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Prediction of Sjögren's Syndrome Years Before Diagnosis and Identification of Patients With Early Onset and Severe Disease Course by Autoantibody Profiling

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Objective. **Autoantibodies are highly characteristic of primary Sjögren's syndrome (SS) and represent important tools for studying its pathogenesis. Nonetheless, thus far, no systematic investigations have assessed the presence of autoantibodies before diagnosis. This study was undertaken to analyze how early and in what order autoantibodies appear, how predictive they are of primary SS, and whether they identify disease subsets.**

Methods. **A nested case–control design linking data from the Malmö primary SS registry and 3 Swedish healthcare biobanks was applied. In all, 175 serum samples obtained from 117 individuals before diagnosis of primary SS and 1 serum sample from each of 117 matched controls were analyzed for antinuclear antibodies (ANAs), rheumatoid factor (RF), and antibodies against Ro 60/SSA, Ro 52/SSA, and La/SSB.**

Results. **Considering all patients with primary SS who were autoantibody positive after diagnosis, at least one autoantibody specificity was detected in 81% up to 20 years (median 4.3–5.1 years) before diagnosis. Those found most often were ANAs, followed by RF, anti–Ro 60/SSA, anti–Ro 52/SSA, and anti-La/SSB. Anti-Ro/SSA and anti-La/SSB antibodies were strongly associated with the risk of developing primary SS, especially early-onset disease and a severe disease course. When Bayesian prior prevalence estimates for primary SS were included in the calculation, prediagnostic anti–Ro 60/SSA and anti–Ro 52/SSA had the highest positive predictive values (25% and 100%, respectively).**

Conclusion. **Our findings indicate that autoantibodies are present for up to 18–20 years before the diagnosis of primary SS, but we cannot exclude even earlier seropositivity, since for most patients, the earliest sample analyzed was positive. In families with multiple cases of autoimmune disease, autoantibody profiling, along with assessment of genetic risk, enables identification of susceptible individuals in a predisease state.**

Primary Sjögren's syndrome (SS) is a systemic autoimmune disease characterized by dryness of mucous membranes throughout the body and inflammation or vasculitis in internal organs. The syndrome entails a spectrum of mild to very severe functional disturbances that overlap with other autoimmune diseases, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) (1). The prevalence in Scandinavia and elsewhere in Europe, when applying strict classification criteria (2), may be as low as 0.05–0.1% (3–5).

Between 5% and 10% of all humans develop some kind of autoimmune disorder during their lifetime (6). The mechanisms that initiate autoimmunity and the conversion from benign or asymptomatic autoimmunity to onset of autoimmune disease are not well understood.

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However, in chronological order, it seems that genetics and prenatal events are the first detectable factors that put an individual at risk of autoimmune disease (1,7). It is conceivable that the next steps comprise interactions between the genes involved and intrinsic or external life events or environmental triggers (8,9), resulting in autoantibody production and the onset of symptoms and disease.

Even though the production of autoantibodies is highly characteristic of primary SS, and autoantibodies against Ro/SSA and La/SSB are particularly important as tools for studying the pathogenesis of this disease (1), there is limited information about the time point at which autoantibodies first appear in primary SS (10). We are well aware that mothers of babies with neonatal lupus syndrome can have anti-Ro/SSA antibodies despite being apparently healthy. However, it has been reported that more than a quarter of such women develop primary SS within 10 years of giving birth, and an even greater percentage of women develop SS if both SSA and SSB autoantibodies are present (11,12). In another study (13), patients with polyarthralgia were screened for various autoantibodies, and it was found that primary SS was starting to develop in 11 of 15 anti-La/SSB–positive patients.

We recently observed that the majority of patients with primary SS had autoantibodies many years before they experienced the first symptoms of Sjögren's syndrome (10). The purpose of this study was to present data from an extended data set of our nested case–control study to determine with greater accuracy the length of time between the initial appearance of antinuclear antibodies (ANAs), rheumatoid factor (RF), or autoantibodies to Ro 60/SSA, Ro 52/SSA, or La/SSB and the diagnosis of primary SS, which autoantibodies appear first, whether the concentration of autoantibodies is higher closer to the time of diagnosis, and whether the presence of prediagnostic autoantibodies identifies patients at higher risk of an unfavorable disease course.

PATIENTS AND METHODS

Study population. At the Department of Rheumatology in Malmö, Sweden, patients with primary SS have been consecutively registered since 1984, both at the time of diagnosis and during subsequent prospective followup conducted at intervals of 6 months to 2 years (14). At the time of the registry linkage for the present study, this registry included a total of 360 patients who fulfilled the 2002 American–European Consensus Group Criteria (2) for primary SS. The cohort for the present study consisted of all registered patients with primary SS from whom serum samples had been obtained before diagnosis and saved in 1 of 3 biobanks. Registry linkage showed that a total of 175 such prediagnostic sera were available from 117 of the patients with primary SS. In addition, 117 control subjects (1 for each patient, with 1 serum sample each) were randomly selected from the same biobank the patient samples were obtained from. The controls were matched for sex and age at the time of acquisition of the respective patient's earliest serum sample, and were matched for the date of sampling (±60 days) as a control for duration of sample storage. Furthermore, we stipulated that the control subjects had to have been alive, living in the Malmö area, and not diagnosed as having primary SS at the time point when primary SS was diagnosed in the index case or later. The study was approved by the Research Ethics Committee at Lund University.

Biobanks. We used serum samples from 3 different biobanks. The first of these was the Southern Sweden Microbiology Biobank (SSMB), which contains samples submitted to Skåne University Hospital in Malmö for clinical microbiologic analyses. The majority of these samples have been obtained for diagnosis of viral infections, viral immunity, or presurgery. The reason for sending a sample for analysis was not revealed in most cases. At the time of the present study, ~1.4 million samples, the oldest from 1969, had been collected from about 560,000 individuals and included in the SSMB. The other 2 biobanks were established by 2 projects: Malmö Preventive Medicine and Malmö Diet and Cancer; these were prospective, population-based studies of ~33,000 men and 30,000 women each. The serum samples used in those 2 projects were collected in 1974–1992 and 1991–1996, respectively. Since healthy individuals were included in these projects, the reason for the sampling was simply to follow the research protocol, and not to monitor any actual symptom or disease manifestation. The 3 biobanks have been described elsewhere (15).

Laboratory investigations. Autoantibodies against Ro 60/SSA, Ro 52/SSA, La/SSB, Sm, RNP, Scl-70, Jo-1, ribosomal P, and chromatin were detected using a multiplex immunobead assay (Quanta Plex SLE Profile 8; Inova Diagnostics) and analyzed with a Luminex 100 instrument, applying the cutoff values recommended by the manufacturer. ANAs were analyzed by indirect immunofluorescence, and as antigen, we used HEp-2 cells grown to confluence on coverslips and fixed in 4% paraformaldehyde. After blocking with 20% fetal bovine serum in phosphate buffered saline for 20 minutes, the cells were stained for 1 hour with patient sera diluted 1:100. Autoantibodies were detected using a mixture of polyvalent goat anti-human IgG, IgA, and IgM antibodies labeled with fluorescein isothiocyanate (#F0202, #F0203, and #F0204; Dako). The coverslips were washed between each step, and the whole procedure was carried out at room temperature. The coverslips were examined by microscopy, and fluorescent staining (above background/autofluorescence) was recorded. IgM-RF was analyzed with an in-house enzyme-linked immunosorbent assay that is used for clinical diagnostics at the Clinical Immunology Laboratory of Skåne University Hospital in Lund, and this analysis was standardized using the World Health Organization RF reference serum with the cutoff set at 10 IU/liter.

Statistical analysis. We used descriptive statistics to analyze the autoantibody profile in relation to the onset of symptoms and the diagnosis of primary SS, and we applied chi-square statistics to compare different age groups with regard to the prediagnostic presence of autoantibodies. Furthermore, we used Friedman's nonparametric repeated-measures test to determine whether the time interval between the initial appearance of autoantibodies and the diagnosis differed for the various antibodies. *P* values less than 0.05 were considered

Table 1. Demographic and disease characteristics of the 117 patients with primary Sjögren's syndrome*

Male/female	8/92
Age at diagnosis, mean ± SD years	53.8 ± 14.1
Duration of followup from diagnosis to 2014, mean ± SD years	18.2 ± 5.4
Anti–Ro 60/SSA positive at or after diagnosis	65
Anti–Ro 52/SSA positive at or after diagnosis	54
Anti–La/SSB positive at or after diagnosis	45
ANA positive at or after diagnosis	87
RF positive at or after diagnosis	68
Labial salivary gland biopsy positive (n = 102)	85
Germinal center–like structures (n = 62)	19
Unstimulated sialometry, mean ± SD ml/15 minutes	0.56 ± 0.94
Schirmer's test, mean ± SD mm/5 minutes (sum of both eyes)	8.1 ± 10.3
Parotid, submandibular, or lacrimal gland swelling	38
Purpura or skin vasculitis	17
Clinically or electromyographically verified polyneuropathy	9
Raynaud's phenomenon	31
Lymphadenopathy	16
First available C3/C4, mean ± SD gm/liter	1.07 ± 0.35/0.25 ± 0.14
First available IgG, mean ± SD gm/liter	17.7 ± 7.7

* Except where indicated otherwise, values are the percent of patients. ANA = antinuclear antibody; RF = rheumatoid factor.

significant. Univariate logistic regression analysis was performed in order to evaluate the predictive value of the autoantibodies for later disease manifestations, and the results are presented as odds ratios (ORs) and 95% confidence intervals. Implementing the Bayesian theorem, we adjusted the positive predictive values (PPVs) of the predisease autoantibody results, taking the prevalence of the disease into account (16). The *t*-test was used for comparison of postdiagnostic levels of laboratory results in patients with or without prediagnostic autoantibodies. The paired *t*-test was applied when comparing early and late autoantibody concentrations in the same patients. Statistical analysis was performed using SPSS version 20.0 for Macintosh.

RESULTS

Serum samples collected before diagnosis of primary SS. The 117 patients with primary SS included 108 women (92%) and 9 men (8%) with a mean ± SD age at diagnosis of 53.8 ± 14.1 years (range 18–80 years). The numbers of prediagnostic serum samples available for the patients were as follows: 1 sample was available for 66% of the patients, 2 samples for 22%, 3 samples for 8%, 4 samples for 2%, and 5 samples for 2%. The earliest available serum sample for each patient had been obtained a mean of 6.4 ± 5.3 years before the diagnosis of primary SS, with a maximum interval of 23.7 years. Patient and disease characteristics are presented in Table 1.

Serum samples and relation to onset of primary SS. Previously, we presented data from a subgroup of 44 patients (38% of those included in this study) whose disease had clearly started after the time point of sampling (10). In the present analysis, all patients with samples obtained before diagnosis were included. We reread all available sources, including the results of immunology tests ordered by other physicians from our region, in order to identify possible earlier suspicion of SS. No information on disease onset was available for 3 (2.6%) of the 117 patients. For 58% of the patients, there were no indications of any kind pointing toward primary SS having started before inclusion in the applied registries. For 16% of the patients, we could assume that symptoms related to primary SS might have been present before sampling, while for the remaining 23%, the information was not specific enough to draw any conclusions.

Prediagnostic presence of autoantibodies. For 88 (75%) of the patients, at least 1 of the autoantibodies analyzed was detected in serum samples obtained before diagnosis. Eight (6.8%) of 117 patients were seronegative for all autoantibodies analyzed (ANAs, RF, anti–Ro 60/SSA, anti–Ro 52/SSA, and anti-La/SSB) during the complete followup including postdiagnostic assessments. ANAs were present in 63% of the patients, RF in 53%, and anti-Ro 60/SSA, anti–Ro 52/SSA, and anti-La/SSB antibodies in 51%, 41%, and 29%, respectively. Anti-RNP antibodies were found in 8 patients (6.8%) and antichromatin antibodies in 2 patients (1.7%), whereas none of the patients had autoantibodies against Sm, Scl-70, Jo-1, or ribosomal P.

In all, 84 of the 88 patients who were seropositive before diagnosis were positive for at least 1 of the autoantibodies analyzed in their first or only available serum sample. Thus, only 33 (28.2%) of all earliest available samples did not display any of the antibodies analyzed (ANAs, RF, anti-Ro 60/SSA, anti-Ro 52/SSA, or anti-La/SSB), and 8 of these 33 patients never developed any autoantibodies during the course of the disease. The

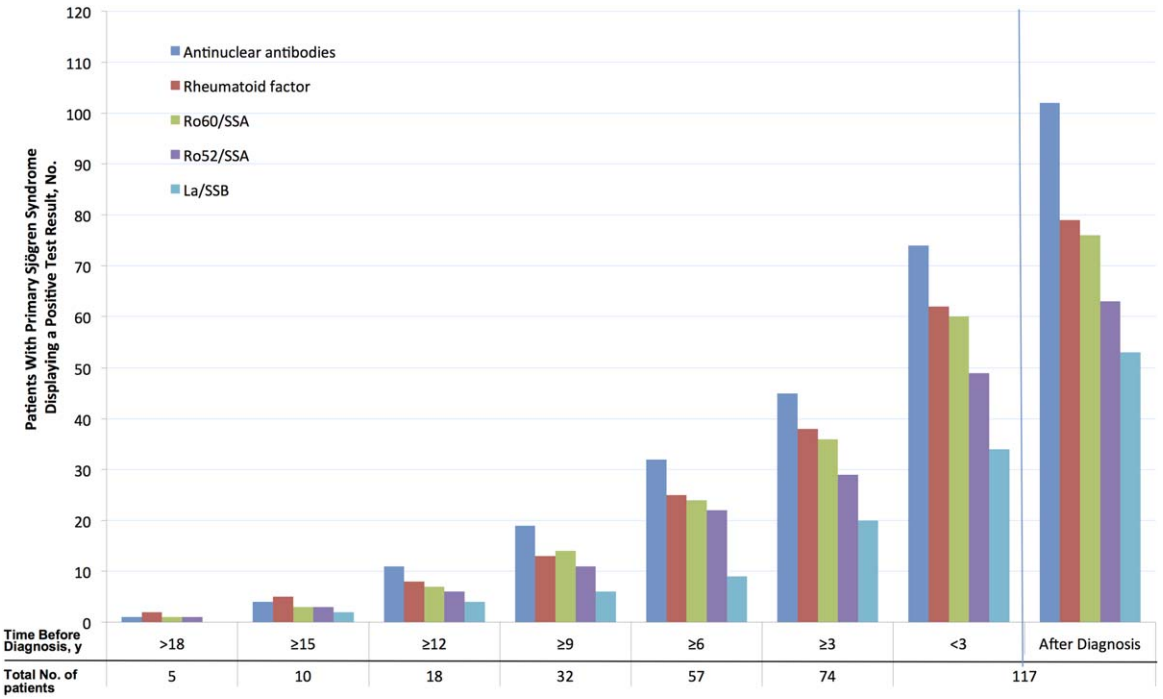

Figure 1. Number of patients with primary Sjögren's syndrome (SS) who were positive for antinuclear antibodies, rheumatoid factor, and autoantibodies against Ro 60/SSA, Ro 52/SSA, and La/SSB during the indicated 3-year periods before diagnosis of the disease. All patients met the American–European Consensus Group Criteria for primary SS and were diagnosed at the Department of Rheumatology in Malmö, Sweden.

earliest available sample in which autoantibodies were detected was obtained 19.5 years before diagnosis and was positive for RF. Also, 1 serum sample obtained as early as 18.8 years before diagnosis was positive for ANAs, anti-Ro 60/SSA, and anti-Ro 52/SSA antibodies, and another sample collected 16.1 years before diagnosis contained anti-La/SSB antibodies. The median time interval between the earliest autoantibody detection and the diagnosis was 4.6 years for ANAs, 4.3 years for RF, 4.5 years for anti-Ro 60/SSA, 5.1 years for anti-Ro 52/SSA, and 3.5 years for anti-La/SSB. No statistically significant differences between these intervals were found.

As indicated in Figure 1, the proportions of patients with prediagnostic serum samples positive for ANAs, RF, or antibodies against Ro 60/SSA, Ro 52/SSA, or La/SSB increased over the years up to the time primary SS was diagnosed. An additional 21 patients first presented with autoantibodies at or after the diagnosis of SS, while 8 never became seropositive.

Predictive value of the autoantibodies. Autoantibodies of any type were detected in 27 (23%) of the 117 matched controls. Of the controls, 13% were positive for RF, 8.5% for ANAs, 1.7% for anti-Ro 60/SSA antibodies, 1.7% for anti–ribosomal P antibodies, and 0.9% for anti-La/SSB (Table 2). No control samples were positive for autoantibodies against Ro 52/SSA, RNP, chromatin, Sm, Scl-70, or Jo-1. The high percentage of positive RF and ANAs among the controls prompted us to investigate the possibility of the presence of an autoimmune disease at the time of sampling or during followup.

Table 2. Predictive value of prediagnostic autoantibodies for primary Sjögren's syndrome*

Autoantibody	No. of patients (n = 117)	No. of controls (n = 117)	OR (95% CI)	Sensitivity, %	Specificity, %	PPV, %†
RF	62	15	4.1 (2.2, 7.7)	53	87	0.41
ANAs	74	10	7.4 (3.6, 15)	63	92	0.34
Anti–Ro 60/SSA	60	2	30.0 (7.2, 126)	51	98	25
Anti–Ro 52/SSA	49	0	Not calculable	42	100	100
Anti-La/SSB	34	1	34.0 (4.6, 253)	29	99	2.8

* OR = odds ratio; 95% CI = 95% confidence interval; RF = rheumatoid factor; ANAs = antinuclear antibodies.
† The positive predictive value (PPV) was prevalence adjusted (0.1%) and calculated as (sensitivity × prevalence)/(sensitivity × prevalence) + (1−specificity) × (1−prevalence) (16).

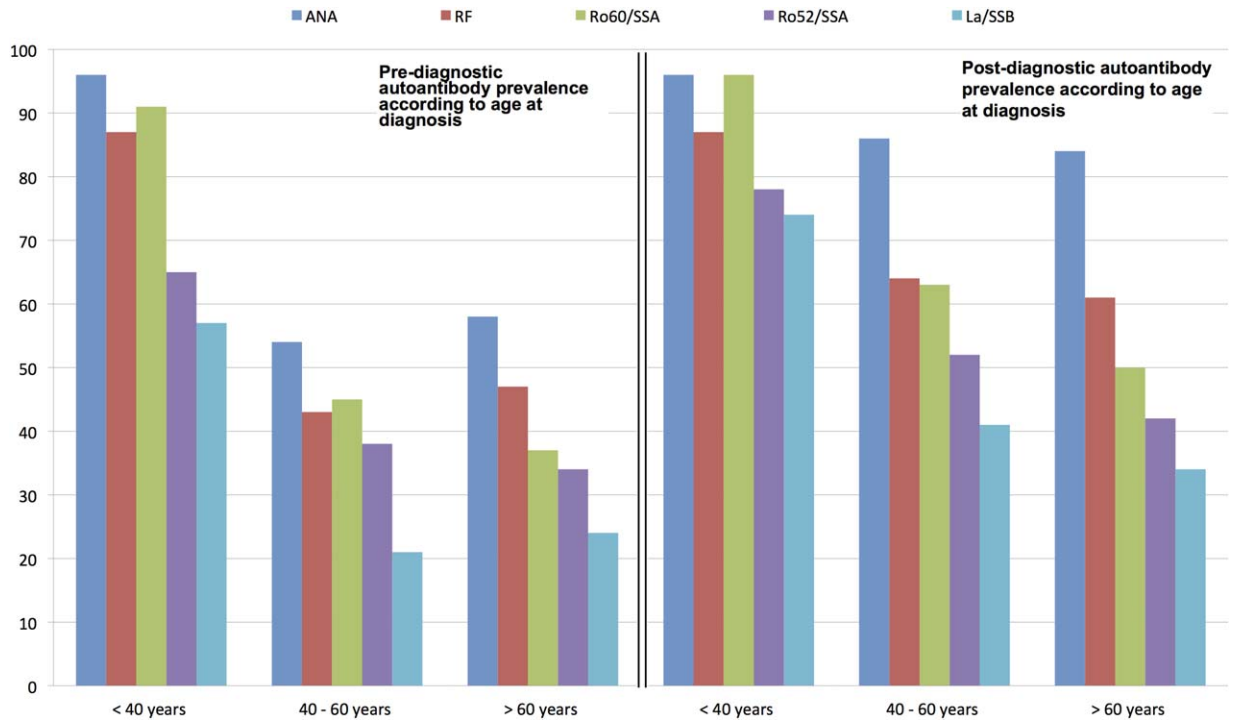

Figure 2. Prediagnostic and postdiagnostic prevalence of antinuclear antibodies (ANAs), rheumatoid factor (RF), and autoantibodies against Ro 60/SSA, Ro 52/SSA, and La/SSB in patients diagnosed as having primary Sjögren's syndrome (SS) according to the American–European Consensus Group Criteria before the age of 40 years (n = 23), at the age of 40–60 years (n = 56), or after 60 years of age (n = 38). Compared with the patients with primary SS who were age 40 or older at diagnosis, those who were diagnosed before age 40 showed significantly higher prediagnostic prevalence of all of the autoantibody specificities studied, and they also had a significantly higher postdiagnostic prevalence of RF and autoantibodies against Ro 60/SSA, Ro 52/SSA, and La/SSB.

One of the RF-positive controls had developed RA, and another with a positive anti-La response was regularly seen by a rheumatologist due to severe erosive osteoarthritis. There were a number of controls with thyroid diseases of different kinds. Most of them, however, had common age-related diseases, such as diabetes mellitus, hypertension, and other cardiovascular events or malignancies. None of the case records studied or routine immunology results revealed signs or suspicion of SLE or SS. However, we had no access to primary care records.

The predictive value for developing primary SS was highest for anti-La/SSB antibodies (OR 34), followed by anti-Ro 60/SSA antibodies (OR 30), ANAs (OR 7.4), and RF (OR 4.1) (Table 2). An OR could not be calculated for anti-Ro 52/SSA antibodies because none of the controls were positive for this autoantibody. Taking into account

Table 3. Comparison of selected important continuous disease variables in patients with primary Sjögren's syndrome with and without antibodies against anti-Ro/SSA or anti-La/SSB before diagnosis*

Variable (no. of patients with/ without antibodies)	Patients with prediagnostic anti-Ro/La	Patients without prediagnostic anti-Ro/La	Difference (95% CI for difference)	*P*
IgG, gm/liter (n = 50/51)	22.2 ± 7.03	13.2 ± 5.3	9.03 (6.6, 11.5)	<0.001
IgA, gm/liter (n = 51/50)	3.4 ± 1.6	2.3 ± 1.4	1.04 (0.43, 1.6)	0.001
C3, gm/liter (n = 51/49)	1.03 ± 0.36	1.10 ± 0.34	−0.07 (−0.21, 0.69)	0.33
C4, gm/liter (n = 51/49)	0.22 ± 0.13	0.28 ± 0.14	−0.05 (−0.11, −0.001)	0.040
CD4+ T cells, % (n = 33/29)	39.7 ± 12.5	48.7 ± 10.9	−9.0 (−15, −3.02)	0.004
CD4+:CD8+ T cell ratio (n = 36/34)	1.9 ± 1.5	2.7 ± 2.0	−0.81 (−1.7, 0.03)	0.058

* Values are the mean ± SD. There were no significant associations between the presence of anti-Ro/La antibodies and measures of eye and mouth dryness, B cell subsets, or IgM levels. 95% CI = 95% confidence interval.

Table 4. Analysis of the predictive ability of prediagnostic antibodies in relation to selected important outcome variables in primary Sjögren's syndrome*

Disease manifestation, predictor	OR (95% CI), by univariate analysis	OR (95% CI), by multivariate analysis†
Systemic disease manifestations		
Ro 60	2.8 (1.3, 6.0)	2.2 (0.61, 7.7)
Ro 52	2.3 (1.1, 5.1)	0.64 (0.17, 2.5)
La	1.9 (0.80, 4.6)	0.55 (0.16, 1.9)
ANAs	2.5 (1.1, 5.4)	–
RF‡	5.11 (2.3, 11.6)	5.9 (2.0, 17.5)
Palpable purpura or skin vasculitis		
Ro 60‡	9.3 (2.5, 34.3)	7.9 (1.3, 48.1)
Ro 52	4.7 (1.6, 13.8)	0.95 (0.2, 4.5)
La	3.2 (1.1, 9.1)	0.89 (0.26, 3.1)
ANAs	16.7 (2.1, 131)	–
RF	4.7 (1.3, 17.5)	1.7 (0.32, 8.85)
Focal sialadenitis in lower lip salivary gland biopsy		
Ro 60	0.76 (0.25, 2.3)	0.22 (0.05, 1.8)
Ro 52	1.3 (0.41, 4.1)	1.9 (0.31, 11.4)
La‡	6.3 (0.78, 50.3)	12.1 (1.3, 112)
ANAs	0.56 (0.17, 1.9)	–
RF	1.29 (0.43, 3.9)	0.87 (0.22, 3.4)
GC-like structures in lower lip salivary gland biopsy		
Ro 60	1.2 (0.33, 4.1)	3.2 (0.52, 9.7)
Ro 52‡	0.46 (0.11, 1.8)	0.08 (0.006, 0.99)
La	1.1 (0.26, 4.8)	2.3 (0.20, 26.7)
ANAs	1.4 (0.36, 5.1)	–
RF	1.1 (0.29, 3.7)	1.9 (0.39, 9.67)
Cytopenia§		
Ro 60	2.5 (0.90, 6.9)	2.2 (0.52, 9.6)
Ro 52	2.4 (0.87, 6.6)	1.6 (0.34, 7.5)
La	1.04 (0.33, 3.2)	0.33 (0.08, 1.5)
ANAs	2.5 (0.70, 6.0)	–
RF	2.5 (0.87, 7.5)	2.3 (0.64, 8.3)
CD4+ T lymphopenia¶		
Ro 60‡	6.3 (1.6, 25.0)	9.8 (1.7, 57.9)
Ro 52	1.75 (0.55, 5.6)	0.37 (0.76, 1.8)
La	2.0 (0.55, 6.9)	0.67 (0.15, 3.1)
ANAs	6.04 (1.2, 29.4)	–
RF	3.5 (0.87, 13.6)	2.2 (0.38, 12.3)
Additional autoantibody production#		
Ro 60	0.77 (0.31, 2.0)	1.2 (0.30, 4.6)
Ro 52	0.71 (0.28, 1.8)	0.75 (0.18, 3.2)
La	0.36 (0.11, 1.2)	0.28 (0.07, 1.1)
ANAs	1.3 (0.46, 3.4)	–
RF	2.2 (0.65, 6.9)	2.1 (0.65, 6.9)

* OR = odds ratio; 95% CI = 95% confidence interval; RF = rheumatoid factor; GC = germinal center.
† Antinuclear antibodies (ANAs) were not included in the multivariate analysis due to collinearity with anti-SSA and anti-SSB.
‡ Predictive of the indicated disease manifestation.
§ Includes all types of cytopenias as described in the EULAR Sjögren's Syndrome Disease Activity Index (33). No associations with parotid swelling, cryoglobulinemia, or lymphadenopathy were found.
¶ Defined as either a CD4+ T cell count of ≤30% of the total T cell count or a CD4+:CD8+ ratio of ≤0.8.
# Including antibodies to thyroid, mitochondria, smooth muscle, mitotic spindle, centromere, DNA, cardiolipin, RNP, Sm, gliadin, transglutaminase, and cyclic citrullinated peptide.

the low prevalence of primary SS (we chose 0.1% according to recent studies [3–5]) and applying the Bayesian theorem (16), we also calculated sensitivity- and specificity-adjusted PPVs for the autoantibodies, illustrating the risk of developing SS conferred by positive serology.

Autoantibody titers in relation to time to diagnosis. For the seropositive patients who had at least 2 available prediagnostic serum samples, we compared the first and last of those samples with regard to the levels of RF and of autoantibodies to Ro 60/SSA,

Ro 52/SSA, and La/SSB. The mean time interval between the 2 samples was 5.6 years (median 6 years). The samples obtained closer to diagnosis displayed a slight trend, which was not statistically significant, toward increased levels of the autoantibodies (data are available upon request from the corresponding author).

There were no significant correlations between levels of autoantibodies to Ro 60, Ro 52, and La and time from sampling to diagnosis, when all first positive samples (i.e., including those patients with only 1 prediagnostic sample available) were included (data not shown). Using the same approach for RF (n = 62), the level of response was slightly higher the closer to diagnosis the sample was obtained (Spearman's $\rho = 0.228$, $P = 0.046$).

Prediagnostic autoantibody prevalence in relation to age at diagnosis. In 23 patients, primary SS was diagnosed before age 40. These patients showed a significantly higher prevalence of prediagnostic autoantibodies (i.e., ANAs, RF, and autoantibodies against Ro 60/SSA, Ro 52/SSA, and La/SSB) compared with those ages 40–60 years (n = 56) or >60 years (n = 38) at diagnosis (Figure 2). ANAs were detected in prediagnostic sera from 96% of the patients diagnosed before age 40 years but only 54% of those ages 40–60 years at diagnosis ($P < 0.001$) and 58% of those age >60 years at diagnosis ($P = 0.001$). RF was detected in 87% of those diagnosed before age 40 years versus 43% of those diagnosed at ages 40–60 years ($P < 0.001$) and 47% of those diagnosed after age 60 years ($P = 0.002$). Anti–Ro 60/SSA antibodies were detected in 91% of those diagnosed before age 40 years versus 45% of those diagnosed at ages 40–60 years and 37% of those diagnosed after age 60 years (both $P < 0.001$). Anti–Ro 52/SSA antibodies were detected in 65% of those diagnosed before age 40 versus 38% of those diagnosed at ages 40–60 years ($P = 0.025$) and 34% of those diagnosed after age 60 years ($P = 0.019$), and anti-La/SSB antibodies were detected in 57% of those diagnosed before age 40 years versus 21% of those diagnosed at ages 40–60 years ($P = 0.002$) and 24% of those diagnosed after age 60 years ($P = 0.010$). The mean ± SD time between sample and diagnosis was 5.1 ± 3.6 years in the early-onset group and 6.8 ± 5.7 years in those in whom SS was diagnosed after age 40 years ($P = 0.16$).

Furthermore, levels of RF were inversely correlated with age at diagnosis, with higher levels of RF the earlier the onset of primary SS (Spearman's $\rho = -0.337$, $P = 0.003$). For anti-Ro/SSA and anti-La/SSB antibodies, this association was not detected. In addition, the numbers of autoantibody specificities per sample were considerably higher in the patients with early-onset disease compared to those with onset later than age 40 years (mean ± SD 3.9 ± 1.3 versus 2.1 ± 1.9; $P < 0.001$).

The prevalence of autoantibodies after diagnosis was also higher in patients with early-onset disease than in those with late-onset disease (Figure 2). These differences were statistically significant for RF ($P = 0.044$ and $P = 0.028$ for patients with early-onset disease versus those diagnosed between 40 and 60 years and versus those older than 60, respectively), and for autoantibodies against Ro 60/SSA ($P = 0.001$ for both comparisons), Ro 52/SSA ($P = 0.029$ and $P = 0.006$), and La/SSB ($P = 0.020$ and $P = 0.007$), but not for ANAs.

Prediagnostic autoantibodies as predictors of unfavorable long-term outcome. The mean ± SD time since diagnosis in the patients included was 18.2 ± 5.4 years. Patients with prediagnostic autoantibodies developed more systemic disease manifestations. Several characteristics associated with more severe disease course, such as the presence of skin vasculitis, higher IgG levels, lower complement levels, disturbance of T cell subsets, or biopsy findings, were more prevalent in patients with prediagnostic autoantibodies (Tables 3 and 4).

The level of postdiagnostic IgG increased significantly with an increasing number of prediagnostic autoantibody specificities ($P < 0.0001$), from a mean ± SD of 13.6 ± 6.3 gm/liter in those without any antibodies to a mean ± SD of 24.3 ± 5.8 gm/liter in those with all 5 subtypes. Pearson's correlation coefficient for the correlation between the number of prediagnostic autoantibodies (0–5) and levels of postdiagnostic variables was 0.649 for IgG ($P < 0.0001$), 0.332 for IgA ($P = 0.001$), −0.339 for C4 ($P = 0.001$), −0.262 for CD4+ T cell count ($P = 0.04$), and −0.262 (Spearman's rho) for CD4:CD8 ratio ($P = 0.028$).

DISCUSSION

Autoantibody production is a dominant feature of primary SS, but very little is known about the time point at which such production begins. To the best of our knowledge, we are the first to systematically investigate this issue, and the findings of this study indicate that autoantibodies are produced many years before clinical onset or diagnosis of the disease.

ANAs, RF, and antibodies against Ro 60/SSA and Ro 52/SSA were detected in samples obtained as early as 19–20 years (median 4.3–5.1 years) before diagnosis, whereas antibodies against La/SSB were detected as early as 16 years (median 3.5 years) before diagnosis. In 75% of the patients in the present cohort, serum samples obtained before diagnosis were found to contain autoantibodies (primarily ANAs, followed by RF, and

anti-Ro 60/SSA, anti-Ro 52/SSA, and anti-La/SS-B, in that order). However, 8 (6.8%) of the patients never became positive for these autoantibodies during the course of their disease. Consequently, 81% of the patients who eventually became seropositive after diagnosis had autoantibodies in prediagnostic serum samples. Even more importantly, for 95% of the patients who expressed autoantibodies before diagnosis and for all of those who expressed autoantibodies before the first symptoms (10), these antibodies were present in the earliest available serum sample. Thus, clearly the antibodies were present earlier than our calculations can prove, i.e., even earlier than 2 decades before diagnosis.

The percentage of patients with prediagnostic autoantibodies may be even higher than demonstrated here, since for a number of patients, only samples taken more than 7 years before diagnosis were available. Those who had negative samples 7 years before diagnosis might have had prediagnostic autoantibodies, which would have been detected if samples had been obtained closer to diagnosis. In summary, the main finding of this study is that the autoimmune process in primary SS often starts a very long time before clinical recognition.

The estimates presented here are conservative and imprecise due to the factors described above. In 95% of the patients with prediagnostic autoantibodies, autoantibody production had started at an unknown time before our first assessment. These facts complicate the analysis of which autoantibody appears first and may explain why we found no statistically significant differences in the median time interval between the detection of the various autoantibody specificities and the diagnosis, even though it did seem that antibodies to La/SSB appeared somewhat later than ANAs, RF, and antibodies to Ro 60/SSA and Ro 52/SSA.

Our findings are consistent with the results of previous studies showing that patients with RA and SLE produced autoantibodies several years before the onset of symptoms and diagnosis (17–20). In the study of SLE by Arbuckle et al (20), at least 1 autoantibody was present before diagnosis in 88% of the patients, and, similar to our findings, a major proportion displayed autoantibodies in their earliest available serum sample (obtained a mean of 4.4 years before the SLE diagnosis, with a maximum interval of 9.4 years between sampling and diagnosis).

Patients diagnosed as having primary SS before the age of 40 seem to represent a separate subgroup of the disease. The prevalence of prediagnostic autoantibodies (specifically ANAs, RF, and anti–Ro 60/SSA, anti–Ro 52/SSA, and anti-La/SSB antibodies) was significantly higher in these patients with early-onset primary SS than in those diagnosed at an older age. They also had higher titers and a higher number of autoantibody specificities in the same samples. This finding is consistent with previous studies showing that patients with disease onset at a young age have higher levels of immunologic/serologic markers and more prominent systemic involvement after diagnosis (21–24). Whether this is a consequence of a more prominent genetic susceptibility or a higher load of environmental or endogenous triggers in early life remains to be studied.

Our results demonstrate that anti-Ro/SSA and anti-La/SSB antibodies in healthy individuals are strongly associated, in terms of odds ratios, with a risk of developing primary SS later. When a Bayesian approach (16) was used, taking into account the low prevalence of doctor-diagnosed primary SS in general populations (3–5), the PPVs were relatively low. However, anticipating the use of autoantibody screening in high-risk populations, there would be higher impacts. Calculating with a presumed prevalence of 5% in high-risk autoimmune families, the PPV for anti-Ro 60/SSA positivity would increase from 25% to 57%, and the PPV for SSB/La positivity from 2.8% to 60%. This difference between using only ORs and also including Bayesian prior estimates (16) in the calculations thus demonstrates the importance of taking disease prevalence in the studied population into consideration when using these predictions in patient counseling.

It has been shown in primary SS that specific HLA subtypes are associated with anti-Ro/SSA and anti-La/SSB antibodies more than with the disease itself (25,26). Genetic predisposition in combination with some kind of environmental trigger may initially result in asymptomatic silent autoimmunity before further events may provoke the development of full-blown disease (8). Alternatively, the high reserve capacity of the exocrine glands does not allow early recognition of the ongoing inflammatory process by the patient. Further studies are needed to reveal whether risk profiling, which combines lifestyle factors, signs of early and persistent infections (microbiomics), hormones, genetic markers, and early autoantibody detection, could be useful as a reliable marker of disease development in individuals.

The different autoantibodies detected in the prediagnostic period seem to be associated with differentiated characteristics in later disease. The presence of the prediagnostic autoantibodies predicted a systemic disease with characteristics of unfavorable outcome. An increasing number of prediagnostic autoantibodies in the same patient was associated with higher IgG and IgA levels, lower C4 levels, decreasing numbers of CD4+ T cells, and a lower CD4+:CD8+ ratio. Thus, prediagnostic autoantibody testing not only reveals the risk of clinical

autoimmune disease, but also mirrors the severity of the future disease in terms of systemic complications, eventually associated with lymphoma development. Given better treatment or detection of modifiable risk factors, autoantibody testing in individuals at high risk of primary SS, for instance those from families with multiple instances of autoimmune disease, could be a potential tool for early intervention.

Autoantibody production requires the presence of autoreactive B cells/plasma cells, possibly formed during adolescence or childhood due to genetic predisposition. A recently published first genome-wide association study confirmed the HLA association and added a number of risk alleles involved in B cell maturation and activation, fitting well with early autoantibody production in these individuals during a scenario of persistent viral infection or other environmental stimuli, possibly in a vulnerable phase of immune maturation (27). Whether this scenario is the actual chain of events in individual patients remains a crucial research question for the future.

The availability and ongoing development of B cell–directed and other immune-modulating therapies potentially interfering with the conversion from asymptomatic seropositivity to clinical disease underscore the importance of studies like ours.

Interestingly, recent studies provided evidence that targeting B cells with rituximab can be an effective treatment strategy for patients with primary SS and severe systemic complications (28,29), and to a certain extent, it may also improve salivary gland function, diminish fatigue, and reduce the number of extraglandular manifestations, especially when given early in the disease course (29–32). This underlines the importance of early diagnosis.

The main drawback of our study is the small sample size of 117 individuals who developed SS and 117 matched controls, which leaves us with imprecise risk calculations. Another important issue is the inherent difficulty of defining the onset or first symptoms of primary SS because of the often nonspecific initial features, such as fatigue and musculoskeletal pain. While all samples were obtained before diagnosis, for 16% of the individuals, the available documentation in case records and immunology databases did not allow us to exclude the possibility that the disease was present but undiagnosed.

In conclusion, our results show that production of autoantibodies characteristic of primary SS frequently occurs many years before both the diagnosis and the clinical onset (10) of the disease and that anti-Ro/SSA and anti-La/SSB antibodies are strong indicators of the development of primary SS, but the early presence of autoantibodies in general seems to point toward more severe disease later on. A future approach in individuals with genetic susceptibility due to a strong family history of autoimmunity might therefore be to perform risk gene analysis and autoantibody profiling for detection of the state of serologic autoimmunity or very early asymptomatic disease. In these cases, at least advice for avoidance of potential environmental triggers (to be identified) might be given. Such strategies might include avoidance of overexposure to ultraviolet light, unnecessary vaccines and toxins, intake of hormones, etc., all depending on the type of autoantibody found. The awareness of potential disease development may then allow early diagnosis in those developing symptoms. The availability and risk/benefit profile of drugs may then contribute to decisions about very early intervention.

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All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Theander had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Theander, Jonsson, Henriksson.

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