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Clinical Phenotyping of Primary Sjögren Syndrome Patients Using Salivary Gland Ultrasonography: Data From the RESULT Cohort

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ABSTRACT. ***Objective.*** To investigate salivary gland ultrasound (SGUS) abnormalities in relation to clinical phenotype and patient characteristics, disease activity, and disease damage in patients with primary Sjögren syndrome (pSS).

Methods. Consecutive outpatients included in our REgistry of Sjögren Syndrome LongiTudinal (RESULT) cohort were selected. Patients with pSS who were included were classified according to the American College of Rheumatology/European League Against Rheumatism (EULAR) criteria and underwent full ultrasonographic examination (Hocevar score 0–48) at baseline. Total SGUS scores of ≥ 15 were considered positive. Patient characteristics, disease activity, and disease damage were compared between the different SGUS groups.

Results. In total, 172 of 186 patients with pSS were eligible, of whom 136 (79%) were SGUS positive. Compared with patients who were SGUS negative, SGUS-positive patients had significantly longer disease duration, higher EULAR Sjögren Syndrome Disease Activity Index, higher Sjögren Syndrome Disease Damage Index, and were more likely to have a positive parotid gland biopsy, anti-SSA/SSB antibodies, and abnormal unstimulated whole saliva (UWS) and ocular staining score (OSS), and higher levels of IgG and rheumatoid factor. Regarding patient-reported outcome measurements (PROM), patients who were SGUS positive scored significantly lower on the EULAR Sjögren Syndrome Patient-Reported Index for fatigue and pain, and more often found their disease state acceptable compared with patients who were SGUS negative. SGUS total score showed significant associations with various clinical and serological variables, and with PROM. Highest associations were found for UWS ($\rho = -0.551$) and OSS ($\rho = 0.532$).

Conclusion. Patients who were SGUS positive show a distinct clinical phenotype in all aspects of the disease compared with patients who were SGUS negative: clinical, functional, serological, and PROM. SGUS could be a helpful tool in selecting patients for clinical trials and estimating treatment need.

Key Indexing Terms: cohort studies, salivary glands, Sjögren syndrome, ultrasound

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Primary Sjögren syndrome (pSS) is a common systemic autoimmune disease[1]. Women are affected 9 times more often than men[2]. pSS is a highly heterogeneous disease, which is reflected by the many different manifestations patients can have. Common symptoms, such as extreme fatigue and sicca symptoms, have a major effect on quality of life[1,3]. This heterogeneity already emerges during the diagnostic examination of pSS (i.e., not every patient with pSS has autoantibodies or a focus score [FS]-positive salivary gland biopsy), which suggests that there are different subgroups of patients. It would be of great value to be able to identify individual patients at high risk for a severe disease outcome. Prospective cohort studies are gaining more and more importance in this quest[4]. Since treatment options for patients with pSS are eagerly awaited, but unfortunately still very limited, the search for patient stratification and proper selection methods for clinical trials is ongoing.

Regarding the care of patients suspected to have pSS, there is a unique collaboration between different departments at the

University Medical Center Groningen (UMCG). The REgistry of Sjögren Syndrome LongiTudinal (RESULT) cohort at the UMCG has been set up to identify biomarkers and clinical variables that determine and predict the longitudinal course of pSS. Observational studies, such as the RESULT cohort, are important as they provide information on long-term outcomes of pSS and reflect daily clinical practice.

Salivary gland ultrasonography (SGUS) is increasingly gaining acceptance as an imaging tool of the salivary glands in pSS and ultrasound (US) is widely accessible in outpatient rheumatology clinics. SGUS is noninvasive and nonirradiating, which makes it patient-friendly and an ideal imaging modality for repeated use[5,6,7].

Previously, we have studied the validity of SGUS and found that a positive US, based on the total Hocevar score[6], predicts classification according to the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria[8]. Subsequently we provided evidence that measuring only hypoechogenic areas in 1 parotid and 1 submandibular gland is sufficient to predict ACR/EULAR classification, increasing the feasibility of SGUS[9]. Although a simpler scoring system suffices for classification purposes, it is not yet known whether SGUS abnormalities can also be used for patient stratification, long-term follow-up, or even as a selection method for clinical trials. Therefore, a full SGUS evaluation according to the Hocevar score is performed in each patient included in the RESULT cohort.

The aim of this study was to investigate SGUS abnormalities in relation to clinical phenotype and patient characteristics, disease activity, and disease damage in patients with pSS.

MATERIALS AND METHODS

The RESULT cohort. The observational RESULT cohort combines up-to-date quality of care with gathering long-term prospective follow-up data in a large cohort of patients. For participation in the RESULT cohort, we consider all consecutive patients with probable or confirmed pSS who visit the outpatient clinic of the Department of Rheumatology and Clinical Immunology at the UMCG, a tertiary referral expertise center. Inclusion in the RESULT cohort is ongoing and duration of follow-up will be 10 years.

The present cross-sectional analysis included the baseline visit of all patients who were included in the RESULT cohort between January 2016 and December 2018. Patients with missing US examination as well as patients who did not fulfill the ACR/EULAR criteria for pSS (i.e., patients with probable pSS)[10,11] were excluded.

This study was conducted in accordance with the Declaration of Helsinki. The research protocol was approved by the Medical Ethics Committee of the UMCG (METC 2014/491). All subjects provided informed consent.

Assessments. Imaging, clinical, functional, histopathological, and serological variables, and patient-reported outcome measurements (PROM) were obtained according to a fixed protocol.

SGUS. B-mode SGUS was performed using the MyLabSeven scanner (Esaote), equipped with a high-resolution linear probe (4–13 MHz). All US images were scored real-time by trained readers (AS, KD, JVN, EM, and RW). Test-retest reliability in our center was demonstrated previously[12]. The scoring system by Hocevar, *et al*[6] was applied (range 0–48), including the components of parenchymal echogenicity, homogeneity, presence of hypoechogenic areas, hyperechogenic reflections, and clarity of the salivary gland border. A total SGUS score of ≥ 15 was considered positive[8].

Other assessments. Demographic characteristics, EULAR Sjögren Syndrome Disease Activity Index (ESSDAI)[13], Disease Activity Score in 28 joints based on erythrocyte sedimentation rate (DAS28-ESR), DAS28 based on C-reactive protein (DAS28-CRP)[14,15], number of tender points, physician global assessment (PGA), Sjögren Syndrome Disease Damage Index (SSDDI)[16], unstimulated whole saliva flow (UWS)[17], Schirmer test, and ocular staining score (OSS)[18] were determined. Two methods were applied for Schirmer test and OSS: when categorizing as normal or abnormal, the worst eye was selected; and when applied as a continuous variable, the mean of both eyes was used. A salivary gland biopsy was not mandatory for participation in the RESULT cohort and therefore, parotid and labial salivary gland FS were recorded if available[19,20,21].

Serological variables were determined, including presence of anti-SSA/SSB antibodies, IgG level, rheumatoid factor (RF) level, complement C3 and C4 levels, and leukocyte count.

Patients completed a questionnaire, which included EULAR Sjögren Syndrome Patient-Reported Index (ESSPRI) dryness, fatigue and pain[22], patient acceptable symptom state (PASS), patient global assessment, and EQ-5D[23].

Statistics. Statistical analyses were performed using IBM SPSS Statistics 23 (SPSS). Descriptive variables were expressed as number (%) of patients for categorical data and mean (SD) or median (IQR) for continuous data.

Patient characteristics, disease activity, and damage were compared between patients who were SGUS negative (score < 15) and positive (score ≥ 15). Subsequently, based on the median score of the SGUS-positive group, patients who were SGUS positive were arbitrarily divided into 2 equal groups: patients with scores ≥ 15 and < 27 were defined as medium-positive and patients with scores ≥ 27 were defined as high-positive.

Fisher exact test or chi-square were used as appropriate to evaluate differences in categorical variables between the US groups. Independent samples *t*-test or Mann-Whitney U test were used as appropriate to evaluate differences in continuous variables between the US groups. ESSDAI subdomains were summarized descriptively.

The association between SGUS total score and continuous variables was analyzed using Spearman correlation coefficient (ρ), and interpreted as poor association (0.0–0.2), fair (0.2–0.4), moderate (0.4–0.6), good (0.6–0.8), or excellent (0.8–1.0)[24]. All variables were also evaluated using univariate logistic regression analysis with SGUS outcome (positive vs negative) as a dependent variable. In the case of residuals with non-Gaussian distribution, variables were transformed (log or square root), before being entered into the model. The explained variance was evaluated using Nagelkerke R^2. *P* values ≤ 0.05 were considered statistically significant.

All analyses were repeated when only taking the average score for hypoechogenic areas in the right parotid and submandibular gland into account[9], instead of the total SGUS score as described by Hocevar, *et al*[6]. For this score, a cutoff value of ≥ 1.5 was considered positive[25].

RESULTS

Between January 2016 and December 2018, there were 186 patients included in the RESULT cohort. Fourteen patients were excluded from the present analysis due to a missing (n = 3) or incomplete (n = 5) US examination, or because they did not fulfill the ACR/EULAR criteria (n = 6). Of the eligible patients (n = 172), mean age was 53 years (SD 13.9), 156 (91%) were female, 136 (79%) were SGUS positive (i.e., SGUS score ≥ 15)[8] and median time since diagnosis was 8 years (Table 1).

Comparison of patients who were SGUS negative and positive. Table 1 shows the characteristics of the total group of patients with pSS, as well as of the patients with a positive or negative SGUS. There were no significant differences in general patient characteristics between the 2 groups, except for disease duration, which was longer in the patients who were SGUS positive.

Table 1. Patient characteristics and comparison of SGUS negative and positive patients.

	Total Group, N = 172	SGUS ≤ 14, n = 36	SGUS ≥ 15, n = 136	*P*
General characteristics				
Age, yrs	52.9 (13.9)	56.0 (14.0)	52.0 (13.8)	0.13
Females	156 (90.7%)	31 (86.1%)	125 (91.9%)	0.29
Disease duration, yrs	8.0 (4.0–13.0)	5.0 (3.0–8.8)	8.5 (5.0–13.8)	**0.003**
Symptom duration, yrs[a]	15.0 (9.0–21.0)	11.0 (6.0–19.0)	15.0 (10.0–22.0)	0.06
BMI, kg/m^2 [b]	24.9 (4.2)	24.6 (3.6)	24.8 (4.3)	0.79
Clinical variables				
ESSDAI total score[b]	4.0 (2.0–8.0)	2.0 (0.0–6.5)	4.0 (2.0–8.0)	**0.028**
ESSDAI categories[b]				**0.024**
0	25 (14.6%)	10 (27.8%)	15 (11.1%)	
1–4	75 (43.9%)	16 (44.4%)	59 (43.7%)	
≥ 5	71 (41.5%)	10 (27.8%)	61 (45.2%)	
DAS28-ESR[c]	3.2 (1.0)	2.9 (0.8)	3.3 (1.0)	**0.027**
DAS28-CRP[c]	2.3 (1.9–2.6)	2.3 (1.9–2.5)	2.3 (1.8–2.7)	0.74
Tender points[c]	1.5 (0.0–8.0)	2.0 (0.0–12.0)	1.0 (0.0–8.0)	0.34
PGA[a]	2.0 (1.0–3.0)	2.0 (1.0–3.0)	3.0 (1.0–4.0)	**0.026**
SSDDI total score[a]	2.0 (1.0–3.0)	1.5 (1.0–2.0)	2.0 (1.0–3.0)	**0.018**
UWS ≤ 0.1 mL/min[c]	111 (68.5%)	16 (45.7%)	95 (74.8%)	**0.001**
UWS flow, mL/min[c]	0.05 (0.01–0.13)	0.12 (0.03–0.27)	0.03 (0.00–0.11)	< **0.001**
Parotid gland biopsy, FS ≥ 11[d]	85 (81.0%)	12 (50.0%)	73 (90.1%)	< **0.001**
Labial gland biopsy, FS ≥ 12[e]	47 (81.0%)	11 (68.8%)	36 (85.7%)	0.14
Schirmer test, ≤ 5 mm/5 min[c]	121 (74.7%)	25 (69.4%)	96 (76.2%)	0.41
Schirmer test ODS, mm/5 min[c]	4.0 (0.9–10.0)	5.5 (2.6–11.1)	3.5 (0.0–9.6)	**0.020**
OSS ≥ 5[b]	58 (34.1%)	3 (8.3%)	55 (41.0%)	< **0.001**
OSS ODS total score[b]	2.5 (0.9–5.0)	0.5 (0.0–2.0)	3.5 (1.0–5.0)	< **0.001**
Serological variables				
Anti-SSA antibodies[b]	154 (90.1%)	27 (75.0%)	127 (94.1%)	**0.001**
Anti-SSB antibodies[b]	92 (53.8%)	9 (25.0%)	83 (61.5%)	< **0.001**
IgG level > 16.0 g/mL[b]	81 (47.4%)	5 (13.9%)	76 (56.3%)	< **0.001**
IgG level, g/mL[b]	15.5 (11.2–20.3)	11.2 (9.3–13.0)	16.9 (12.1–21.8)	< **0.001**
RF level > 5.0 IU/mLb	115 (67.3%)	12 (33.3%)	103 (76.3%)	< **0.001**
RF level, IU/mL[b]	15.0 (2.6–42.0)	2.1 (0.6–10.6)	21.0 (5.2–51.0)	< **0.001**
C3 level, g/L[b]	1.12 (0.23)	1.20 (0.24)	1.10 (0.22)	**0.012**
C4 level, g/L[b]	0.19 (0.15–0.24)	0.20 (0.18–0.24)	0.18 (0.14–0.24)	**0.015**
Leukocyte count, 10^9/L[b]	5.4 (1.9)	6.3 (2.0)	5.2 (1.8)	**0.002**
PROM				
ESSPRI, total score[b]	6.0 (4.3–7.0)	6.7 (5.0–7.7)	5.7 (4.3–7.0)	0.**016**
Dryness[b]	6.0 (5.0–8.0)	6.0 (4.0–8.0)	7.0 (5.0–8.0)	0.26
Fatigue[b]	7.0 (5.0–8.0)	8.0 (5.0–8.0)	7.0 (4.3–8.0)	**0.024**
Pain[b]	5.0 (2.0–7.0)	7.0 (5.0–8.0)	4.5 (2.0–7.0)	< **0.001**
PtGA[c]	6.0 (4.0–8.0)	7.0 (4.3–8.0)	6.0 (4.0–8.0)	0.15
EQ-5D[f]	0.77 (0.14)	0.73 (0.17)	0.80 (0.12)	0.23
PASS, acceptable[c]	117 (71.8%)	21 (58.3%)	96 (75.6%)	**0.042**

Data are expressed as n (%), mean (SD), or median (IQR. Schirmer test ≤ 5 mm/min and OSS ≥ 5 were considered positive if criteria were met in at least 1 eye. For Schirmer test, ODS and OSS ODS, the mean score of both eyes was calculated. Values in bold are statistically significant. [a] < 5% missing data. [b] 5–10% missing data. [c] 10–15% missing data. [d] 22% missing data. Data available for [e] 61% and [f] 34% of patients. DAS28-CRP: Disease Activity Score in 28 joints based on C-reactive protein; DAS28-ESR: Disease Activity Score in 28 joints based on erythrocyte sedimentation rate; ESSDAI: EULAR Sjögren Syndrome Disease Activity Index; ESSPRI: EULAR Sjögren Syndrome Patient-Reported Index; EULAR: European League Against Rheumatism; FS: focus score; ODS: ocular discomfort score; OSS: ocular staining score; PASS: patient acceptable symptom state; PROM: patient-reported outcome measurement; PGA: physician global assessment; PtGA: patient global assessment; RF: rheumatoid factor; SGUS: salivary gland ultrasonography; SSDDI: Sjögren Syndrome Disease Damage Index; UWS: unstimulated whole saliva.

Patients who were SGUS positive had significantly higher ESSDAI scores, higher DAS28-ESR, and higher PGA compared with patients who were SGUS negative, indicating higher disease activity (Table 1, Figures 1A,B; Supplementary Figure 1, available with the online version of this article). Moreover, a parotid gland FS ≥ 1, UWS ≤ 0.1 mL/min, and OSS ≥ 5 were more often seen in patients who were SGUS positive (Table 1). SSDDI, UWS, Schirmer test, and OSS also differed significantly between both groups, with more damage and worse salivary and lacrimal gland function in patients who were SGUS positive (Table 1; Figure 1C–E).

Regarding the serological variables, anti-SSA and anti-SSB

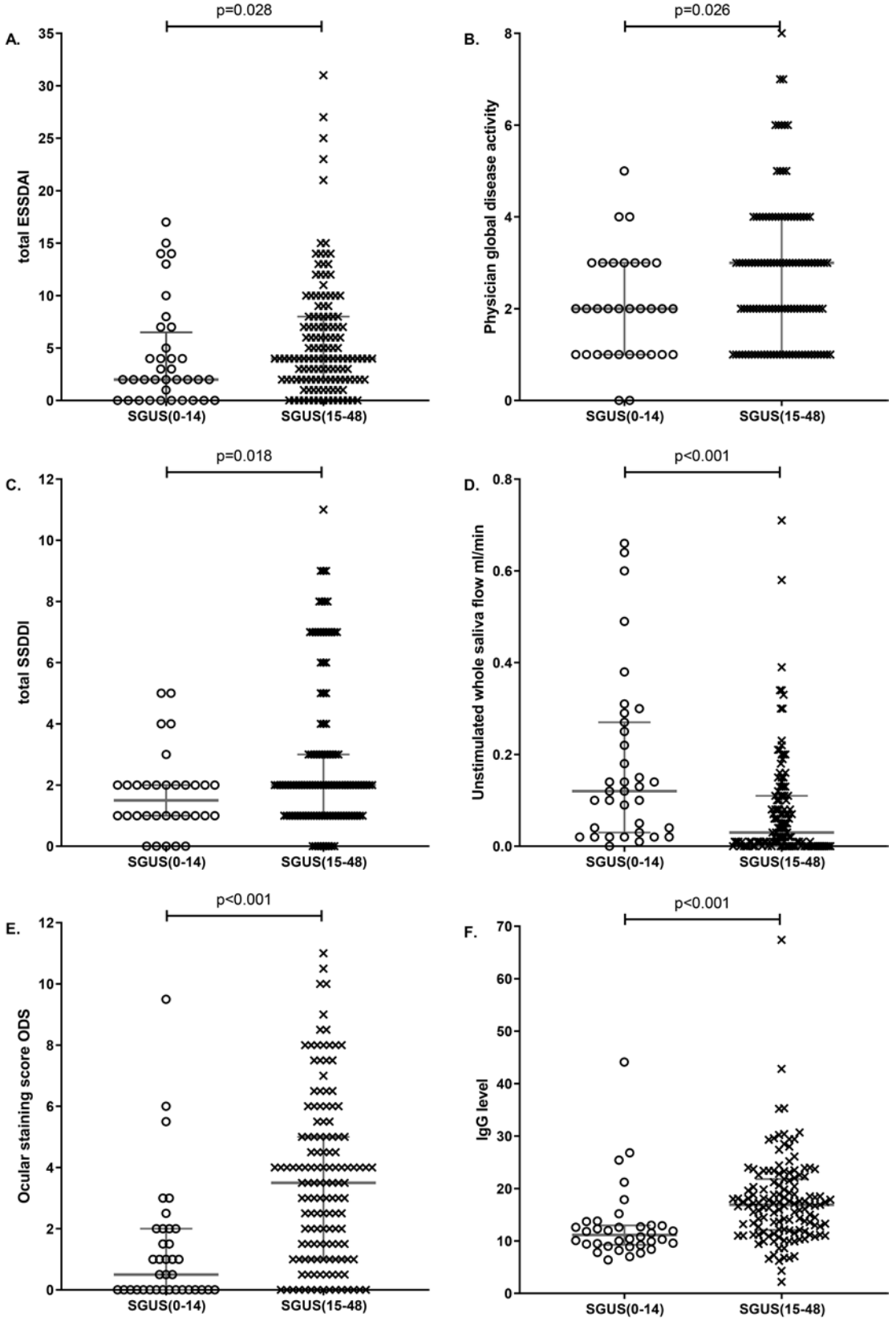

Figure 1. Ultrasound total score (negative/positive) compared with (A) total ESSDAI; (B) physician global assessment of disease activity; (C) total SSDDI; (D) unstimulated whole saliva flow; (E) ocular staining score; and (F) total IgG level. ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; EULAR: European League Against Rheumatism; ODS: ocular discomfort score; SGUS: salivary gland ultrasonography; SSDDI: Sjögren's Syndrome Disease Damage Index.

antibodies were more often present in patients who were SGUS positive. Further, patients who were SGUS positive showed higher levels of IgG and RF, lower complement C3 and C4 levels, and lower leukocyte counts compared with patients who were SGUS negative (Table 1, Figure 1F).

Regarding PROM, patients who were SGUS positive scored significantly lower on ESSPRI fatigue and pain and more often found their disease state acceptable, which indicates that patients who were SGUS positive experienced fewer symptoms (Table 1).

Results were confirmed with univariate logistic regression analyses (Table 2). The explained variance of individual variables varied from 0.1% for BMI to 22.4% for parotid gland biopsy (FS ≥ 1).

As an overview of the available data, a heatmap of the characteristics of the individual patients with pSS is shown in Supplementary Figure 2 (available with the online version of this article). The patients' order has been determined based upon the total SGUS score. Overall, our data show that patients who were SGUS positive have a distinct clinical phenotype compared with patients who were SGUS negative. These findings illustrate the results described above in another way.

Table 2. Logistic regression analyses of demographic, clinical, serological, and patient-reported outcome variables to predict ultrasound outcome.

	Univariate Analysis OR (95% CI)	*P*	R^2
General characteristics			
Age, yrs	0.979 (0.952–1.007)	0.13	0.021
Females	1.833 (0.593–5.662)	0.29	0.009
Disease duration, yrs	1.108 (1.028–1.195)	0.007	0.082
Symptom duration, yrs[a]	1.036 (0.991–1.083)	0.12	0.028
BMI, kg/m^2 [b]	0.988 (0.906–1.078)	0.79	0.001
Clinical variables			
ESSDAI total score[b*]	1.438 (1.040–1.988)	0.028	0.046
DAS28-ESR[c]	1.607 (1.047–2.466)	0.030	0.048
DAS28-CRP[c]	1.276 (0.738–2.205	0.38	0.008
Tender points[c]	0.976 (0.924–1.032)	0.40	0.007
PGA[a]	1.473 (1.062–2.043)	0.020	0.064
SSDDI total score[a]	1.357 (1.053–1.748)	0.018	0.079
UWS ≤ 0.1 mL/min[c]	3.525 (1.622–7.663)	0.001	0.094
UWS flow, mL/min[c]	0.010 (0.001–0.138)	0.001	0.120
Parotid gland biopsy, FS ≥ 11[d]	9.125 (3.089–26.953)	< 0.001	0.224
Labial gland biopsy, FS ≥ 12[e]	2.727 (0.696–10.684)	0.15	0.049
Schirmer test ≤ 5 mm/5 min[c]	1.408 (0.621–3.194)	0.41	0.006
Schirmer test ODS, mm/5 min[c**]	0.658 (0.459–0.942)	0.022	0.051
OSS ≥ 5[b]	7.658 (2.236–26.227)	0.001	0.141
OSS ODS total score[b]	1.598 (1.274–2.005)	< 0.001	0.212
Serological variables			
Anti-SSA antibodies[b]	5.292 (1.872–14.956)	0.002	0.084
Anti-SSB antibodies[b]	4.788 (2.088–10.984)	< 0.001	0.136
IgG level > 16.0 g/mL[b]	7.986 (2.927–21.795)	< 0.001	0.192
IgG level, g/mL[b]	1.129 (1.049–1.215)	0.001	0.121
RF level > 5.0 IU/mL[b]	6.438 (2.897–14.305)	< 0.001	0.192
RF level, IU/mL[b]	1.020 (1.004–1.036)	0.012	0.094
C3 level, g/L[b]	0.132 (0.026–0.672)	0.015	0.055
C4 level, g/L[b]	0.026 (0.000–1.991)	0.10	0.024
Leukocyte count 10^9/L[b]	0.756 (0.622–0.919)	0.005	0.075
PROM			
ESSPRI total score[b]	0.814 (0.662–1.001)	0.051	0.038
Dryness[b**]	1.680 (0.795–3.550)	0.17	0.016
Fatigue[b]	0.837 (0.701–0.998)	0.047	0.040
Pain[b**]	0.380 (0.179–0.803)	0.011	0.075
PtGA[b*]	0.808 (0.427–1.529)	0.51	0.004
EQ-5D[f]	10.489 (0.483–227.980)	0.14	0.026
PASS, acceptable[c]	2.212 (1.018–4.809)	0.045	0.036

[a] < 5% missing data. [b] 5–10% missing data. [c] 10–15% missing data. [d] 22% missing data. Data available for [e] 61% and [f] 34% of patients. * Square root transformation; ** LN transformation. DAS28-CRP: Disease Activity Score in 28 joints based on C-reactive protein; DAS28-ESR: Disease Activity Score in 28 joints based on erythrocyte sedimentation rate; ESSDAI: EULAR Sjögren Syndrome Disease Activity Index; ESSPRI: EULAR Sjögren Syndrome Patient-Reported Index; EULAR: European League Against Rheumatism; FS: focus score; ODS: ocular discomfort score; OSS: ocular staining score; PASS: patient acceptable symptom state; PGA: physician global assessment; PtGA: patient global assessment; PROM: patient-reported outcome measurements; RF: rheumatoid factor; SSDDI: Sjögren Syndrome Disease Damage Index; UWS: unstimulated whole saliva.

Comparison of patients with medium-positive or high-positive SGUS scores. When subdividing the group of patients who were SGUS positive into medium- and high-positive patients, we observed that compared with patients with a medium-positive SGUS score, patients with a high-positive SGUS score significantly more often had an UWS ≤ 0.1 mL/min, Schirmer test ≤ 5 mm/5 min, and OSS ≥ 5 (Table 3). Further, SSDDI, UWS, Schirmer test, and OSS differed significantly between medium- and high-positive patients with SGUS, showing more damage and worse salivary and lacrimal gland function in the high-positive patients (Table 3).

Patients with high-positive SGUS scores experienced

Table 3. Comparison of SGUS positive patients with medium or high SGUS scores.

	SGUS 15–26, n = 67	SGUS 27–41, n = 69	*P*
General characteristics			
Age, yrs	53.1 (13.6)	51.0 (13.9)	0.39
Females	63 (94.0%)	62 (89.9%)	0.53
Disease duration, yrs	8.0 (4.0–14.0)	9.0 (6.0–13.5)	0.35
Symptom duration, yrs[a]	14.5 (8.0–21.8)	16.0 (11.0–22.0)	0.22
BMI, kg/m^2[b]	24.8 (4.7)	24.8 (4.0)	0.99
Clinical variables			
ESSDAI total score[b]	4.0 (2.0–8.0)	4.0 (2.0–8.0)	0.76
ESSDAI categories[b]			0.92
0	7 (10.6%)	8 (11.6%)	
1–4	30 (45.5%)	29 (42.0%)	
≥ 5	29 (43.9%)	32 (46.4%)	
DAS28-ESR[c]	3.3 (1.0)	3.3 (1.0)	0.88
DAS28-CRP[c]	2.3 (1.7–2.7)	2.3 (2.0–2.7)	0.59
Tender points[c]	2.0 (0.0–9.0)	0.0 (0.0–5.8)	0.19
PGA[a]	2.0 (1.0–3.0)	3.0 (1.0–4.0)	0.28
SSDDI total score[a]	2.0 (1.0–3.0)	2.0 (2.0–5.8)	**0.001**
UWS ≤ 0.1 mL/min[c]	40 (60.6%)	55 (90.1%)	**< 0.001**
UWS flow, mL/min[c]	0.08 (0.01–0.15)	0.01 (0.00–0.04)	**< 0.001**
Parotid gland biopsy, FS ≥ 11[d]	36 (85.7%)	37 (94.9%)	0.27
Labial gland biopsy, FS ≥ 12[e]	18 (81.8%)	18 (90.0%)	0.67
Schirmer test ≤ 5 mm/5 min[c]	41 (67.2%)	55 (84.6%)	**0.022**
Schirmer test ODS, mm/5 min[c]	5.0 (1.0–12.0)	2.0 (0.0–5.3)	**0.017**
OSS ≥ 5[b]	17 (25.8%)	38 (55.9%)	**< 0.001**
OSS ODS total score[b]	2.0 (1.0–4.0)	4.0 (2.5–6.4)	**< 0.001**
Serological variables			
Anti-SSA antibodies[b]	60 (90.9%)	67 (97.1%)	0.16
Anti-SSB antibodies[b]	38 (57.6%)	45 (65.2%)	0.36
IgG level > 16.0 g/mL[b]	37 (56.1%)	39 (56.5%)	0.96
IgG level, g/mL[b]	16.8 (12.0–19.9)	17.4 (12.1–22.6)	0.57
RF level > 5.0 IU/mL[b]	47 (71.2%)	56 (81.2%)	0.17
RF level, IU/mL[b]	15.5 (3.0–36.3)	32.0 (8.5–57.5)	**0.037**
C3 level, g/L[b]	1.10 (0.23)	1.10 (0.22)	0.88
C4 level, g/L[b]	0.19 (0.15–0.24)	0.18 (0.13–0.22)	0.16
Leukocyte count, 10^9/L[b]	5.3 (1.6)	5.1 (2.0)	0.64
PROM			
ESSPRI total score[b]	6.0 (4.3–7.2)	5.7 (4.0–6.7)	0.30
Dryness[b]	6.0 (4.0–8.0)	7.0 (5.0–8.0)	**0.050**
Fatigue[b]	7.0 (5.0–8.0)	6.0 (4.0–7.0)	**0.042**
Pain[b]	6.0 (3.0–7.0)	4.0 (2.0–6.0)	**0.019**
PtGA[c]	6.0 (4.0–7.5)	6.0 (4.0–8.0)	0.80
EQ-5D[f]	0.78 (0.14)	0.78 (0.11)	0.94
PASS, acceptable[c]	45 (73.8%)	51 (77.3%)	0.65

Data are expressed as n (%), mean (SD), or median (IQR). Schirmer test ≤ 5 mm/min and OSS ≥ 5 were considered positive if criteria were met in at least 1 eye. For Schirmer test ODS and OSS ODS, the mean score of both eyes was calculated. Values in bold are statistically significant. [a] < 5% missing data. [b] 5–10% missing data. [c] 10–15% missing data. [d] 22% missing data. Data available for [e] 61% and [f] 34% of patients. DAS28-CRP: Disease Activity Score in 28 joints based on C-reactive protein; DAS28-ESR: Disease Activity Score in 28 joints based on erythrocyte sedimentation rate; ESSDAI: EULAR Sjögren Syndrome Disease Activity Index; ESSPRI: EULAR Sjögren Syndrome Patient-Reported Index; EULAR: European League Against Rheumatism; FS: focus score; ODS: ocular discomfort score; OSS: ocular staining score; PASS: patient acceptable symptom state; PGA: physician global assessment; PROM: patient-reported outcome measurements; PtGA: patient global assessment; RF: rheumatoid factor; SGUS: salivary gland ultrasonography; SSDDI: Sjögren Syndrome Disease Damage Index; UWS: unstimulated whole saliva.

significantly more dryness, but less fatigue and pain compared with patients with a medium-positive SGUS score (Table 3).

Correlations of SGUS total score. Significant associations were found between SGUS total score and disease duration (ρ = 0.279), symptom duration (ρ = 0.234), ESSDAI (ρ = 0.196), DAS28-ESR (ρ = 0.159), PGA (ρ = 0.217), SSDDI (ρ = 0.398), UWS (ρ = −0.551), Schirmer's test (ρ = −0.349), and OSS (ρ = 0.532; Supplementary Table 1, available with the online version of this article; Figure 2A–F and Figure 3A).

Further, significant associations were found between SGUS total score and IgG level (ρ = 0.264), RF level (ρ = 0.343), complement C4 level (ρ = −0.200), and leukocyte count (ρ = −0.244; Supplementary Table 1, available with the online version of this article; Figures 3B,C).

Moreover, SGUS total scores showed significant association with PROM; ESSPRI total score (ρ = −0.157), dryness (ρ = 0.223), fatigue (ρ = −0.209), and pain (ρ = −0.314; Supplementary Table 1, available with the online version of this article; Figure 3D–F).

To summarize, an increase in SGUS abnormalities is associated with longer disease duration, increased damage, and worse gland function, and with increased dryness symptoms.

SGUS–hypoechogenic areas only. When using only hypoechogenic areas to define SGUS positivity[9], multiple variables showed similar results as when total Hocevar score was applied, except that no significant differences were found for ESSDAI, DAS28-ESR, PGA, complement C3 and C4 levels, leukocyte counts, and PASS (Supplementary Tables 2 and 3, available with the online version of this article).

DISCUSSION

In our prospective observational RESULT cohort, we showed that patients who were SGUS positive had a distinct clinical phenotype compared with patients who were SGUS negative. This difference was found in all aspects of the disease: clinical, functional, serological, and PROM. SGUS could give an overall indication about the observable and experienced severity of pSS.

Patients who were SGUS positive had higher systemic disease activity, measured by ESSDAI, DAS28-ESR, and PGA, compared with patients who were SGUS negative. Of interest, patients who were SGUS positive score significantly worse on all individual items of the ACR/EULAR criteria (i.e., parotid gland biopsy, anti-SSA antibodies, Schirmer test, OSS, and UWS) compared with patients who were SGUS negative. Overall, total SGUS score showed the strongest association with OSS and UWS. In addition to these differences, patients who were SGUS positive scored worse on SSDDI and serological variables. These results show that SGUS enables us to identify patients with higher clinical and serological disease activity and more damage due to pSS.

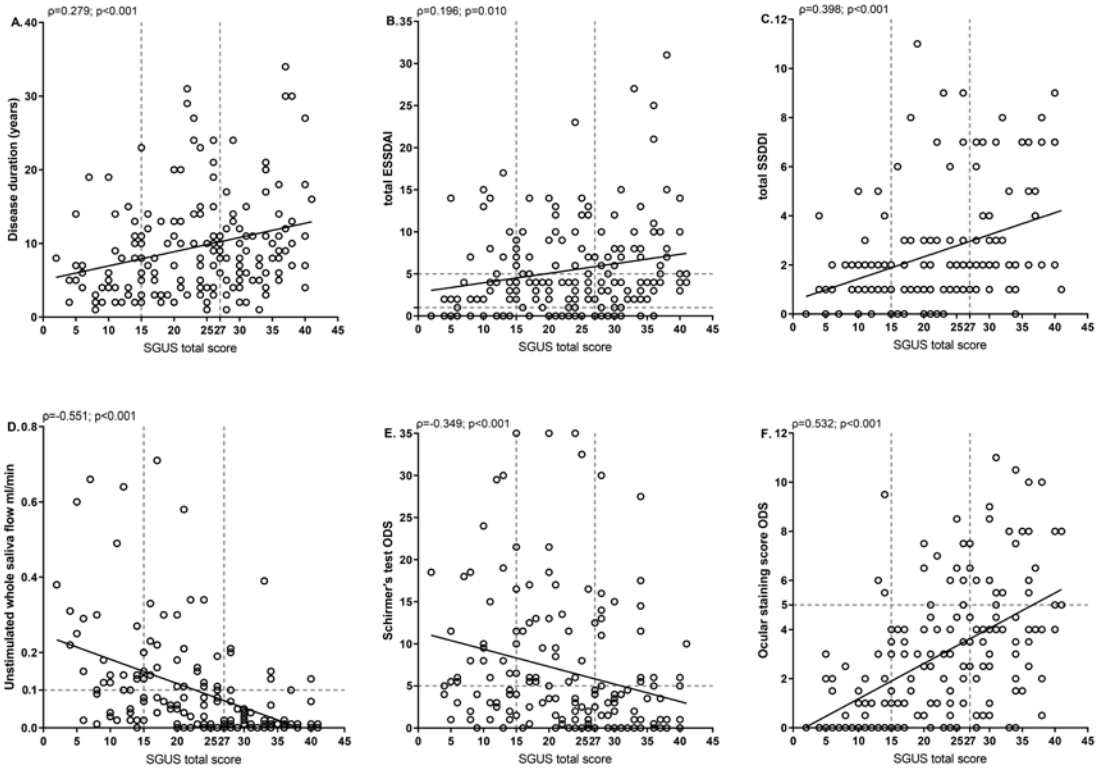

Figure 2. Scatterplots of ultrasound total score compared with (A) disease duration; (B) total ESSDAI; (C) SSDDI; (D) unstimulated whole saliva flow; (E) Schirmer test; and (F) ocular staining score. For Schirmer test and ocular staining scoare, the mean score of both eyes was calculated. ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; EULAR: EULAR: European League Against Rheumatism; ODS: ocular discomfort score; SGUS: salivary gland ultrasonography; SSDDI: Sjögren's Syndrome Disease Damage Index.

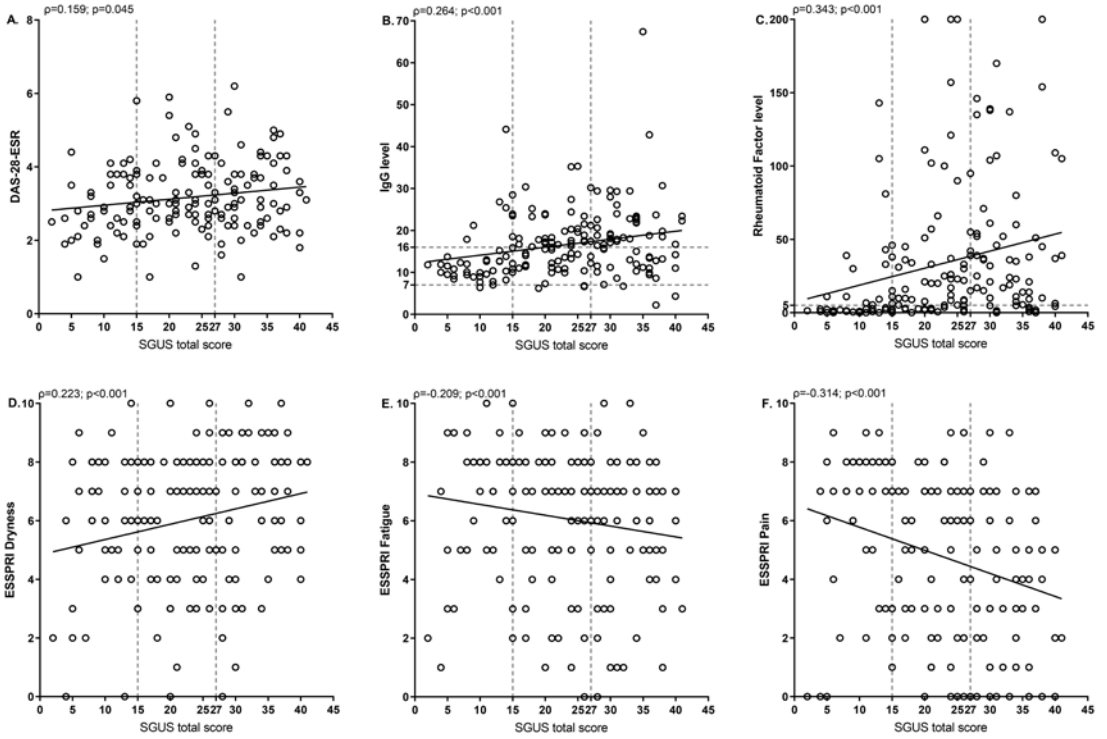

Figure 3. Scatterplots of ultrasound total score compared with (A) DAS28-ESR; (B) IgG level; (C) rheumatoid factor level; (D) ESSPRI dryness; (E) ESSPRI fatigue; and (F) ESSPRI pain. DAS28: Disease Activity Score in 28 joints; ESR: erythrocyte sedimentation rate; ESSPRI: EULAR Sjögren Syndrome Patient-Reported Index; EULAR: European League Against Rheumatism; SGUS: salivary gland ultrasonography.

Interestingly, patients who were SGUS positive experienced less fatigue and pain, both measured by ESSPRI, and more often found their disease state acceptable, which implies that these patients have a lower symptom burden. Perhaps patients who have already had pSS (or symptoms) for several years are more accustomed to it and have developed their own coping strategies or they have adjusted their expectations. Another possibility for the differences between SGUS-negative and -positive patients is that there are indeed different phenotypic clusters of patients with pSS. Previously, Tarn, *et al*[26] defined 4 subgroups of patients with pSS based upon the PROM of dryness, fatigue, pain, anxiety, and depression. Our data suggest that patients with high SGUS scores belong to a subgroup of patients with low symptom burden. Unfortunately, the Hospital Anxiety and Depression Scale is not part of the questionnaires within our RESULT cohort. Therefore, we were unable to verify whether SGUS scores also differ within these 4 subgroups of patients.

In the current study, we not only compared patients who were SGUS negative and positive based on a previously defined diagnostic cutoff point[8] but also focused on the broad range of patients who were SGUS positive. As expected, patients with a high-positive SGUS score showed more pSS-related damage (SSDDI), lower salivary and lacrimal gland function, and more glandular damage, compared with patients with a medium-positive SGUS score. Interestingly, there were no differences in the percentage of patients with a positive biopsy or presence of anti-SSA antibodies between both groups. This could be because most patients within our cohort score positive on these items, which makes it more difficult to see differences within subgroups of patients. Moreover, both FS and anti-SSA antibodies were collected as absent or present rather than on a continuous scale. Further, the differences in ESSPRI fatigue and pain remain, with fewer patient symptoms in the high-positive group. In contrast, however, high-positive patients with SGUS do indeed experience more dryness compared with the medium-positive patients, which is logical considering the relationship between SGUS and glandular function.

The association between SGUS and disease duration suggests that there is an increase in US abnormalities over time. In contrast, when looking solely at the SGUS-positive patients, there is no difference in disease duration between patients with medium-positive or high-positive scores. This raises the question of how long it takes for these SGUS abnormalities to develop and how long these abnormalities continue to worsen. Gazeau, *et al*[27] showed that a nearly 2-year interval between consecutive SGUS examinations was not enough to see significant progression over time in a group of 49 suspected patients with pSS. A possible explanation for the lack of difference in disease duration in medium-positive and high-positive patients with SGUS might be interobserver differences, as it was previously shown that SGUS scores between different observers show more variability when total score exceeds 20[12]. Alternatively, it could be postulated that after a certain disease duration, SGUS lesions stabilize, as is the case with the production of saliva[28].

In our previous studies, we have shown that for diagnostic purposes, it suffices to measure only hypoechogenic areas in 1 parotid and 1 submandibular gland[9] and that optimal cutoff for a positive SGUS is ≥ 1.525. Since the use of SGUS to stratify patients with pSS is essentially different from the use of SGUS for diagnostic purposes, we assessed whether results would be similar when using total SGUS score compared with measuring only hypoechogenic areas. Regarding UWS, Schirmer test, OSS, and disease damage measured by SSDDI, results were the same when only the component hypoechogenic areas were taken into account. This suggests that evaluation of hypoechogenic areas can be used to identify patients with glandular dysfunction and overall pSS-related damage. However, no differences in ESSDAI, PGA, and DAS28-ESR were found when SGUS positivity was based solely on hypoechogenic areas, although there were significant differences in serological activity. Therefore, the US component hypoechogenic areas should not be used to identify patients with high disease activity. For this purpose, a more comprehensive scoring system, such as the Hocevar scoring system[6], may be preferred above a scoring system including only 1 component.

Previously, several groups studied associations between SGUS and clinical, serological, and patient-reported variables[29–37]. However, there are considerable differences between some of these studies and our current study. The most important difference is that most studies focus on the possible diagnostic purposes of SGUS rather than its possible use for stratification of already-classified pSS patients[30,33,34,35]. In our study, differences between the patients who were SGUS negative and positive cannot be attributed to the fact that there are non-Sjögren syndrome (SS) sicca controls included, as we included only patients with pSS in this study. In comparison with the previous studies, we included a considerably higher number of patients with pSS. Nevertheless, previous studies found significant differences between patients who were SGUS negative and positive, regarding ESSDAI[31], tear and saliva production[29,30,31,32], presence of anti-SSA antibodies and/or anti-SSB antibodies[29,30,31,32], RF positivity[30,31], visual analog scale dry mouth[32], and ESSPRI dryness[29], and, with the exception of the patient-reported dryness symptoms, we were able to confirm these results. In contrast, other studies did not find differences in ESSDAI[29,30] and SSDDI[30] between patients who were SGUS negative and positive. In a study including pSS as well as non-SS sicca controls, patients who were SGUS positive had higher labial gland FS and more often had an OSS ≥ 3, UWS ≤ 0.1 mL/min, were anti-SSA/SSB and RF positive, and had hypergammaglobulinemia, compared with SGUS-negative patients[33]. In a large, mixed population of patients with pSS and healthy controls, Milic, *et al*[36] found significant correlations between SGUS score and age, minor salivary gland biopsy, SSDDI, and ESSDAI. However, in contrast to our findings, the authors did not find a significant correlation between SGUS and disease duration and ESSPRI. Other studies also found associations between SGUS and ESSDAI[34] and several serological variables[34,35,37], but again in a mixed population of pSS and non-SS sicca controls.

Other differences between previously performed studies and our current study relate to the applied SGUS scoring system and the criteria set used for classification. Some studies, including this current study, applied the Hocevar scoring system[6], but different cutoff points were applied[29,30]. Further, we applied the ACR/EULAR classification criteria, as did Kim, *et al*[33] and La Paglia, *et al*[37], whereas in all other studies, including the more recent ones, the American-European Consensus Group criteria were applied[29–32,34–36].

To confirm our results in different populations, a consensus scoring system with a validated cutoff is needed. Previously, the first steps in reaching international expert consensus have indeed been taken by the Outcome Measures in Rheumatology task force on SS[38]. Further, the development of an SGUS endpoint for use in future clinical trials is part of the Innovative Medicines Initiative project (NECESSITY)[39]. Two previous studies showed that the addition of SGUS improves the performance of the ACR/EULAR classification criteria[25,40]. In addition to the potential value of SGUS for diagnostic purposes, our results indicate that SGUS could also be used for patient stratification (e.g., for the selection of subgroups of patients for clinical trials). Although our results seem promising, the value of SGUS for patient stratification needs to be confirmed by other research groups. Currently, within the European Union, initiatives (e.g., the HarmonicSS research project) have already been taken to improve stratification of patients with pSS, also including the use of SGUS[41].

Our prospective observational cohort revealed that the majority of patients are SGUS positive. These patients have a longer disease duration, a higher disease activity, and more pSS-related damage compared with patients who were SGUS negative, whereas patients who were SGUS negative experience more fatigue and pain. In the future, SGUS hopefully can be used as a valid selection method for clinical trials, as it gives an overall indication of the disease.

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ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this article.

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