less β2m clearance. These findings imply that the decreased risk of CTS in our study period may be associated with the reduction of immunological reaction—usually stronger among younger patients—affected by the improvement in dialysis. But the immunological reaction may not be represented by CRP, since serum CRP level was not associated with the first-time CTS in this or in previous studies [15]. It had always been thought that lowering the level of pre-dialysis serum β2m, which is a main component of DRA, was a treatment goal for preventing DRA and improved biocompatible membranes could have resulted in lower production of β2m with the consequence of slower DRA production [10]. This explanation was based on *in vitro* studies showing that monocytes in culture released more β2m after exposure to Cuprophan than with exposure to the synthetic membrane polymethyl methacrylate [23]. However, based on our findings, lowering pre-dialysis β2m is not associated with reduced risk of CTS. Once the nucleus is formed, the growth phase proceeds through the incorporation of the monomers into the ends of seed fibrils in a template-dependent manner [24]. In addition, advanced glycation end-products, inflammation and oxidant stress, both of which are considered risk factors for DRA, play an important role in developing amyloid fibrils [25–27]. Garbar *et al.* [28] reported that tissue alteration caused by advanced glycation end-products and other modifications linked to the uremic state may favor β2m amyloid deposition following macrophage activation and result in juxta-articular bone erosions. Therefore, given our findings—in conjunction with previous knowledge—to prevent the onset of DRA, it seems important to reduce immunological reaction by using more biocompatible dialysis membrane and purer dialysis solution, not by decreasing pre-dialysis β2m. And β2m clearance may be efficacious in dialysis for removing more uremic toxins, cytokines and other immunogens.

In conclusion, our study shows a dramatic reduction in DRA risk in the last two decades: the risk of first-time CTS, as proxy for the onset of DRA, was about halved. Although the mechanisms by which β2m amyloid fibrils develop are still not fully known, it is clear at present that the efforts aimed to improve dialysis quality paid off in the now *proven*, statistically significant decrease in DRA risk, based on a large-size dialysis patient cohort. Yet such high quality dialysis is still not universally available. We hope that the present evidence of the delayed DRA onset will lead to the implementation of improved dialysis technology wherever available, both from an economic and a logistic point of view, for the benefit of dialysis patients worldwide.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

ACKNOWLEDGEMENTS

We thank the members of the committee of the JSDT Renal Data Registry for all their efforts, the staff members of dialysis facilities who participated in the survey and responded to the questionnaires, and Ms Chiho Iseki (PhD) for data management. This study was supported in part by J.H.'s research grant from the Okinaka Memorial Institute for Medical Research from Toranomon hospital, and in part by a Grant-in-Aid for Amyloidosis research, Research on Intractable Disease, from the Ministry of Health, Labor, and Welfare of Japan (H23-intractable-general-012).

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Jadoul M. Dialysis-related amyloidosis: importance of biocompatibility and age. Nephrol Dial Transplant 1998; 13(Suppl. 7): 61–64
2. Koch KM. Dialysis-related amyloidosis. Kidney Int 1992; 41: 1416–1429
3. Gejyo F, Suzuki S, Arakawa M. Dialysis-related amyloidosis and clinical significance of extracorporeal removal of beta2-microglobulin. Ther Apher 1997; 1: 126–128
4. Schwarz A, Keller F, Seyfert S *et al.* Carpal tunnel syndrome: a major complication in long-term hemodialysis patients. Clin Nephrol 1984; 22: 133–137
5. Kopec J, Gadek A, Drozdz M *et al.* Carpal tunnel syndrome in hemodialysis patients as a dialysis-related amyloidosis manifestation—incidence, risk factors and results of surgical treatment. Med Sci Monit 2011; 17: CR505–9
6. Gejyo F, Narita I. Current clinical and pathogenetic understanding of beta2-m amyloidosis in long-term haemodialysis patients. Nephrology (Carlton) 2003; 8(Suppl): S45–S49
7. Cruz DN, de Cal M, Ronco C. Oxidative stress and anemia in chronic hemodialysis: the promise of bioreactive membranes. Contrib Nephrol 2008; 161: 89–98
8. Busch M, Schwenzky A, Franke S *et al.* Advanced glycation end products and beta(2)-microglobulin as predictors of carpal tunnel syndrome in hemodialysis patients. Blood Purif 2012; 34: 3–9
9. Lonnemann G, Koch KM. Beta(2)-microglobulin amyloidosis: effects of ultrapure dialysate and type of dialyzer membrane. J Am Soc Nephrol 2002; 13(Suppl 1): S72–S77
10. van Ypersele de Strihou C, Jadoul M, Malghem J *et al.* Effect of dialysis membrane and patient's age on signs of dialysis-related amyloidosis. The Working Party on Dialysis Amyloidosis. Kidney Int 1991; 39: 1012–1019
11. Schwalbe S, Holzhauer M, Schaeffer J *et al.* Beta 2-microglobulin associated amyloidosis: a vanishing complication of long-term hemodialysis? Kidney Int 1997; 52: 1077–1083
12. Nakai S, Iseki K, Itami N *et al.* An overview of regular dialysis treatment in Japan (as of 31 December 2010). Ther Apher Dial 2012; 16: 483–521

13. Iseki K, Nakai S, Yamagata K *et al.* Tachycardia as a predictor of poor survival in chronic haemodialysis patients. Nephrol Dial Transplant 2011; 26: 963–969
14. Shoji T, Masakane I, Watanabe Y *et al.* Elevated non-high-density lipoprotein cholesterol (non-HDL-C) predicts atherosclerotic cardiovascular events in hemodialysis patients. Clin J Am Soc Nephrol 2011; 6: 1112–1120
15. Hoshino J, Yamagata K, Nishi S *et al.* Carpal tunnel surgery as proxy for dialysis-related amyloidosis: results from the Japanese society for dialysis therapy. Am J Nephrol 2014; 39: 449–458
16. Nakai S, Sinzato T, Sanaka T *et al.* An overview of dialysis treatment in Japan (as of Dec. 31, 1999). Ther Apher Dial 2001; 34: 1–13
17. Baz M, Durand C, Ragon A *et al.* Using ultrapure water in hemodialysis delays carpal tunnel syndrome. Int J Artif Organs 1991; 14: 681–685
18. Koda Y, Nishi S, Miyazaki S *et al.* Switch from conventional to high-flux membrane reduces the risk of carpal tunnel syndrome and mortality of hemodialysis patients. Kidney Int 1997; 52: 1096–1101
19. Kleophas W, Haastert B, Backus G *et al.* Long-term experience with an ultrapure individual dialysis fluid with a batch type machine. Nephrol Dial Transplant 1998; 13: 3118–3125
20. Kuchle C, Fricke H, Held E *et al.* High-flux hemodialysis postpones clinical manifestation of dialysis-related amyloidosis. Am J Nephrol 1996; 16: 484–488
21. Schiffl H, Lang SM, Fischer R. Ultrapure dialysis fluid slows loss of residual renal function in new dialysis patients. Nephrol Dial Transplant 2002; 17: 1814–1818
22. Watanabe Y, Yamagata K, Nishi S *et al.* Japanese society for dialysis therapy clinical guideline for "hemodialysis initiation for maintenance hemodialysis". Ther Apher Dial 2015; 19(Suppl 1): 93–107
23. Zaoui PM, Stone WJ, Hakim RM. Effects of dialysis membranes on beta 2-microglobulin production and cellular expression. Kidney Int 1990; 38: 962–968
24. Naiki H, Hashimoto N, Suzuki S *et al.* Establishment of a kinetic model of dialysis-related amyloid fibril extension in vitro. Amyloid 1997; 4: 223–232
25. Niwa T, Katsuzaki T, Momoi T *et al.* Modification of beta 2 m with advanced glycation end products as observed in dialysis-related amyloidosis by 3-DG accumulating in uremic serum. Kidney Int 1996; 49: 861–867
26. Miyata T, Inagi R, Iida Y *et al.* Involvement of beta 2-microglobulin modified with advanced glycation end products in the pathogenesis of hemodialysis-associated amyloidosis. Induction of human monocyte chemotaxis and macrophage secretion of tumor necrosis factor-alpha and interleukin-1. J Clin Invest 1994; 93: 521–528
27. Matsuo K, Ikizler TA, Hoover RL *et al.* Transforming growth factor-beta is involved in the pathogenesis of dialysis-related amyloidosis. Kidney Int 2000; 57: 697–708
28. Garbar C, Jadoul M, Noel H *et al.* Histological characteristics of sternoclavicular beta 2-microglobulin amyloidosis and clues for its histogenesis. Kidney Int 1999; 55: 1983–1990

Received for publication: 16.4.2015; Accepted in revised form: 15.6.2015

［VI］班構成員名簿