What is the clinical significance of anti-Sm antibodies in systemic lupus erythematosus? A comparison with anti-dsDNA antibodies and C3

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Abstract

Objective

To investigate the clinical value of anti-Sm antibodies in diagnosis and monitoring of systemic lupus erythematosus (SLE) and their ability to predict lupus flares compared with that of anti-dsDNA antibody and complement (C3) assays.

Methods

Autoantibodies against Smith antigen (Sm) and double-stranded DNA (dsDNA) in sera from SLE (n=232), myositis (n=26), systemic sclerosis (n=81), Sjögren's syndrome (n=88), and rheumatoid arthritis patients (n=165) and healthy donors (n=400) were determined by using enzyme-linked immunosorbent assays (both from Euroimmun). New thresholds for both autoantibodies were calculated by receiver operating characteristics (ROC) curve analysis. Cross-sectional, longitudinal and predictive analyses of anti-Sm and disease activity were also performed.

Results

Sensitivities of 25.9% for anti-Sm (cut-off: 3.6 relative units/ml) and 30.2% for anti-dsDNA (cut-off 157.4 international units/ml) were obtained at a specificity of 99%. 14.8% of anti-dsDNA-negative patients were positive for anti-Sm, and more than half (51.4%) of anti-dsDNA-positive patients were also positive for anti-Sm. Anti-Sm antibodies were associated with age (p=0.0174), the number of ACR criteria (p=0.0242), the ACR criteria renal (p=0.0350) and neurologic disorder (p=0.0239), the BILAG category constitutional symptoms (p=0.0227), fatigue (p=0.0311) and cross-sectional disease activity (r=0.2519, p=0.0224). Although no correlations with lupus activity were observed in the longitudinal and predictive analysis, a remarkable association was found between anti-Sm and proteinuria.

Conclusion

Anti-Sm antibodies are essential for diagnosis of SLE, especially in anti-dsDNA-negative patients. However, our data suggest that anti-Sm monitoring is only helpful in SLE patients with active lupus nephritis.

Key words

systemic lupus erythematosus, anti-Smith antibodies, anti-dsDNA antibodies, biomarker, lupus nephritis

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C. Dähnrich and W. Schlumberger are employed by EUROIMMUN, which provided test results for all included, commercially available autoantibodies (Anti-dsDNA ELISA, Anti-Sm ELISA).
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Introduction

Systemic lupus erythematosus (SLE) is a chronic multifactorial autoimmune disease that can affect nearly every organ system (1). It is characterised by different autoantibodies that are predominantly directed against nuclear proteins and nucleic acids (2). These autoantibodies are not only decisive factors in the pathogenesis of SLE, but also useful tools for SLE diagnosis since the disease can present with a diversity of different manifestations (3). Lupus affects mostly young women of childbearing age (4). The 10-year survival rate is still only about 90% (5), which underscores the need for further improvement of early diagnosis and treatment.

Antibodies to double-stranded DNA (dsDNA) are already well-known and well-studied. Both anti-dsDNA and anti-Smith (anti-Sm) antibodies are included in the American College of Rheumatology (ACR) and Systemic Lupus International Collaborating Clinics (SLICC) criteria for the classification of SLE (6-8). Among the earliest identified autoantibodies in lupus, anti-Sm antibodies are highly specific for SLE (6, 9). They were found to occur at the same frequencies in patients positive and negative for anti-dsDNA antibodies (10), but their additional value in the diagnosis of SLE, especially in anti-dsDNA-negative patients, is still not entirely clear.

The reported prevalence rates of autoantibodies to Sm range from less than 10% to over 80%; these discrepancies might be due to the different ethnicities of the patient cohorts (11-14). Especially African American patients show anti-Sm antibody prevalences of 40% and higher (15, 16). Patients of Asian origin are also more often anti-Sm-positive than Caucasians (17).

Opinions diverge widely as to the associations between these autoantibodies and different disease manifestations. Previous studies suggest that anti-Sm antibodies are often related to renal involvement and proteinuria (18, 19), and they seem to be associated with juvenile-onset SLE (20), central nervous system dysfunction (21) and serositis (22). Different studies investigating the relationship between anti-Sm antibodies and SLE disease activity indicate that not only cross-sectional but also longitudinal associations exist (23-25). Moreover, Barada *et al*. reported that anti-Sm antibodies predict lupus flares in 50% (26).

Nevertheless, the clinical value of autoantibodies to Sm, especially compared to that of standard biomarkers like anti-dsDNA antibodies and complement component 3 (C3), still remains unclear. Thus, the present study was designed to compare the value of anti-Sm antibodies for diagnosing and monitoring SLE and for predicting lupus flares to that of standard biomarkers. We demonstrate that anti-Sm antibodies play an essential role in diagnosis of SLE and provide a useful tool for follow-up of patients with lupus nephritis.

Materials and methods

Study design

The study consisted of four different parts (Fig. 1). First, we determined optimal anti-Sm and anti-dsDNA antibody thresholds by receiver operating characteristics (ROC) curve analysis. Second, these thresholds were used to define the added value of anti-Sm antibodies in diagnosis of SLE. Third, the patients were divided into anti-Sm^+ and anti-Sm^- subgroups and compared to each other regarding characteristics in their medical records. Fourth, we investigated associations between antibodies to Sm and disease activity in contrast to anti-dsDNA-antibodies and C3 in a cross-sectional (n=82) and longitudinal analysis (n=26). Additionally, 33 clinically quiescent patients were monitored for future lupus flares over a period of 180 days to evaluate the prognostic value of anti-Sm antibodies.

A total of 992 serum samples were obtained from 232 SLE patients who fulfilled the American College of Rheumatology (ACR) revised criteria for the classification of SLE (6) and 760 controls consisting of 400 healthy donors and 360 patients with other rheumatic diseases. Among those were patients with myositis (n=26) (27), systemic sclerosis (SSc, n=81) who fulfilled the ACR criteria for systemic sclerosis (28), primary Sjögren's syndrome (pSS, n=88) who met the revised European classification criteria (29) and rheuma-

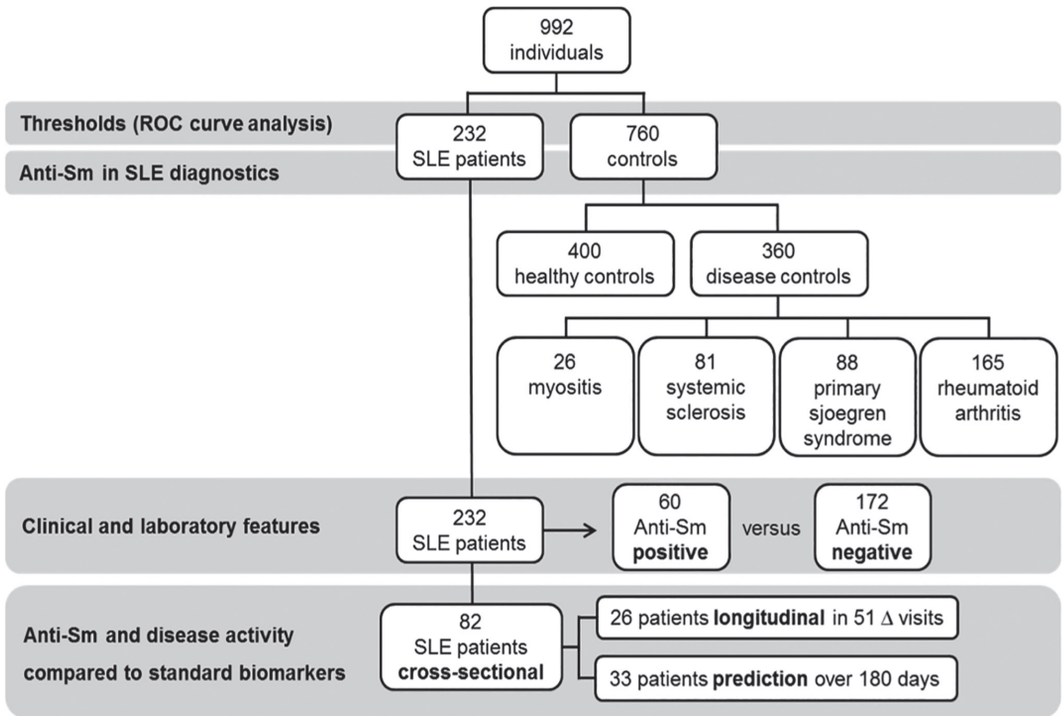

Fig. 1. Flow chart showing the study design.

toid arthritis (RA, n=165) who fulfilled the revised ACR criteria (30). The detailed characteristics of the SLE patients are given in Supplementary file 1.

All patients were recruited between August 2003 and December 2009 at the Department of Rheumatology and Clinical Immunology, Charité Universitätsmedizin Berlin, Germany. According to the Pediatric Rheumatology International Trials Organisation, juvenile-onset SLE was diagnosed when the age at diagnosis was 18 years or younger (31). Written informed consent was obtained from all participants prior to participation. The study was approved by the ethics committee of the Charité Universitätsmedizin Berlin.

Disease activity

SLE disease activity was determined using the modified Systemic Lupus Erythematosus Disease Activity Index 2000 (mSLEDAI 2000), which includes neither antibodies nor complement components. Additionally, the British Isles Lupus Assessment Group 2004 Index (BILAG-2004), which is based on an ordinal scale and includes 9 systems, was applied (32). Evaluation with the letters A – E depends on the physician's intention to treat and can be summarised by a numerical index to an overall disease activity score (A=12, B=8, C=1, D/E=0) (33). A lupus flare was defined as a new A or B score in any BILAG-2004 category. SLE patients with no A or B score in BILAG-2004 were classified as clinically quiescent.

Detection of serum biomarkers

All autoantibody titers were determined using commercially available test systems (Anti-Sm ELISA and Anti-dsDNA ELISA from Euroimmun) and processed according to the manufacturer's instructions. All assays were run in duplicate.

The Anti-Sm ELISA included in this study allows the monospecific, quantitative determination of antibodies against the Sm antigen. The Sm antigen was purified by affinity chromatography from calf thymus and it was verified via Maldi-TOF/TOF Mass Spectrometry (MS) that it consists of all 7 core proteins (B/B', D1, D2, D3, E, F, G) (Supplementary file 2). No indications for the presence of RNP proteins were seen in SDS-PAGE and MS analysis. Absence of RNP proteins was further confirmed via ELISA using a panel of human sera samples with known reactivity against RNP 68 kD, A and C.

Complement component 3 concentrations were measured in the local laboratory by nephelometry, and thresholds were defined as recommended by the manufacturer.

Statistical analysis

Statistical analyses were performed with GraphPad Prism 5.0 (GraphPad Software, La Jolla, California, USA). Thresholds for anti-Sm and anti-dsDNA antibodies were calculated by ROC curve analysis. Cross-sectional cut-offs were chosen at a comparable specificity of 99%. Individual thresholds were determined for predictive value assessment. The correlation between the biomarkers and metric variables was assessed using the Spearman rank test. The Mann-Whitney U-test was used to compare patients with positive and negative biomarkers. The Fisher's exact test was applied for analysis of biomarker-positive and -negative patients and categorical variables. The strength of association between autoantibodies and lupus nephritis was evaluated using odds ratio (OR), and precision using 95% confidence interval. *P*-values <0.05 were considered significant for all tests performed.

Results

Derivation of optimal thresholds

Anti-Sm reactivity was detected by the Anti-Sm ELISA in 232 SLE patients,

400 healthy donors and 360 patients with other rheumatic diseases (Fig. 2). To ensure optimal comparability of the antibody test systems, cut-off thresholds for anti-Sm and anti-dsDNA antibodies were defined by ROC curve analysis of 992 individuals (Table I). Above a threshold of 11.5 relative units/ml (RU/ml), anti-Sm antibodies already achieved a specificity of 100%, but with a low sensitivity of 13.8%. At the manufacturer's threshold of 20 RU/ml, the sensitivity further decreased to 10.8%.

For all subsequent analyses using Anti-Sm and Anti-dsDNA ELISA, thresholds were chosen at a specificity of 99%. At this specificity, three healthy controls and four disease controls were false-positive in the Anti-Sm ELISA (Fig. 2). Despite critical review of medical records, none of these individuals could be diagnosed as having SLE, and all were negative for anti-dsDNA antibodies in ELISA, radioimmunoassay and Crithidia luciliae immunofluorescence test.

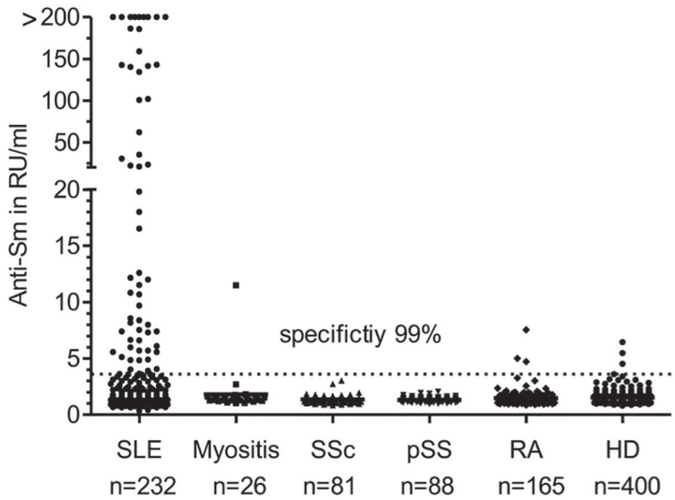

Fig. 2. Scatterplot showing anti-Sm antibodies in SLE, other rheumatic diseases and healthy donors. 992 sera measured using enzyme-linked immunosorbent assay. Dotted line represents distinct threshold (3.6 RU/ml) based on ROC curve analysis at a specificity of 99%. Values >200 RU/ml were set to 200 RU/ml for a clearer arrangement of the figure. SSc: systemic sclerosis; pSS: primary Sjögren's syndrome; RA: rheumatoid arthritis; HD: healthy donors.

Table I. Test values of anti-Sm and anti-dsDNA antibodies calculated in ROC analysis.

Criteria	Anti-Sm	Anti-dsDNA
Area under curve	0.6452	0.7986
95% CI	0.59 to 0.70	0.76 to 0.84
p-value	<0.0001	<0.0001
Sensitivity at 95% specificity (cut-off)	41.0 (2.1)	48.7 (58.8)
Sensitivity at 98% specificity (cut-off)	32.3 (3.0)	36.6 (104.8)
Sensitivity at 99% specificity (cut-off)	25.9 (3.6)	30.2 (157.4)
Maximum sum of specificity and sensitivity	136.9	153.3

95% CI, 95% confidence interval. Test criteria for Anti-Sm and Anti-dsDNA enzyme-linked immunosorbent assay (ELISA) were calculated using a ROC curve analysis based on test readings of 992 samples from 232 lupus patients, 360 disease controls and 400 healthy donors. Outcome parameters of ROC curve analysis were diagnosis *versus* no diagnosis of SLE.

Anti-Sm antibodies provide added benefit in SLE diagnosis

Anti-Sm antibodies are highly specific for SLE and, like anti-dsDNA antibodies, they are included in the ACR classification criteria (6). Although all ACR criteria have the same weight, the presence of high-specific antibodies is most helpful. However, little is known about the frequency of anti-Sm antibodies in SLE patients with and without anti-dsDNA antibodies. As high specificity is required for SLE diagnosis, we selected anti-Sm and anti-dsDNA antibody cut-offs with a specificity of 99% in order to guarantee comparability. At this specificity, the Anti-Sm ELISA yielded a sensitivity of 25.9% (cut-off 3.6 RU/ml) while the Anti-dsDNA ELISA provided a slightly higher sensitivity of 30.2% (cut-off 157.4 IU/ml) in identical samples.

A ring diagram was created to visualise the distribution of reactivity for both autoantibody species (Fig. 3). These data can be described from different perspectives. First, 14.8% of all anti-dsDNA-negative samples (n=162) were positive for anti-Sm antibodies (n=24), – or seen from the opposite perspective: 40.0% of all anti-Sm-positive samples (n=60) were negative for anti-dsDNA antibodies. In these cases (10.3% of all SLE patients), classification can be verified exclusively by detection of anti-Sm antibodies. Furthermore, only 19.8% of all anti-Sm-negative sera (n=172) were positive for anti-dsDNA antibodies (n=34), showing only a moderate superiority of anti-dsDNA over anti-Sm antibodies. In a subsequent investigation, we found that anti-Sm antibodies are more frequent in the first years of disease underlying its importance especially in ensuring SLE diagnosis (Fig. 4).

Comparison of disease features in anti-Sm-positive vs. -negative SLE patients

To further determine the special characteristics of lupus patients with elevated anti-Sm antibodies, we evaluated their medical records for general patient characteristics, ACR criteria, mSLEDAI 2000 criteria, laboratory parameters and SLE medications and compared the results to those of their anti-Sm$^-$ counterparts. The obtained results using Mann-Whitney U-test were further related to those of standard biomarkers to reveal any additional benefit of anti-Sm antibodies. All clinical laboratory results and detailed demographic information are given in Supplementary file 3.

In contrast to their anti-Sm-negative counterparts, anti-Sm$^+$ patients were younger (p=0.0174), had a shorter disease duration (p=0.0279) and more severe SLE, as reflected by a higher number of ACR criteria (p=0.0242). Serious lupus manifestations according to the

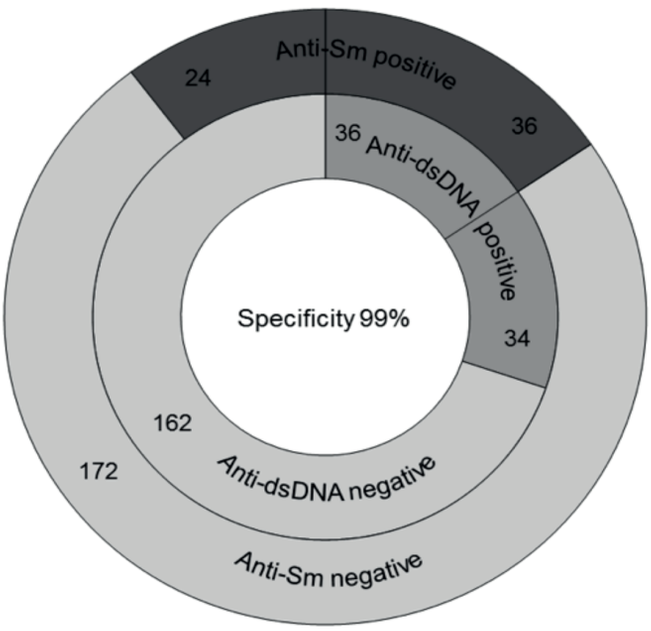

Fig. 3. Additional diagnostic benefit of anti-Sm antibodies in SLE patients.
The ring diagram aims to demonstrate the additional diagnostic value of anti-Sm antibodies in lupus patients positive and negative for anti-dsDNA antibodies.
The cut-offs of both test systems were set at a specificity of 99% to allow optimal comparability (compare Table I for cut-offs).

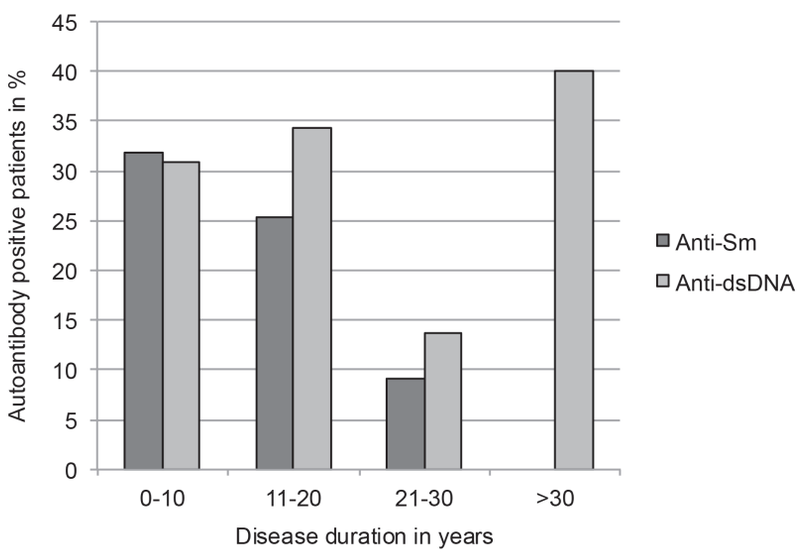

Fig. 4. Relation of disease duration and frequency of autoantibodies. Division of 232 SLE patients into four groups of disease duration revealed a higher frequency of anti-Sm antibodies in early years of disease.

ACR criteria – *e.g.* renal (p=0.0350) and central nervous system (p=0.0239) involvement – were more frequent in the anti-Sm$^+$ subgroup. Moreover, anti-Sm antibodies were significantly more prevalent in the small number of Asians included in the sample than in Caucasians (p=0.0004).

Even though the subgroups with elevated anti-dsDNA or decreased C3 levels had significantly higher disease activity (mSLEDAI) than their reference groups, higher disease activity was not observed in the anti-Sm$^+$ subgroup.

Due to the association of anti-Sm antibodies with renal involvement in the ACR criteria, we also investigated current renal impairment. Proteinuria was significantly higher in patients with elevated anti-Sm antibodies than in the anti-Sm$^-$ subgroup (p=0.0340). C3 showed similar behaviour (p=0.0171), but, surprisingly, anti-dsDNA antibodies did not. Proteinuria above 500 mg per day was more frequent in the anti-Sm$^+$ (48.5%) than in the anti-Sm$^-$ subgroup (23.1%). Similar results were found for anti-dsDNA antibodies. However, odds ratio was higher (OR=3.13) for anti-Sm antibodies than for anti-dsDNA antibodies (OR=2.48). Furthermore, patients positive for both antibodies had significantly higher rates of high proteinuria than patients negative for both autoantibodies (OR=4.97; p=0.0009, Supplementary file 4). No associations were observed between SLE medications (azathioprine, cyclophosphamide, prednisolone, hydroxychloroquine, mycophenolate mofetil) and any of the studied parameters.

Anti-Sm and lupus activity – presence, progress and prognosis

Next, we studied associations of anti-Sm antibodies to disease activity measured by BILAG-2004 in prospectively well characterised SLE patients. The relation between biomarkers and disease activity can be sub-classified into cross-sectional, longitudinal and prognostic correlations. In order to evaluate anti-Sm antibodies as biomarkers of lupus activity, we studied them in these three time categories and related the results to those of the standard biomarkers to allow a comparison (Fig. 5).

In the cross-sectional analysis, the presence of antibodies to dsDNA (r=0.320; p=0.0034) and, especially, decreased C3 (r=0.431; p<0.0001) strongly correlated with BILAG-2004, whereas anti-Sm antibodies showed a weaker correlation (r=0.252, p=0.0224). In the further check for correlations of anti-Sm antibodies with distinct BILAG categories, we only found a significant association with the subcategory constitutional symptoms (p=0.0227). Further analysis revealed that fatigue was responsible for this association (p=0.0099). However, anti-dsDNA and C3 also correlated with fatigue (anti-dsDNA: p=0.0209, C3: p=0.0242), but to a lower degree, as determined using Spearman's rank test.

Monitoring the progression of disease is of prime importance in the management of SLE patients. In order to test whether anti-Sm antibodies correlate with lupus activity over time, we calculated the changes in BILAG-2004 scores and anti-Sm titers at different time points. Data were obtained from 51 differential visits of 26 SLE patients. Based

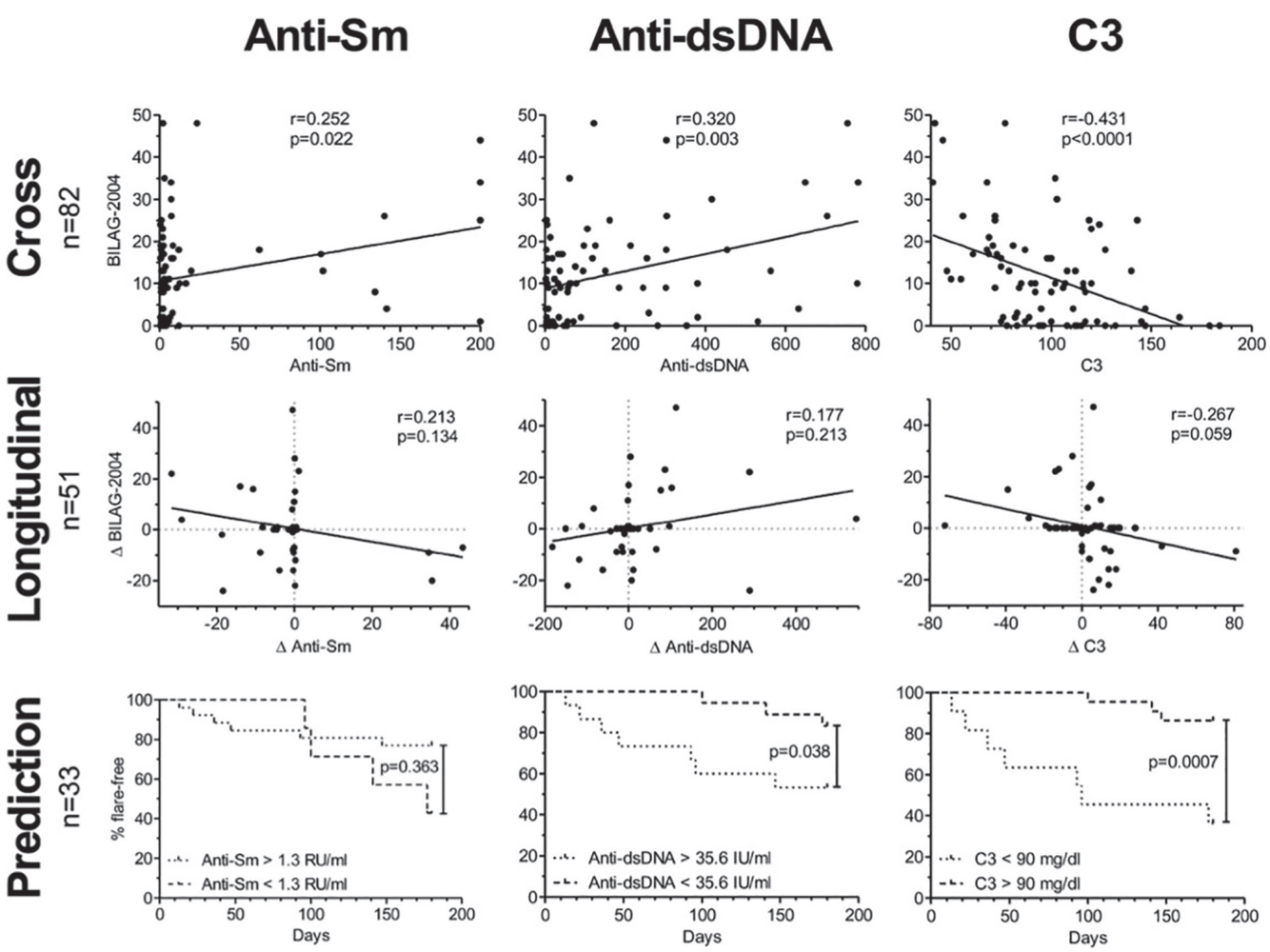

Fig. 5. Anti-Sm antibodies in cross sectional, longitudinal and predictive study compared to anti-dsDNA antibodies and C3 in SLE patients. The cross section was based on 82 different systemic lupus erythematosus (SLE) patients. The autoantibody cut-offs were set at a specificity of 99% (compare Table I) and *p*-values were calculated using Spearman's rank test. The results of the longitudinal study were based on 26 SLE patients in all together 51 visits. Delta values were calculated by subtracting values for a defined parameter from an actual visit from a defined parameter from the last visit. *p*-values were obtained using linear regression weighted for the number of visits. The optimal cut-offs in the predictive study, which was based on 33 SLE patients, were defined using ROC curve analysis and *p*-values were calculated using Mantel-Cox test.

on linear regression weighted for the number of visits, changes in anti-Sm antibodies and standard biomarkers did not correlate with the observed changes in disease activity over time (anti-Sm: p=0.134; anti-dsDNA: p=0.213; C3: p=0.059). To further exclude the possibility that the low prevalence of anti-Sm antibodies might disguise a correlation to disease activity, we studied whether changes in anti-Sm titers above 5 RU/ml were significantly associated with changes in BILAG-2004. Again, no significant associations were observed.

To evaluate the ability of anti-Sm antibodies to predict lupus flares, 33 SLE patients with inactive or mild active disease were monitored for disease exacerbations over a period of 180 days. Ten patients developed a SLE flare, defined as a new A or B score in any BILAG category. The best cut-off point for anti-Sm antibodies (1.3 RU/ml, sensitivity 40%, and specificity 85.7%) was determined using ROC curve analysis. Regarding the frequency of flares, no statistically significant difference between anti-Sm^+ and anti-Sm^- patients was observed within the investigation period. Patients positive for anti-dsDNA antibodies (threshold: 35.5 IU/ml) or with decreased C3 were more likely to develop SLE flares (anti-dsDNA: p=0.0378, C3: p=0.0007). Conclusively, anti-Sm antibodies were not able to predict lupus flares in contrast to the standard biomarkers.

Longitudinal changes in anti-Sm antibodies and proteinuria

Anti-Sm antibodies were associated with renal involvement (ACR criteria), and anti-Sm^+ patients had higher proteinuria than their anti-Sm-negative counterparts. Therefore, independent of the missing longitudinal correlation of anti-Sm antibodies with lupus activity, we studied whether anti-Sm levels correlated with the extent of proteinuria over time. As presented in Figure 6, changes in anti-Sm titers were indeed accompanied by changes in proteinuria in 13 patients with active lupus nephritis over two consecutive visits (p=0.0035). Similar results were found for anti-dsDNA antibodies (p=0.0032), but not for C3 (p=0.1979).

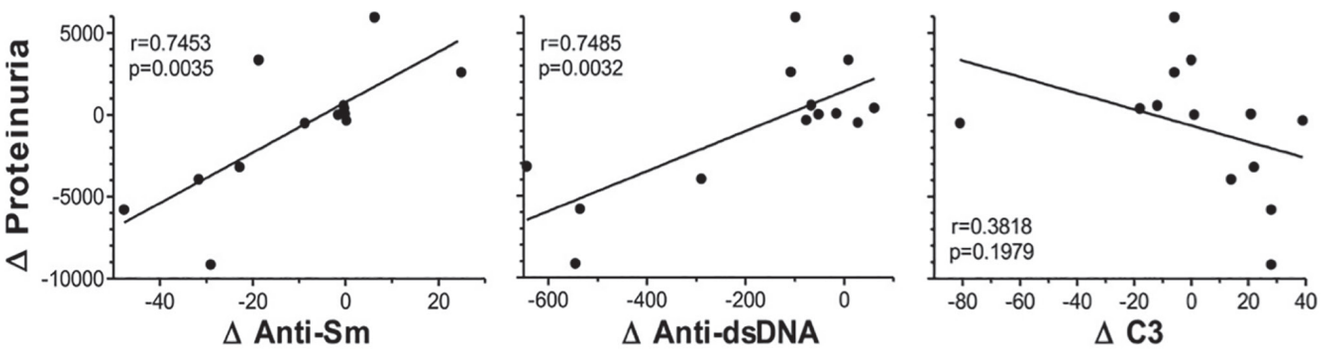

Fig. 6. Changes in proteinuria *versus* changes of anti-Sm antibodies compared to anti-dsDNA antibodies and C3 in SLE patients with active lupus nephritis. All results are based on 13 differential visits of different systemic lupus erythematosus (SLE) patients. Delta values were calculated by subtracting values for a defined parameter from an actual visit from a defined parameter from the last visit. *p*-values were obtained using linear regression.

Discussion

This study was designed to investigate the clinical utility of anti-Sm antibodies in comparison with anti-dsDNA-antibodies and C3 in SLE. First, we determined anti-Sm cut-offs optimal for SLE diagnosis. Therefore, levels of anti-Sm antibodies in a large cohort of 232 SLE patients were compared to those in 400 healthy donors and 360 rheumatic disease controls.

Remarkably, the manufacturer's threshold of 20 RU/ml for the Anti-Sm ELISA appeared to be set too high since our ROC curve analysis already revealed a specificity of 100% above a threshold of only 11.5 RU/ml (sensitivity=13.8%). Using the manufacturer's threshold, the sensitivity declined to 10.8%. Thus, the diagnostic test misses at least 3% of anti-Sm-positive SLE patients besides those with definite SLE according to anti-Sm-reactivity.

When we tried to compare the results of our ROC curve analysis with those of previous studies, we were unable to identify any comparable studies. Furthermore, only a few investigators have stated which threshold they used in the Anti-Sm ELISA (34-38). Although the authors of five different studies reported sensitivities and specificities for anti-Sm, these results were not derived from ROC curve analysis (10, 11, 22, 39, 40). Most of the studies in the literature neither mention the threshold nor the test characteristics (12, 13, 16, 18-21, 25, 26, 41-46). This was unexpected since anti-Sm antibodies have been used as classification criteria for SLE since 1982 (6). Conclusively and to our surprise, this is to our knowledge the first study using ROC curve analysis to identify an optimal cut-off for the Anti-Sm ELISA in the diagnosis of SLE.

Several thresholds with distinct sensitivities and specificities were determined in the ROC curve analysis. At a specificity of 99%, a sensitivity of 25.9% was obtained in our Caucasian cohort. This value was in the upper range of previously reported sensitivities, which lie between 5–30% (6, 12-14). Further comparison of this finding with data in the literature is hampered by the fact that reports of specificity data are often missing – in many cases, due to the lack of healthy as well as disease controls.

In contrast to Caucasians, it is well known that patients of other ethnicity, especially Africans and African Americans, have a higher prevalence of antibodies to Sm (15, 16). Moreover, SLE patients of Asian descent often have higher anti-Sm titers than Europeans (17). We could reproduce those findings even though only 10 Asian patients were included in our study.

Anti-dsDNA and anti-Sm antibodies were included for the first time as classification criteria for SLE in 1982 because "*their inclusion was found essential, since the ability to use the greater sensitivity of the antinuclear antibody test and the considerable specificity of tests for antibody to DNA or Sm greatly improved performance of the 1982 criteria*" (6). However, no data were shown that supported this conclusion or approach. Therefore, the diagnostic value of anti-Sm antibodies in relation to anti-dsDNA antibodies with comparable cut-offs was addressed in the present study. We demonstrated that not only 51.4% of the anti-dsDNA-positive SLE patients but also 14.8% of the anti-dsDNA-negative patients had anti-Sm antibodies. These results are in contrast to those of Sanchez-Guerrero *et al*. (10), who found nearly identical anti-Sm positivity of 33% and 34% in SLE patients with and without anti-dsDNA-antibodies. These discrepancies are most likely explained by undetermined specificities of the two test systems and the different ethnic backgrounds of the included patients. Our analysis conclusively showed that the diagnostic value of anti-Sm antibodies is nearly equal to that of anti-dsDNA antibodies. Thus, we demonstrated for the first time evidence that both autoantibodies are essential for the classification and diagnosis of SLE.

Opinions concerning the associations between anti-Sm antibodies and the clinical and serological features of SLE are divided, even though this has been the subject of many studies in different patient cohorts. Like others before us (20, 42), we found equal frequencies of anti-Sm antibodies in men and women, but gender differences have also been reported (43, 44, 47). As age is known to influence the autoantibody profile of lupus, we agree with Arroyo-Ávila *et al*. (48) and Ni *et al*. (41) that patients with anti-Sm antibodies tend to be younger and have shorter disease duration. Furthermore, our findings confirm the results of Webb *et al*. (49) showing no difference in anti-Sm levels between juvenile- and adult-onset SLE.

Isenberg *et al*. (25) also investigated the relationship between anti-Sm antibodies and SLE disease activity measured by BILAG. Even though they did

not observe the association between anti-Sm and the global score found in our study, they came to the same conclusion that anti-Sm antibodies are associated with the BILAG category constitutional symptoms. This finding could be explained by the strong correlation between anti-Sm antibodies and fatigue, which has only been described for low C3 and fatigue before (50).

Although a weak correlation with BILAG-2004 was found in the cross-sectional analysis, no association between changes in anti-Sm autoantibodies and disease activity could be observed in our longitudinal analysis which was limited due to small number of 51 differential visits, even though this was previously suggested (23, 24). Similarly, we could not confirm the ability of anti-Sm antibodies to predict lupus flares, as was proposed by Barada *et al*. (26), who found that antibodies to Sm predicted disease flares in 50% of cases.

As suggested by Arroyo-Ávila *et al*. (48), who investigated 2322 SLE patients enrolled in the PROFILE study, we also found central nervous system involvement more often in anti-Sm positive patients.

However, we did find higher levels of anti-Sm antibodies in patients with renal involvement. This association has been described by Alba *et al*. (18), Varela *et al*. (46) and Arroyo-Ávila *et al*. (48). Moreover, our anti-Sm^+ patients had proteinuria more often than their anti-Sm^- counterparts. This was previously discussed by Homma *et al*. (19). Notably, proteinuria above 500 mg/day occurred in patients positive for both anti-Sm and anti-dsDNA antibodies. In contrast to Bastian *et al*. (45), who determined predictive factors for new or worsening proteinuria in 529 SLE patients within the scope of the LUMINA study, we were able to show that anti-Sm antibody titers do in fact change with proteinuria over the time.

Roughly 15% of our anti-dsDNA-negative SLE patients were positive for anti-Sm-antibodies, as determined using thresholds with a specificity of 99%. However, since we included pre-treated SLE patients with relatively long-term disease, this does not reflect the circumstances at the time of diagnosis. This is, of course, a weakness of our study. Thus, it is possible that the percentage of anti-dsDNA-positive patients might be much higher in untreated patients at the time of diagnosis. Though, based on our findings in treated SLE patients, it is not only justifiable but also very useful to include anti-Sm antibodies in the ACR criteria for SLE. Because the exact influence of SLE medications on anti-Sm titers is not known, the results should be verified in further studies in untreated patients.

Summary

Anti-Sm antibodies should always be determined if SLE is suspected and they are also found in SLE patients without anti-dsDNA antibodies. The probability of the correct diagnosis of SLE increases with the titer of anti-Sm antibodies. The specificity of an individual test result can be estimated from the included ROC curve analysis (Table I). Compared to anti-dsDNA antibodies or C3, repeated determinations of anti-Sm antibodies offer only advantage in patients with active lupus nephritis. In this subgroup, anti-Sm antibodies correlate with proteinuria (as indicator for renal inflammation) cross sectional and over time.

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