Original article

A single center retrospective analysis of AECG classification criteria for primary Sjögren's syndrome based on 112 minor salivary gland biopsies in a Japanese population

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***Running title*: Application of AECG criteria for Japanese SS patients**

***Key words:* Sjögren's syndrome, classification criteria, minor salivary gland biopsy**

Abstract

Objective: To assess the usefulness and performance of the American European Consensus Group (AECG) criteria based on minor salivary gland biopsy (MSGB) in Japanese patients with primary Sjögren's syndrome (SS).

Methods: Among 208 MSGB cases, we retrospectively selected 112 subjects who satisfied the complete set of AECG classification criteria. Of the 112 subjects studied, 63 primary SS patients and 49 non-SS group subjects were classified according to the AECG criteria. The contribution of subjective and objective components was statistically analyzed.

Results: Sex, dry eye, Saxon test, Schirmer's test anti-SS-A/Ro antibody, MSGB grading and sialography statistically contributed to the diagnosis. Multiple logistic regression analysis showed that positive MSGB (OR; 105, 95% CI; 13-849), positive anti-SS-A/Ro antibody (OR; 96, 95% CI; 10-923), a positive Saxon test (OR; 46, 95% CI; 6-340) and the existence of dry eye (OR; 8, 95% CI; 2-43) were associated with the diagnosis of primary SS. Among the components of the AECG criteria, MSGB and anti-SS-A/Ro antibody were very strong contributors. Furthermore, the abnormal-finding-positive rate in sialography significantly correlated with MSGB grading (p for trend = 0.0006),

although other subjective and objective components were not associated with MSGB grading.

Conclusion: The usefulness of the AECG criteria for Japanese primary SS patients was confirmed.

Introduction

Sjögren's syndrome (SS) is an autoimmune disorder characterized by chronic T lymphocytic infiltration of the salivary glands or lacrimal glands, leading to exocrine gland dysfunction **(1-3)**. Although various classification criteria for SS have been proposed, no international criteria have been established. Preliminary European classification criteria **(4)** published in 1993 have been proposed; however, the inclusion of non-autoimmune diseases was a major problem. Since the Sjögren's Syndrome Foundation organized the American European Study Group in order to propose new classification criteria, the American European Consensus Group (AECG) revised the European criteria in 2002 **(5)** based on an analysis of 180 cases from 16 centers in 10 European countries.   The usefulness of the novel criteria was determined by a detailed analysis based on a receiver operating characteristic curve. These new classification criteria also include 6 components with revised rules in which a histopathology of focus score (FS) ≥1 or the presence of serological positivity is required. On the other hand, the AECG criteria proposed that 3 of 4 objective items are indicative of primary SS, even when ocular symptoms or oral symptoms were not considered.   Among the components of the AECG criteria,

the usefulness of minor salivary gland biopsy (MSGB) has been reported by European institutions **(6)**. In the present study, we evaluated the usefulness and performance of the AECG criteria, focusing on MSGB, for Japanese primary SS patients at a single center.

Patients and Methods

Patients

We performed 208 MSGBs at our institution for suspected SS between 1995 and 2009. Among these cases, we retrospectively selected 112 subjects who had complete sets of the 6 essential items in the AECG classification criteria. The study was conducted in accordance with the human experimental guidelines of our institution. According to the AECG criteria, the 112 subjects were divided into a primary SS group (n=63; male/female: 3/60) and a non-SS group (n=49; male/female: 9/40). The ages and background information of the subjects are summarized in Table 1.

Serological tests and exocrine dysfunction

Antibodies to SS-A (Ro) and SS-B (La) antigens were determined by enzyme-linked immunosorbent assay (Mesacup SS-A/Ro test; normal range: 10-30 and Mesacup SS-B/La test; normal range: 15-25, MBL). To evaluate the secretion of tears and saliva, Schirmer's test and the Saxon test were employed. Schirmer's test was considered positive when less than 5 mm of the strips of filter paper was wet after 5 minutes. In the original setting in the AECG criteria, unstimulated saliva is shown to be less than 1.5 ml in 15 minutes. As a modified method for measuring the unstimulated saliva volume, we employed the Saxon test. For the Saxon test, the patients chewed gauze slowly for 2 minutes; an increase of less than 2 g was defined as a positive outcome. Positivity of sialography was evaluated using the classification determined by Rubin and Holt **(7)**.

Minor labial salivary gland biopsy and sialography

Minor salivary glands were obtained from the inner surface of the lower lip under local anesthesia. The grading of mononuclear cell (MNC) infiltration was defined according to Chisholm and Mason **(8)**. The presence of at least one

focus of mononuclear cells in 4 mm^2 sections was defined as grade 3 (FS=1) sialadenitis. MSGB was performed with informed consent from all participants.

Sialography was conducted using a catheter, which was inserted into Stensen's duct **(9)**. After cannulation, a contrast fluid was slowly injected, then an image was taken.

Statistical analysis

Data were analyzed using the Statistical Analysis System Version 9.1.3 (SAS Institute Inc. Cary, NC). Student's *t* test was used to compare continuous variables. Fisher's exact test was used for categorical variables. A logistic regression model was used to explore the simultaneous effects of parameters on the diagnosis of primary SS. Starting with a full model including variables in the univariate analysis, the most appropriate model was selected on the basis of Akaike's information criteria (AIC). The odds ratio (OR) and the 95% confidence interval (CI) were calculated for each covariate included in the model. The Cochran-Armitage test was used to test for a trend in the prevalence of AECG components according to the grades of minor salivary gland biopsy in patients

with primary SS.

Results

Contribution of background elements and the AECG components for SS

We initially confirmed the contribution of each of the AECG components as well as the background information in the present study. Positive SMGB grading and the existence of anti-SS-A/Ro antibody showed the highest statistical significance among the components **(Table 1)**. Salivary gland involvement, the Saxon test and sialography also demonstrated statistical significance. For lacrimal function, the existence of dry eye and Schirmer's test showed significance.

Multiple logistic analysis of the AECG components

Multiple logistic regression analysis showed that positive MSGB (OR; 105, 95% CI; 13-849), positive anti-SS-A/Ro antibody (OR; 96, 95% CI; 10-923), a positive Saxon test (OR; 46, 95% CI; 6-340) and the existence of dry eye (OR; 8, 95% CI; 2-43) were associated with the diagnosis of primary SS **(Table 2)**. Among the 6 components of the AECG criteria, MSGB was the most significant

contributor and anti-SS-A/Ro antibody was the second-most significant. The ORs

of the Saxon test and dry eye were also significant, although their significance

was less than that of MSGB grading or anti-SS-A/Ro antibody.

Relationship between positive rates of the AECG components according to

SMGB grading

Since SMGB positivity was the variable with the greatest significance in the

diagnosis of primary SS, we next verified the relationship between SMGB

grading and 7 items in the AECG classification criteria. As a result, the only

relationship was shown between SMGB grading and abnormal sialography

findings; the latter significantly increased with MSGB grading (p for trend =

0.0006) **(Table 3)**. Other positive items in the logistic analysis including

anti-SS-A/Ro antibody, the Saxon test and dry eye had no significant relationship

with SMGB grading.

Discussion

In the present study, we demonstrated the usefulness of the AECG classification

criteria for Japanese subjects. Although the criteria were originally based on data

from European subjects, multiple components of the AECG criteria are considered to be reasonably applicable to the Japanese population according to the present analytical result. The revision of the AECG criteria from the preliminary European criteria has improved accuracy of the classification of primary SS.

Among the items in the AECG criteria, MSGB as well as serological examinations made important contributions to the classification of primary SS. Although Schirmer's test and the Saxon test were also important contributors to the classification, a high specificity of MSGB for SS has been reported in Turkey **(10)**. In an analysis of 502 MSGB cases among Italian subjects **(6)**, 94.5% of the cases met the AECG criteria. Furthermore, Scardina et al. **(11)** demonstrated that the FS of SS patients remained unchanged in the histological sections at 3 different depths, suggesting that MNC infiltration in a single section has little impact on the results of the classification determined by the AECG criteria. Since reproducibility error that is dependent on the operators should be considered with regard to Schirmer's test or the Saxon test, MSGB may be the most reliable item for detecting the involvement of exocrine glands.

Notably, the radiographic component of the criteria, sialography, also made a major contribution to the classification of primary SS. Originally, it was reported that conventional sialography showed high sensitivity and specificity compared to MSGB in an analysis of 150 subjects **(12)**. Although sialography was not calculated as a high OR item in our logistic analysis, the severity of MSGB grading correlated well to the rate of abnormality found in sialography, suggesting that sialography well represents the histological damage due to SS. We should note that MSGB grading was adopted in the logistic analysis because the stronger variable was allowed to remain when the relevance of both MSGB grading and sialography was calculated. For future use, less-invasive magnetic resonance sialography **(13)** could be used in place of conventional sialography.

In the application of the AECG criteria, the following points should be considered. One is an exclusion criteria including viral infection or use of anti-cholinergic drugs as revised rules for classification and other, alternative diagnostic tools found in the AECG criteria. Another consideration is that the application of ocular dry scores such as the Rose Bengal score or salivary scintigraphy should also be considered. Although the AECG criteria include the Rose Bengal score or salivary scintigraphy, we did not perform both items. Thus,

the bias for the classification of primary SS might occur according to no use of these items.

In summary, the present study has shown the usefulness and validity of the AECG criteria for Japanese subjects. Furthermore, the contributions of MSGB, serology and sialography were shown. As a next step, internationally approved diagnostic criteria should be developed that utilize the advantages of the various diagnostic components. Among the diagnostic items, MSGB could be the most significant component.

Key messages:

Of the AECG criteria, MSGB made the greatest contribution to diagnosis in the Japanese population.

The severity of MSGB grading correlated to the rate of abnormality found by sialography.

Abbreviations

AECG; American European Consensus Group, CI; confidence interval, FS; focus score, MSGB; minor salivary gland biopsy, OR; odds ratio, SS; Sjögren's syndrome

The authors declare no conflict of interest.

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Table 1. Background information and contribution of each of the AECG criteria to the diagnosis of primary Sjögren's syndrome

	SS (n=63)	Non SS (n=49)	P value
Age	59.4 ± 12.5	59.9 ± 12.7	0.85
Sex (M/F)	3/60	9/40	0.03
Dry eye	42 (66.7)	19 (38.8)	0.004
Dry mouth	50 (79.4)	34 (69.4)	0.27
Saxon test	53 (84.1)	25 (51.0)	0.0002
Schirmer's test	47 (74.6)	25 (51.0)	0.017
Anti-SS-A Ab	37 (58.7)	7 (14.3)	<0.0001
Anti-SS-B Ab	11 (17.5)	4 (8.2)	0.17
Grades 3 and 4	58 (92.1)	19 (38.8)	<0.0001
Sialography	31 (49.2)	8 (16.3)	0.0003

Data are shown as means ± SD or as numbers and percentages (parentheses). Student's *t* test was used to compare continuous variables. Fisher's exact test was used for categorical variables. P value <0.05: statistically significant. Ab; antibody, AECG; American-European Classification Criteria, SS; Sjögren's syndrome

Table 2. Logistic analysis of AECG components for the diagnosis of primary Sjögren's syndrome

	OR[*]	95% CI	P value
Grade	105	13-849	<0.0001
Anti-SS-A Ab	96	10-923	<0.0001
Saxon test	46	6-340	0.0002
Dry eye	8	2-43	0.0112

[*]OR; odds ratio, 95% CI; 95 % confidence interval, P value <0.05: statistically significant. Ab; antibody, AECG; American-European Classification Criteria

Table 3. Positive rates of AECG components according to the grades of minor salivary gland biopsies in patients with primary Sjögren's syndrome

MSGB grade	Positive			P value for trend
	1, 2	**3**	**4**	
	(n=5)	**(n=24)**	**(n=34)**	
Dry eye	4 (80.0)	17 (70.8)	21 (61.7)	0.33
Dry mouth	4 (80.0)	19 (79.2)	27 (79.4)	0.99
Anti-SS-A Ab	4 (80.0)	13 (54.2)	20 (58.8)	0.68
Anti-SS-B Ab	3 (60.0)	1 (4.2)	7 (20.6)	0.58
Saxon test	4 (80.0)	20 (83.3)	29 (85.3)	0.74
Schirmer's test	5 (100)	17 (70.8)	28 (73.5)	0.46
Sialography	1 (20.0)	6 (25.0)	24 (70.6)	0.0006

Data are shown as numbers and percentages (parentheses). P value was calculated by the Cochran-Armitage trend test. P value <0.05: statistically significant. MSGB grade 3 represents at least one focus of more than 50 counts of mononuclear cell infiltration in 4 mm^2 of salivary gland area. Ab; antibody, AECG; American-European Classification Criteria, MSGB; minor salivary gland biopsy