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Time-Dependent Analysis of Sicca Symptoms and Anti-Ro/SSA and Anti-La/SSB Antibodies in Patients with AQP4-IgG-Positive Neuromyelitis Optica Spectrum Disorder

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Anti-aquaporin-4 antibody (AQP4-IgG)-positive neuromyelitis optica spectrum disorder (NMOSD) and Sjögren syndrome (SS) are likely comorbidities. However, the exact effects of age and disease duration on the positivity rates of serum anti-Ro/SSA and anti-La/SSB (anti-SSA/SSB) antibodies and the presence of sicca symptoms in patients with AQP4-IgG remain unknown. In the present study, we evaluated the data from patients with suspected NMOSD who had neurological episodes and tested for serum AQP4-IgG. Associations between the presence of serum AQP4-IgG and SS-related findings were evaluated. The presence of anti-SSA/SSB antibodies [odds ratio (OR), 7.34; 95% confidence interval (CI), 5.71-9.43; $p < 0.0001$] and that of sicca symptoms (OR, 2.08; 95% CI, 1.67-2.58; $p < 0.0001$) were both higher in patients with AQP4-IgG (n = 1,651) than in those without AQP4-IgG (n = 2,796). Meanwhile, neither age nor the elapsed time from neurological onset was linked to the prevalence of anti-SSA/SSB antibodies or sicca symptoms, and the prevalence rates of the SS-related factors were elevated since the onset of neurological episodes in those with AQP4-IgG. The frequency of sicca symptoms among those with anti-SSA/SSB antibodies was irrespective of AQP4-IgG (OR, 1.11; 95% CI, 0.67-1.85; $p = 0.6892$). The measured AQP4-IgG titers did not differ significantly according to the presence of anti-SSA/SSB antibodies ($p = 0.2386$; Mann-Whitney U test). In summary, age and duration of NMOSD were not the factors producing an elevated prevalence of anti-SSA/SSB antibodies and sicca symptoms in patients with AQP4-IgG, implying that the occurrence of comorbid SS is likely to temporarily precede or synchronize with the onset of AQP4-IgG-positive NMOSD.

Keywords: anti-aquaporin-4 antibodies (AQP4-IgG); anti-Ro/SSA and anti-La/SSB antibodies; neuromyelitis optica spectrum disorder (NMOSD); sicca symptoms; Sjögren syndrome
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Introduction

Neuromyelitis optica spectrum disorder (NMOSD) is an autoimmune relapsing neurological disease of the central nervous system (CNS) that typically affects the optic nerves, medulla oblongata, and spinal cord. The disease is characterized by the presence of serum anti-aquaporin-4 antibodies (AQP4-IgG), which are observed in most patients with NMOSD (Lennon et al. 2005; Wingerchuk et al. 2015). The comorbidity rate between AQP4-IgG-positive NMOSD and Sjögren syndrome (SS) is significantly higher than the expected rate based on coincidental comorbidities (Pittock et al. 2008; Akaishi et al. 2021). Moreover, patients with AQP4-IgG-positive NMOSD and comorbid SS are suggested to be more likely to experience higher relapse activity and faster progression of neurologi-

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cal disturbances (Lin et al. 2022; Yao et al. 2022). Since neurological disturbances in AQP4-IgG-positive NMOSD primarily increase with relapse episodes, elucidating the profiles and characteristics of comorbidities between NMOSD and SS is essential. The exact relationship between AQP4-IgG, anti-Ro/SSA, and anti-La/SSB (anti-SSA/SSB) antibodies and sicca symptoms has not been fully elucidated yet. Furthermore, the relationship between demographic data, such as age and disease duration, and the presence of anti-SSA/SSB antibodies or sicca symptoms among patients with AQP4-IgG-positive NMOSD remains unknown. By evaluating data from a large number of individuals, who were tested for serum AQP4-IgG after having neurological episodes involving CNS, we aimed to clarify the associations between age, disease duration, sicca symptoms, and presence of AQP4-IgG and anti-SSA/SSB antibodies.

Methods

Study design

This cross-sectional observational study focusing on the relationship between serum AQP4-IgG positivity and SS-related factors was conducted between 2005 and 2020. The individuals who were evaluated had single or repeated neurological episodes suggestive of NMOSD (e.g., optic neuritis, acute myelitis, and other episodes attributable to cerebral or brainstem lesions), and clinicians decided to check the positivity of serum AQP4-IgG. Therefore, the participants in this study included both those with serum AQP4-IgG and those without it, but all of them had neurological episodes suggestive of NMOSD. All serum samples from the participants were tested for serum AQP4-IgG positivity at Tohoku University using a live cell-based assay method. From the evaluated individuals, data regarding the following variables were comprehensively collected: age at testing AQP4-IgG, age at the onset of neurological episodes, sex, positivity for serum anti-SSA/SSB antibodies, and the presence of sicca symptoms (dry eye and/or dry mouth). Information regarding the presence of serum anti-SSA/SSB antibodies and sicca symptoms was collected at the timing of testing serum AQP4-IgG.

Serum AQP4-IgG titration

A microscopic live cell-based assay for AQP4 was performed. Human HEK293 cells expressing the M23-AQP4 protein were incubated with 1:16 diluted serum samples, and serum samples from the tested individuals were added to the medium (Takahashi et al. 2006, 2007; Sato et al. 2013; Waters et al. 2014). The mixtures were stained with an Alexa 488-conjugated secondary antibody. Among the participants with serum AQP4-IgG, 90 were randomly selected for additional measurements of AQP4-IgG titers. Titers were semi-quantitatively calculated based on consecutive two-fold endpoint dilutions.

Statistical analysis

The distributions of age and duration from the onset of neurological episodes are described as median and interquartile range (IQR; 25-75 percentiles). Distributions between the two groups were compared using the Mann-Whitney U test. $P < 0.05$ was considered to be statistically significant. Frequency of a factor between two groups was compared using a chi-squared test, and an unadjusted odds ratio (OR) with the 95% confidence interval (CI) was further obtained. To visually evaluate the relationships between age, disease duration, sicca symptoms, and the presence of serum AQP4-IgG and/or serum anti-SSA/SSB antibodies, 10-year moving average charts showing the prevalence of sicca symptoms or anti-SSA/SSB antibodies are depicted. For each time point of age and duration in the moving average charts, the prevalence rate of anti-SSA/SSB antibodies or sicca symptoms within ± 5 years and the 95% CI were obtained.

Ethics

This study was approved by the institutional review board of Tohoku University Graduate School of Medicine. Written informed consent was obtained from all participants. The study was conducted according to the latest version of the Helsinki declaration for medical research involving humans, as revised in 2013 (World Medical Association 2013).

Results

Participants

We initially recruited 4,447 individuals (1,208 male and 3,239 female participants) who were examined for serum AQP4-IgG at Tohoku University. By the time of checking the serum AQP4-IgG, 1,078 (24.24%) individuals had both episodes of acute optic neuritis and acute myelitis; 1,798 (40.43%) had at least one episode of acute myelitis but never had optic neuritis; 769 (17.29%) had at least one episode of optic neuritis but never had acute myelitis; the other 802 (18.03%) were without these types of clinical episodes or without reliable data regarding the clinical types of neurological episodes. Among the 4,447 individuals, 1,651 (37.13%; 173 male and 1,478 female participants) had serum AQP4-IgG, and the other 2,796 (62.87%; 1,035 male and 1,761 female participants) did not. Data regarding the presence of anti-SSA/SSB antibodies were available for 3,011 individuals; 393 (13.05%) were with anti-SSA/SSB antibodies and the other 2,618 (86.95%) were without them. Data regarding the presence of sicca symptoms were available for 4,186 individuals; 365 (8.72%) were with sicca symptoms and the other 3,821 (91.28%) were without them. Anti-SSA/SSB antibodies were detected in 83.84% (n = 306/365) of those with sicca symptoms and in 68.33% (n = 2,611/3,821) of those without sicca symptoms. Presence of sicca symptoms was reported in 96.18% (n = 378/393) of those with anti-SSA/SSB antibodies, 96.98% (n = 2,539/2,618) of those without anti-SSA/SSB antibodies,

and 88.37% (n = 1,269/1,436) of those who were with unknown anti-SSA/SSB positivity. The prevalence rates of sicca symptoms among those with anti-SSA/SSB antibodies, irrespective of the presence of AQP4-IgG, were 35.98% (n = 136/378) among those with anti-SSA/SSB antibodies, 6.70% (n = 170/2,539) among those without anti-SSA/SSB antibodies, and 4.65% (n = 59/1,269) among those with unknown anti-SSA/SSB positivity. Between those with serum AQP4-IgG and those without it, the prevalence rates of anti-SSA/SSB antibodies [26.64% (n = 305/1,145) vs. 4.72% (n = 88/1,866); unadjusted OR, 7.34; 95% CI, 5.71-9.43; p < 0.0001] and sicca symptoms [12.56% (n = 194/1,544) vs. 6.47% (n = 171/2,642); unadjusted OR, 2.08; 95% CI, 1.67-2.58; p < 0.0001] were significantly higher in the former group with AQP4-IgG.

AQP4-IgG titers by the presence of anti-SSA/SSB antibodies

Among the 1,651 participants with serum AQP4-IgG levels, 90 were randomly selected to measure AQP4-IgG titers. The median (IQR) of the measured titers was 512 × (128 × − 4,096 ×) and the range was 16 × − 262,144 ×. The distribution of AQP4-IgG titers in the presence of anti-SSA/SSB antibodies is shown in Fig. 1A. The measured titers did not significantly differ between those with and without anti-SSA/SSB antibodies (p = 0.2386; Mann-Whitney U test).

Relationship between AQP4-IgG, anti-SSA/SSB antibodies, and sicca symptoms

Of the overall 4,447 participants, 2,917 had a complete dataset of serum AQP4-IgG, anti-SSA/SSB antibodies, and sicca symptoms. Among these 2,917 individuals, the numbers of individuals by the combination of these factors are listed in Table 1. The overlap among these three factors is shown in Fig. 1B. Among the AQP4-IgG-positive patients with anti-SSA/SSB, the prevalence of sicca symptoms was 36.52% (n = 107/293). The same prevalence among the AQP4-IgG-negative patients with anti-SSA/SSB was 34.12% (n = 29/85). The prevalence of sicca symptoms in those with anti-SSA/SSB antibodies did not differ by the presence of AQP4-IgG (unadjusted OR, 1.11; 95% CI, 0.67-1.85; p = 0.6892, chi-square test). Collectively, these results suggest a common mechanism underlying the emergence of AQP4-IgG and anti-SSA/SSB antibodies. Moreover, anti-SSA/SSB antibodies were linked with sicca symptoms, whereas AQP4-IgG was not directly linked with sicca symptoms. Co-occurring anti-SSA/SSB antibodies in patients with AQP4-IgG could be the key factor explaining the higher prevalence of sicca symptoms in those with AQP4-IgG than in those without AQP4-IgG.

Impact of age and duration of AQP4-IgG NMOSD on SS

The effect of age and elapsed time from the onset of AQP4-IgG-positive NMOSD on the prevalence of anti-SSA/SSB antibodies or sicca symptoms was evaluated according to the presence of serum AQP4-IgG. Among the 1,651 individuals with AQP4-IgG, the age distribution at the time of AQP4-IgG testing was slightly lower among those with anti-SSA/SSB antibodies than among those without the antibodies [median (IQR): 50 (40-62) years vs. 54 (43-63) years; p = 0.0071, Mann-Whitney U test], and the duration since the onset of neurological condition at the time of AQP4-IgG testing did not differ significantly between patients with and without anti-SSA/SSB antibod-

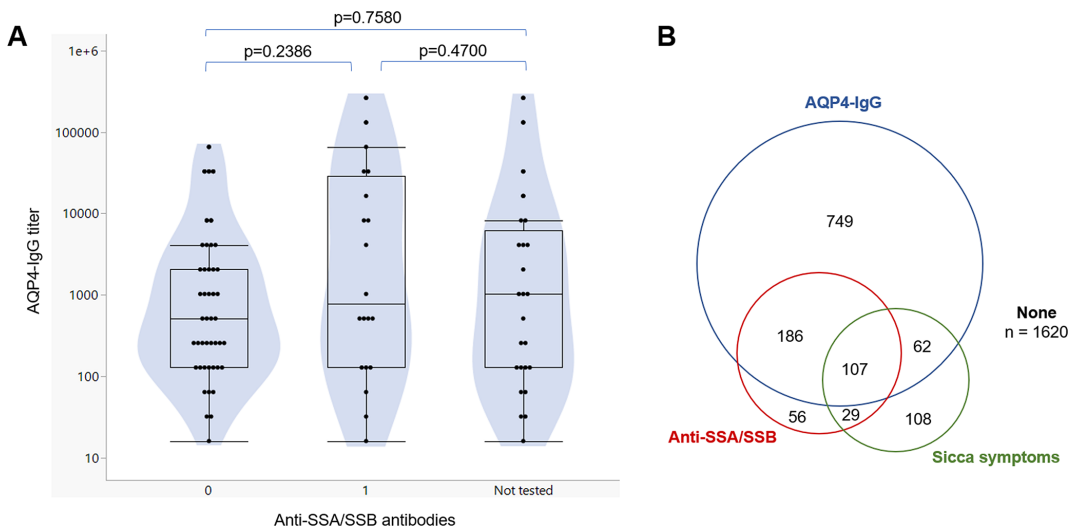

Fig. 1. AQP4-IgG titer distribution according to the presence of anti-SSA/SSB antibodies and overlap between the antibodies and sicca symptoms.
(A) A violin plot showing the distributions of the measured serum AQP4-IgG titers from randomly selected 90 AQP4-IgG-positive individuals, by the presence of serum anti-SSA/SSB antibodies. The distributions did not significantly differ by the presence of anti-SSA/SSB antibodies. (B) A Venn diagram showing the prevalence of AQP4-IgG, anti-SSA/SSB antibodies, and sicca symptoms. It can be estimated from this diagram that the prevalence of anti-SSA/SSB antibodies is higher in patients with AQP4-IgG than in those without AQP4-IgG (irrespective of sicca symptoms).

Table 1. Anti-SSA/SSB antibodies and sicca symptoms by the presence of AQP4-IgG.

Anti-SSA/SSB antibody positivity by the presence of AQP4-IgG and sicca symptoms				
	anti-SSA/SSB (+)	anti-SSA/SSB (−)	OR (95% CI)	p-values
AQP4-IgG (+), total	293	811	7.34 (5.69-9.48)	< 0.0001
AQP4-IgG (−), total	85	1,728		
AQP4-IgG (+), sicca (+)	107	62	6.43 (3.84-10.76)	< 0.0001
AQP4-IgG (−), sicca (+)	29	108		
AQP4-IgG (+), sicca (−)	186	749	7.18 (5.26-9.81)	< 0.0001
AQP4-IgG (−), sicca (−)	56	1,620		
AQP4-IgG (+), sicca (+)	107	62	6.95 (4.89-9.88)	< 0.0001
AQP4-IgG (+), sicca (−)	186	749		
AQP4-IgG (−), sicca (+)	29	108	7.77 (4.76 -12.67)	< 0.0001
AQP4-IgG (−), sicca (−)	56	1,620		
Presence of sicca symptoms by the presence of AQP4-IgG and anti-SSA/SSB antibodies				
	Sicca (+)	Sicca (−)	OR (95% CI)	p-values
AQP4-IgG (+), total	169	935	2.21 (1.74-2.81)	< 0.0001
AQP4-IgG (−), total	137	1,676		
AQP4-IgG (+), anti-SSA/SSB (+)	107	186	1.11 (0.67-1.85)	0.6892
AQP4-IgG (−), anti-SSA/SSB (+)	29	56		
AQP4-IgG (+), anti-SSA/SSB (−)	62	749	1.24 (0.90 -1.72)	0.1897
AQP4-IgG (−), anti-SSA/SSB (−)	108	1,620		
AQP4-IgG (+), anti-SSA/SSB (+)	107	186	6.95 (4.89-9.88)	< 0.0001
AQP4-IgG (+), anti-SSA/SSB (−)	62	749		
AQP4-IgG (−), anti-SSA/SSB (+)	29	56	7.77 (4.76-12.67)	< 0.0001
AQP4-IgG (−), anti-SSA/SSB (−)	108	1,620		

This study included 2,917 participants who had complete data regarding the positivity of serum AQP4-IgG, anti-SSA/SSB antibodies, and the presence of sicca symptoms. The upper half shows the prevalence of anti-SSA/SSB antibodies according to the presence of AQP4-IgG and sicca symptoms, suggesting a common mechanism underlying the emergence of AQP4-IgG and anti-SSA/SSB antibodies. The lower half shows the prevalence of sicca symptoms by the presence of AQP4-IgG and anti-SSA/SSB antibodies, suggesting that the presence of anti-SSA/SSB antibodies is linked with sicca symptoms, whereas AQP4-IgG presence is not directly linked with sicca symptoms.

ies. [2 (1-8) years vs. 4 (1-10) years; p = 0.1207]. Ten-year moving average charts for anti-SSA/SSB antibody positivity rates according to age, disease duration, and AQP4-IgG positivity in the overall participants are shown in Fig. 2. These charts demonstrated that the prevalence rates of anti-SSA/SSB antibodies or sicca symptoms were higher in those with AQP4-IgG than in those without it regardless of age and duration of neurological condition. Next, the same relationships were evaluated among those with sicca symptoms at serum AQP4-IgG testing. The obtained 10-year moving average charts for the anti-SSA/SSB antibody positivity rates among those with sicca symptoms are shown in Fig. 3. Even when focusing to the individuals with sicca symptoms, the prevalence rate of anti-SSA/SSB antibodies was higher in those with AQP4-IgG than in those without it regardless of age and years from the neurological onset. For reference, among the 194 individuals with AQP4-IgG and sicca symptoms, the age distribution [54 (41-65) years vs. 55 (47-64) years; p = 0.3899] and duration of neurological conditions [2 (1-7) years vs. 4 (1-11) years; p = 0.1689] did not significantly differ according to the presence of anti-SSA/SSB antibodies. Finally, the prevalence rates of sicca symptoms according to age, disease duration, and AQP4-IgG positivity was evaluated in those with anti-SSA/SSB antibodies. The obtained 10-year moving average charts for the prevalence of sicca symptoms are shown in Fig. 4. These charts demonstrated that the frequency of sicca symptoms among those with anti-SSA/SSB antibodies did not differ by the presence of AQP4-IgG, suggesting that AQP4-IgG would not contribute to the development of sicca symptoms. For reference, among the 305 individuals with AQP4-IgG and anti-SSA/SSB antibodies, the distribution of age [54 (41-65) vs. 49 (39-59) years, p = 0.0703] and duration of neurological conditions [2 (1-8) vs. 2 (1-7) years, p = 0.8397] did not differ significantly according to the presence of sicca symptoms.

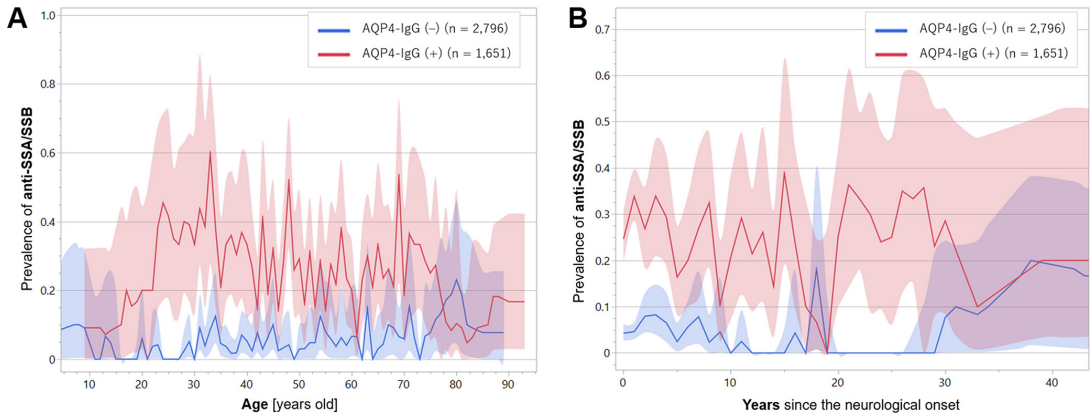

Fig. 2. Anti-SSA/SSB antibodies positivity according to age or disease duration by the presence of AQP4-IgG.
For the overall participants, 10-year moving average charts for anti-SSA/SSB antibody positivity rate by the presence of AQP4-IgG according to age (A) or disease duration since the onset of neurological episodes (B) are depicted. The presence of anti-SSA/SSB antibodies and sicca symptoms were checked at the time of AQP4-IgG testing. The filled areas around the lines represent 95% confidence intervals for the moving averages. The moving averages took values between 0.0 (prevalence of 0%) and 1.0 (prevalence of 100%). Elevated prevalence rates of anti-SSA/SSB antibodies and sicca symptoms in those with AQP4-IgG were already seen in young patients aged < 30 years and from the neurological onset. Anti-SSA/SSB, anti-Ro/SSA and anti-La/SSB; AQP4-IgG, anti-aquaporin-4 antibodies.

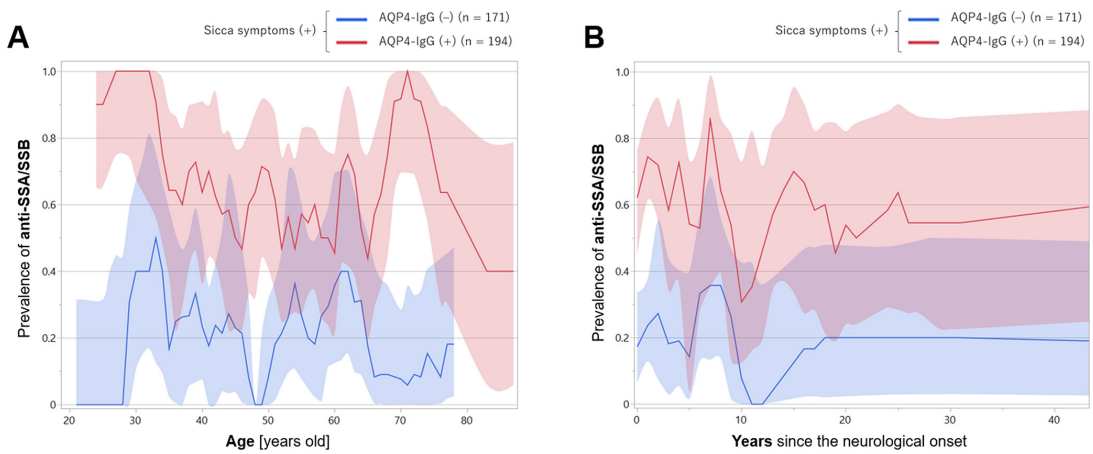

Fig. 3. Anti-SSA/SSB antibodies positivity according to age or disease duration in those with sicca symptoms.
For the participants with sicca symptoms at the time of AQP4-IgG testing, 10-year moving average charts for anti-SSA/SSB antibody positivity rates by the presence of AQP4-IgG according to age (A) or disease duration since the onset of neurological episodes (B) are depicted. The filled areas around the lines represent 95% confidence intervals for the moving averages. Majority of AQP4-IgG-positive patients with sicca symptoms possessed anti-SSA/SSB antibodies regardless of age and disease duration.

Discussion

The results of the present study demonstrated that age or duration of AQP4-IgG-positive NMOSD did not significantly influence the prevalence of anti-SSA/SSB antibodies or sicca symptoms, although SS and AQP4-IgG-positive NMOSD showed a significantly high comorbidity rate. Furthermore, the presence of sicca symptoms in patients with anti-SSA/SSB antibodies did not differ with the presence of serum AQP4-IgG. These findings collectively suggest that the associations between the emergence of serum AQP4-IgG, anti-SSA/SSB antibodies, and sicca symptoms are not time-dependent, and overlaps between these factors are observed before or within the early stages of AQP4-IgG-positive NMOSD. These findings differed from the expected findings before conducting the present study, as the median age at diagnosis of SS is known to be above 40 years of age and the prevalence of sicca symptoms would increase with age (Baer and Walitt 2017; Ramírez Sepúlveda et al. 2017; Anquetil et al. 2019; Goules et al. 2020; Negrini et al. 2022). If the emergence of serum AQP4-IgG and anti-SSA/SSB antibodies were completely independent of each other and they continue to exist in the serum, the overlap of these antibodies would increase with the age or duration of disease, which was not the finding of this study. The obtained result suggests a common mechanism for the emergence of AQP4-IgG and anti-SSA/SSB antibodies. A conceivable theory includes the presence of common epitopes in the bodies of patients that simultaneously trigger the emergence of these autoantibodies. For

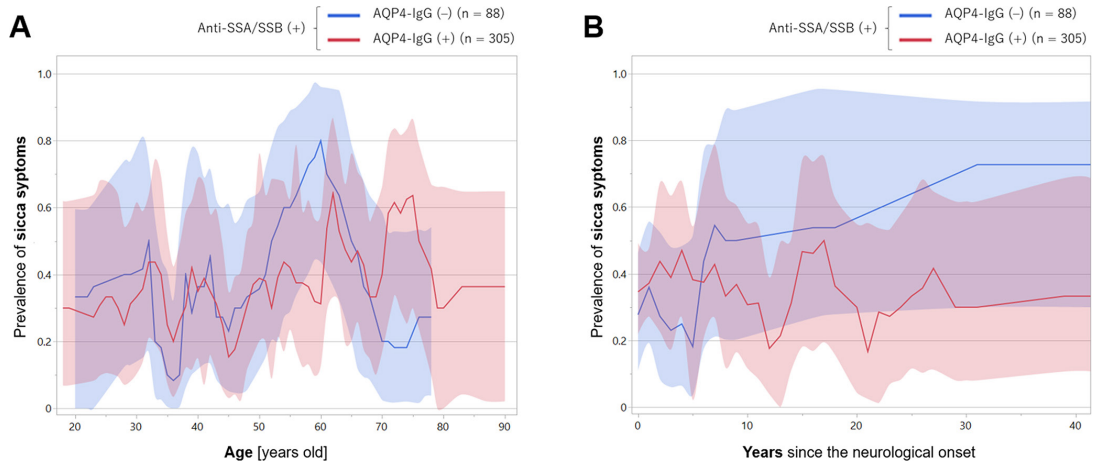

Fig. 4. Prevalence of sicca symptoms according to age or disease duration in those with anti-SSA/SSB antibodies. For the participants with anti-SSA/SSB antibodies, 10-year moving average charts for the prevalence of sicca symptoms by the presence of AQP4-IgG according to age (A) or disease duration since the onset of neurological episodes (B) are depicted. The filled areas around the lines represent 95% confidence intervals for the moving averages.

example, anti-aquaporin-5 antibodies (AQP5-IgG) appear in the blood of many patients with SS (Alam et al. 2016; Chivasso et al. 2023), with an abnormal distribution of AQP5 water channel proteins in the salivary glands (Steinfeld et al. 2001). Phylogenetic tree reconstructions of the AQP family (i.e., AQP1-AQP12B) in previous studies have demonstrated a proximal phylogenetic origin between AQP4 and AQP5, both of which are orthodox AQPs (Tzartos et al. 2017; Sandhya et al. 2023). It is reasonable to assume that the production of AQP4-IgG from circulating lymphocytes is triggered by abnormally expressed AQP proteins (other than AQP4) in the lacrimal and salivary glands of patients with SS together with the production of anti-SSA/SSB antibodies. Further studies are required to determine the presence and types of abnormally expressed AQP proteins in patients with AQP4-IgG.

Another notable finding of this study was the relatively low prevalence of sicca symptoms among patients with anti-SSA/SSB antibodies. Among the all participants, only 30-40% of those with anti-SSA/SSB antibodies reported sicca symptoms at the AQP4-IgG testing. These findings suggest that the majority of individuals with anti-SSA/SSB antibodies do not experience sicca symptoms compatible with SS. Based on previous studies, the prevalence of anti-SSA antibodies in the general population has been estimated to be 2-15% (Hayashi et al. 2008; Guo et al. 2014; Smeele et al. 2021), although the prevalence would change by the cutoff level to decide the seropositivity. All studies agreed that most individuals with anti-SSA antibodies in the general population did not have sicca symptoms compatible with SS. In one of these studies, which checked for the serum anti-SSA antibody in 2,181 residents living in a town in Japan, only four (10.0%) of 40 anti-SSA-positive individuals confirmed to have SS, whereas SS was suspected in other four individuals (Hayashi et al. 2008). Compared to the estimated prevalence of anti-SSA/SSB antibodies based on these previous studies, the prevalence of anti-SSA/SSB antibodies among those without AQP4-IgG in this study (4.72%) was not remarkably different, whereas that among individuals with AQP4-IgG (26.64%) was significantly higher. These findings support the validity of the obtained results regarding the frequency of anti-SSA/SSB positivity and sicca symptoms in the present study.

This study had several limitations. First, the age of SS onset among participants were not evaluated in the present study. Therefore, the distribution of the intervals between the onsets of SS and AQP4-IgG-positive NMOSD among participants remains uncertain. Secondly, the titers of anti-SSA/SSB antibodies were not evaluated, and the titers of AQP4-IgG were measured in randomly selected 90 individuals among the 1,651 individuals with serum AQP4-IgG. Consequently, the relationship between the titers of AQP4-IgG and anti-SSA/SSB antibodies and the level of sicca symptoms remains unknown.

In conclusion, SS and AQP4-IgG-positive NMOSD were likely to be comorbid; however, age or disease duration from neurological onset in patients with AQP4-IgG did not significantly influence the prevalence of anti-SSA/SSB antibodies or sicca symptoms, suggesting the absence of time dependency in the emergence of anti-SSA/SSB antibodies or sicca symptoms in AQP4-IgG-positive NMOSD. The prevalence rates of anti-SSA/SSB antibodies or sicca symptoms in those with AQP4-IgG were higher than those without it from the neurological onset. Comorbid SS in AQP4-IgG-positive NMOSD would be likely to precede or synchronize with the onset of NMOSD.

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Conflict of Interest

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