Sjögren's syndrome in Systemic Lupus Erythematosus

-a subset characterized by a systemic inflammatory state

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Sjögren´s syndrome in SLE

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Short running head: Sjögren´s syndrome in SLE

Abstract

Objective: Secondary Sjögren's syndrome (SLE-sSS) is an often-neglected subset of patients with Systemic lupus erythematosus (SLE). Furthermore, primary Sjögren's syndrome overlaps and can be difficult to delineate from SLE. To shed light on the SLE-sSS subset, we investigated a large and well-characterized SLE cohort comparing patients with SLE-sSS versus SLE patients without SS (SLE-nonsSS) and controls.

Methods: We included 504 consecutive SLE patients, fulfilling the 1982 revised ACR criteria, and 319 controls from the general population, matched for age and gender to the first 319 patients. SLE-sSS was defined according to the American-European Consensus Criteria (AECC). A thorough clinical investigation, including subjective and objective quantifications of sicca symptoms, was performed in all participants. Autoantibodies and 20 selected cytokines were measured by luminex and multiplex analysis, respectively.

Results: SLE-sSS, as defined by AECC, occurred in 23% of the SLE patients. In comparison to SLE-nonsSS, the SLE-sSS group was older, more enriched in females. Leucopenia and peripheral neuropathy was more and nephritis less frequent. Circulating levels of 6/20 investigated pro-inflammatory cytokines (TNF-α, IL-6, MCP-4, MIP-1β, IL-12/IL23p40 and IP-10), total IgG, anti-SSA/Ro52, anti-SSA/Ro60, anti-SSB/La antibodies and rheumatoid factor (IgM and IgA) were higher in the SLE-sSS group ($p<0.05$ for all comparisons).

Conclusion: The frequency of SLE-sSS increased with age and affected roughly ¼ of all SLE patients. Despite less internal organ involvement, a systemic inflammatory state with high levels of pro-inflammatory cytokines is present in the SLE-sSS subgroup. This is a novel observation which may impact future understanding and treatment of the SLE-sSS subset.

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune systemic disease characterized by enhanced autoantibody production and formation of immune complexes. SLE is also a very heterogeneous condition involving many organ systems and disease activity varies from persistently mild to life-threatening(1). A state of systemic inflammation, often associated with complement consumption, enhanced activity in the type-1 interferon system, as well as high levels of pro-inflammatory cytokines, e.g. TNF-α, IL-6, IL-8 and IP-10 are common in SLE(2-5). While the general prognosis of SLE has improved, mortality rates remain more than two-fold higher than in the general population, and cardiovascular diseases (CVD) constitutes a growing share of mortality causes(6-8).

It has become increasingly clear that different subsets of the SLE population exist. Already in 1959 Heaton suggested that Sjögren´s syndrome (SS) is a chronic and relatively benign form of SLE(9). More recently several studies have identified autoantibody clusters/immune phenotypes, which vary with regard to clinical symptoms, biomarkers and prognosis(10-14). A SLE phenotype characterized by antibodies to Sjögren's syndrome A and B antigens (SSA/SSB, also referred to as Ro/La) consistently appeared in these studies, but this, supposedly milder, SLE subset has so far achieved limited scientific attention.

The diagnosis SS is a clinical entity, based on dryness of eyes and mouth due to destructive inflammation in the exocrine glands, especially tear and salivary glands. SS can exist isolated, primary SS (pSS), or together with other rheumatic diseases, referred to as secondary SS (sSS). A major difference according to the 2002 Revised American-European Consensus Criteria (AECC) is the classification where the serologic item (SSA/SSB antibodies) is included for pSS, but not for sSS.(15) In SLE these autoantibodies are common, usually stable over time and they appear early, even several years before disease onset(16, 17).

The clinical SLE-sSS phenotype has been described as a mild version of SLE with dominance

of skin and joint manifestations and with less severe internal organ involvement especially nephritis(9, 18, 19). Differences and similarities between pSS and SLE with SS (SLE-sSS) have been studied(20), but to what extent the inflammatory pattern differs between SLE-sSS and SLE patients without SS (SLE-nonsSS) is not known, and this information may be important with regard to treatment perspectives.

In the present study subjective and objective symptoms of sSS, defined according to AECC(15), from a large and well-characterized cohort of consecutive SLE patients and matched controls are presented. To our knowledge, no previous study has investigated SLE patients and matched population controls for both subjective and objective symptoms of SS and associated SSA/SSB autoantibodies. The primary outcomes were occurrence, clinical and immunological characteristics of the SLE-sSS subgroup. As secondary outcomes, we performed stratified analyses based on anti-SSA/SSB profiles.

Patients and methods

Patients and controls

Patients with SLE managed at the Department of Rheumatology, Karolinska University Hospital and Danderyd´s Hospital who fulfilled four or more items of the American College of Rheumatology (ACR) 1982 revised classification criteria for SLE(21), were invited to participate during the inclusion period February 2004 to December 2014.

Population controls were individually matched for sex, age and region of living to the first 319 SLE patients. The remaining 185 SLE patients did not have matched controls. Matching was performed through use of the national registration number, which includes date of birth and is coded for sex. The only exclusion criteria were a diagnosis of SLE or Sjögren´s syndrome.

Clinical and routine laboratory characterization

A structured protocol, similar for patients and controls was used. Medical charts were reviewed. A rheumatologist performed a clinical examination and evaluated general health and features of SLE including all items according to the ACR 1982 classification criteria(21). SLE disease activity was determined with both Systemic Lupus Activity Measure (SLAM)(22) and SLE Disease Activity Index 2000 (SLEDAI-2K)(23). Permanent organ damage was evaluated with Systemic Lupus International Collaborating Clinics /ACR Damage index (SLICC/ACR DI)(24).

All participants were evaluated for the individual items of SS according to the AECC(15). Subjective ocular and oral symptoms were recorded as defined by the validated questionnaire. Objective measurements were Schirmer's test and whole unstimulated salivary flow (WUSF) over 15 minutes(25). For controls these objective measurements were only performed if subjective symptoms were present. Data on fulfilment of each item was collected. The amount of tear and saliva were recorded. If a salivary gland biopsy had been performed earlier, results

were retrieved from the patient´s records. Patients who had not performed a salivary gland biopsy, when this was needed to determine if they fulfilled the sSS criteria or not, were regarded as not having sSS. The attending rheumatologist assessed these patients as not having sSS, and hence that a salivary gland biopsy was not necessary.

Laboratory measurements

Fasting blood samples were drawn at inclusion. Laboratory tests were performed at the SWEDAC (www.swedac.se) accredited Clinical Chemistry and Immunology Laboratories at the Karolinska University Hospital. Routine laboratory tests and analyses of complement factors and immunoglobulins were performed on fresh samples according to clinical routine.

Antinuclear antibodies (ANA) were analysed by indirect immunofluorescence (IFL) on HEp-2 cells (Immunoconcepts, Sacramento, CA, USA). Antibodies to specific nuclear antigens (dsDNA, SSA/Ro52, SSA/Ro60, SSB/La, Sm, RNP) and phospholipid related antigens (cardiolipin IgG, IgM and β_2-glycoprotein1 IgG, IgM) were analysed by multiplexed bead technology (Luminex) using BioPlex 2200 system (Bio-Rad, Hercules, CA, USA) according to the specifications of the manufacturer. Patients who are simultaneously positive for SSA/Ro52, SSA/Ro60 and SSB/La are referred to as triple SSA/SSB positive. The cut off for anti-cardiolipin (aCL) and anti-β_2-glycoprotein1 (aβ_2GP1) fulfills the 99th percentile of the general population in Stockholm, as described (26). Lupus Anticoagulant was determined using a modified Dilute Russel Viper Venom method (Biopool, Umea, Sweden) using Bioclot lupus anticoagulant.

IgA, IgG and IgM RF were measured with a Phadia2500 instrument at Phadia Thermofisher Uppsala. Cutoffs for RF isotypes were determined as >95% specificity compared with 100 blood donors for IgA and IgM RF and for 285 population controls for IgG RF.

Mesoscale Discovery (MSD) multiplex analysis of cytokines EDTA-plasma samples were analyzed on the MSD V-PLEX™ Human Cytokine 30-plex kit (K15054D; Mesoscale Discovery, Gaithersburg, MD) according to manufacturer's instruction, as previously reported.(4) In short, the plasma samples were thawed at room temperature and diluted twice for pro-inflammatory- and cytokine-, and four times for chemokine-analysis in sample diluents. The diluted samples were incubated on the MSD plates for two hours at room temperature with shaking. Plates were washed and incubated an additional two hours with detection antibodies. After washing, 2x Read buffer T was added and the plates were analyzed in a Sector Imager 6000. Calibrator and plasma samples were analyzed in duplicates. Using the MSD Workbench software, the responses of the calibrator concentrations were plotted as log signal unit on the vertical (Y) axis versus log concentration on the horizontal (X) axis. A weighted four parameter logistic fit (4PL) equation was used for curve fitting and back calculation of plasma sample concentrations.

Statistical analysis

Patient and control characteristics are presented as mean ± standard deviation (SD), median (interquartile range, IQR) or percentages, depending on data type and distribution.

Groups were compared with the Students t-test, Wilcoxon rank sum test or Chi-square tests as appropriate. We used non-parametric tests when log transformation of continuous variables did not give an approximately normal distribution. Adjustment for age was performed by multiple logistic regression.

Calculations were performed using JMP software (SAS Institute, Carey, NC, USA). A two-sided p-value < 0.05 was considered statistically significant.

The local Ethics Committee at Karolinska Institutet approved the study (Dnr 03-556, Dnr 2017/1570-32). All study subjects gave written informed consent to participate in the study.

Results

The 504 patients and 319 matched controls were well matched for age, but the female percentage was slightly lower among the patients (86.3% vs. 91.9%; p=0.01). The SLE patients were 33.9 ± 15.5 years at diagnosis, and their disease duration was 12.1 ± 12.3 years. Basic characteristics are presented in table 1.

Patients with SLE-sSS versus SLE nonsSS

SLE-sSS was present in 23.2% of the SLE patients. Patients with SLE-sSS were older at inclusion (54.6±13.6 vs. 43.4±14.7 years; p= <0.0001) and at SLE onset (40.4±15.6 vs. 31.9±14.9 years; p= <0.0001). The percentage of SLE-sSS patients rose with rising age, as demonstrated in Figure 1. The frequency of females was higher in the SLE-sSS group compared to SLE-nonsSS (95.7% vs. 83.4%; p= 0.0007, Table 1).

We performed additional calculations to illustrate potential bias regarding the 132 SLE patients who were regarded as SLE-nonSS by their attending rheumatologist, but could have been reclassified after a biopsy. Results are presented in supplementary table 1.

Autoantibodies

Sjögren associated autoantibodies SSA-Ro52, SSA-Ro60 and SSB/La were more common in patients with SLE-sSS versus SLE-nonsSS (47.9% vs. 21.8 %; p= <0.0001, 59% vs. 35.9%; p= <0.0001 and 37.6% vs. 18%; p= <0.0001 respectively), but 39.3 % of the SLE-sSS patients were negative for all three SSA-Ro52, SSA-Ro60 and SSB/La antibodies. The frequency of SSA-Ro52 and SSA-Ro60 was highest among young patients, age 21-30 (Figure 2). Furthermore, RF of the IgM (38.6% vs. 19.9%; p=0.0005) and the IgA (45.9% vs. 28.0%; p=0.004) isotypes were more frequent in SLE-sSS as compared to SLE-nonsSS. Anti-dsDNA autoantibodies were non-significantly less prevalent in SLE-sSS than among SLE-nonsSS patients (31.3% vs. 41% p= 0.06) (Table 1).

Lupus manifestations, disease activity and damage

In the SLE-sSS group leucopenia and peripheral neuropathy were more common than in the SLE-nonsSS (57.3% vs. 45.2%; p= 0.02 and 15.4% vs. 7.5%; p= 0.01 respectively), while nephritis was less frequent (31.9% vs. 42.6%; p= 0.03).

Organ damage (SLICC/ACR-DI score >1), was more frequent in the SLE-sSS group (73% vs. 59.9%; p=0.01), but this difference did not remain after age adjustment. High disease activity, as measured by SLAM (score >6) was more common in the SLE-sSS group (67.2% vs. 57.1%; p=0.05), whereas SLEDAI scores were similar in both groups (Table 1).

Cytokines and other biomarkers

Higher levels of total IgG characterized the SLE-sSS group (p= 0.009). Cytokines were measured in 432 SLE patients and 315 controls. Of 30 investigated cytokines 20 were reliably detectable and evaluated, of these 19/20 were higher in SLE than in controls, as previously reported.(4) When comparing the SLE-sSS to the SLE-nonsSS group 6/20 cytokines (TNF-a, IL-6, MCP-4, MIP-1β, IL12/IL-23p40 and IP-10) were upregulated in SLE-sSS (Table 1). Exclusion of the 132 patients, who could have been reclassified after biopsy, yielded similar results except for IL12/IL-23p40 and IP-10, which no longer differed significantly.

Sjögren associated autoantibodies and their clinical associations

SSA-Ro52

SLE-sSS (p=<0.0001), leucopenia (p=0.04) and lymphopenia (p=0.05) were more common among SLE patients who were positive for SSA-Ro52, whereas malar rash (p=0.04) and seizures (p=0.02) were less frequent than in patients negative for SSA-Ro52 (Table 2).

SSA-Ro60

SSA-Ro60 positive patients were more often affected by sSS (p=<0.0001), leucopenia (p=0.0006) and photosensitivity (p=0.02) and less affected by discoid lesions (p= 0.02), nephritis (p=0.05), vasculitis (p=0.03) and peripheral neuropathy (p=0.02) (Table 2).

SSB-La

In the SSB/La positive patient group sSS (p=<0.0001), arthritis (p=0.002), leucopenia (p=0.0003) and lymphopenia (p=0.05) were more common, but nephritis (p=0.0005) was less common in this group (Table 2).

Sicca symptoms

In all investigated groups (SLE-sSS, SLE-nonsSS and controls) subjective symptoms of ocular and/or oral sicca symptoms were less frequent than objective measurements of reduced tear and saliva production. While subjective symptoms clearly differed between SLE-nonsSS and controls in all age spans, objectively measured tear production was similar in the age spans >40 years, and differences were small also for WUSF after the age of 50 years. (Figures 3 and 4)

Ocular Sicca Symptoms

In the SSA-Ro52, SSA-Ro60 and SSB/La positive groups, 41.4% 39.3% and 44.2%, respectively, presented with subjective ocular sicca symptoms. Figures were similar for the triple SSA/SSB positive patients, 44.9% (Supplementary Figure 1 A).

Oral Sicca Symptoms

In the SSA-Ro52, SSA-Ro60 and SSB/La positive groups, 49.2%, 52.7% and 50.9 % respectively, presented with subjective oral sicca symptoms. Figures were similar for the triple SSA/SSB positive patients, 53.9 % (Supplementary Figure 1B).

Sicca symptoms in triple SSA/SSB negative SLE patients and controls

Among patients who were negative for all three SSA/SSB antibodies 36.9% reported subjective oral sicca symptoms, 24.6% reported ocular sicca symptoms and 16.8 % were diagnosed with sSS. In the control group 7.5% reported oral, and 7.1% ocular symptoms (Supplementary Figure 1A and B).

Discussion

According to the revised AECC 5(15) SLE-sSS occurred in 23 % of patients in this large and well-defined cohort of consecutive SLE patients. It is a novel observation that, despite less internal organ involvement, higher levels of pro-inflammatory cytokines were present in the SLE-sSS group than in the SLE-nonsSS. We also confirm that older age, female gender and SSA/SSB antibodies, not included in the AECC definition of sSS, were positively associated with SLE-sSS. But notably a large minority (39%) of SLE-sSS patients were negative for all three SSA/SSB antibodies.

To our knowledge this is the first study to investigate if systemic inflammation, as measured by cytokine levels, differs between SLE-sSS and SLE-nonsSS. Despite the fact that SLE-sSS is often considered to be a less severe form of lupus the levels of several pro-inflammatory cytokines, TNF-α, IL-6, MCP-4, MIP-1β, IL-12/IL23p40 and IP-10, were higher in SLE-sSS than in SLE-nonsSS patients. As previously reported the investigated cytokines were also upregulated in SLE versus controls, and they were positively associated with SLE disease activity (4). TNF-α and IL-6 have also been reported to be high in pSS (27). Interesting an IP-10 antagonist ameliorated the progression of autoimmune sialoadenitis in MRL/lpr mice.(28) Furthermore, hypergammaglobulinemia, a well-known feature of SS (29) was in our study consequently more common among SLE-sSS patients, although the levels of IgG were usually below 20 mg/ml. We previously reported that low total IgM levels were associated with an SSA/SSB positive profile in SLE(29), but using the AECC IgM levels were similar in SLE-sSS and SLE-nonsSS subgroups.

The occurrence of SLE-sSS in our study, 23 %, is higher than reported by most previous studies, 6 % -14 % (18, 20, 30-32). An important reason for this discrepancy is likely attributable to our meticulous investigation procedures. In contrast to most studies we measured tear and saliva production in all SLE patients, regardless if subjective symptoms were present. It was a general

finding that objectively decreased production of tear and saliva is more common than subjectively reported sicca symptoms. The high frequency of SLE-sSS is likely also affected by ethnicity. Our study population is mostly of European Caucasian origin and *Baer et al* previously reported that in Caucasians, as compared to other American ethnicities, frequencies of SLE-sSS were higher, 18% versus 14 % (18).

The frequencies of SLE-sSS increased with age. Patients with SLE-sSS were on average nine years older than SLE-nonsSS both at disease onset and at inclusion. Similar age differences were also reported previously.(18, 20, 30, 33, 34) The difference in age at SLE onset did however not remain after controlling for present age. Comparable findings were reported by *Baer et al*. who investigated a large multiethnic SLE cohort (18) and by *Manoussakis et al.*(30) In line with these observations *Nossent et al.* showed a rising percentage of SLE-sSS during a four year follow up of patients with SLE(19). To further clarify whether sicca symptoms correlated with age, we stratified SLE-sSS, SLE-nonsSS and controls by age. The results demonstrate that the prevalence of both subjective and objective sicca symptoms, rise with increasing age also in the SLE-nonsSS group and in controls. Thus, in some of the older SLE-sSS patients, the AECC criteria may diagnose sSS patients, who have more age related than to immunological aberrations. Taken together the present and previous studies demonstrate that sSS is an age related complication among patients with SLE(18, 19).

The presence of SSA/SSB autoantibodies and their positive association to sicca symptoms is well recognized. Earlier studies reported that these antibodies occur together.(12, 16) We observed that the prevalence of oral and ocular dryness was more pronounced in patients who are positive for each and all three SSA/Ro52 and Ro60 and SSB/La antibodies. Our study thus supports the presence of an autoantibody cluster related to the SLE-sSS subset(10, 11, 29). Although the presence of the SSA/SSB antibodies was associated with sicca symptoms, a considerable fraction of patients with SLE-sSS (39%) were negative for all three SSA/SSB

antibodies. We also report higher frequencies of IgM and IgA RF in patients with SLE-sSS. Thus, the presence of sicca symptoms is not exclusively related to SSA/SSB antibodies, but also to increasing age, RF, and possibly to other unidentified factors.

Overall organ damage was more severe in the SLE-sSS group, but this difference did not remain after age adjustment. Regarding disease activity, we performed two validated indices. SLEDAI,(23) a qualitative (presence versus absence) recording of symptoms and laboratory aberrations, and SLAM, (22) which grades symptom severity and includes subjective symptoms such as fatigue, headache and arthralgia. SLEDAI measurements did not differ, whereas with SLAM higher disease activity scores were observed in the SLE-sSS than in the SLE-nonsSS subgroup. This observation could possibly be explained by the inflammatory state, which may cause subjective and general symptoms, such as muscle and joint pain, headache and fatigue, which are included in the SLAM but not in the SLEDAI index.

Consistent with earlier studies (18, 30, 34) the female predominance was more pronounced among SLE-sSS patients as compared to SLE-nonsSS, 96% vs. 84 %. The very high percentage of female patients with SLE-sSS, 96%; is similar to reports in pSS (30). The average age of disease-onset for SLE-sSS was higher than for SLE-nonsSS, 40,4 +/-15.6 vs 33,9+/-14.9 years, but still considerably younger than the average age of onset for pSS, which is 55 years(35, 36).

In clinical practice, it is often difficult to delineate pSS from SLE-sSS. Organ manifestations commonly reported in pSS are fever, lymphadenopathy, parotid gland enlargement, Raynaud´s phenomenon, interstitial lung disease, peripheral neuropathy and vasculitis(37-39). All these clinical features, except parotid gland enlargement, were investigated in the present study, but only peripheral neuropathy differed and was more frequent in SLE-sSS than in SLE-nonsSS, demonstrating that the majority of these manifestations are shared between SLE and pSS. Of lupus manifestations, leucopenia was more common in the SLE-sSS group and

there was also a positive trend for photosensitivity, both of which have been associated with the type 1 interferon signature. Nephritis was, in accordance with previous studies less frequent and anti-dsDNA positivity showed a similar trend(18, 20, 30, 34). We conclude that SLE-sSS and patients with pSS have many similarities and it is not surprising that among the patients with SLE-sSS according to AECC, 21% also fulfilled the criteria for pSS, if we disregard the fact that they are diagnosed with SLE.

Strengths of this study are the objective measurements of sicca symptoms in all SLE patients, according to the AECC criteria and recommended praxis in Sweden, in a well-characterized consecutively collected cohort of patients with SLE. However, if the investigating rheumatologist did not consider sSS to be present, we did not refer to an ophthalmologist for Rose Bengal staining or other ocular dye scores to achieve full potential for all patients to fulfill Item III (Ocular signs) in the classification criteria. Similarly, salivary gland biopsies were only performed when the investigating rheumatologist suspected that sSS could be present, despite not fulfilling item III and item V (Salivary gland involvement) according to USWSF. We are aware that previous studies have reported a lower sensitivity if only Schirmer's test and USWSF are used(40), and we have considered the risk for misclassification but rely on the fact that the rheumatologist's assessed these patients clinically as SLE-nonsSS. To further illustrate this issue, we excluded the 132 patients, in whom a biopsy could have made a difference, but this did not essentially change the characteristic differences between SLE-sSS and SLE-nonsSS. Excluding these patients would also have changed the study's cross-sectional and consecutive design. Finally, we did not measure saliva and tear production in controls without sicca symptom. Since positive objective measures were more common than subjective complaints we may have underestimated the number of controls with impaired saliva/tear production.

Our investigations of the SLE-sSS subset demonstrate that it affects roughly ¼ of SLE patients, and the frequency increases with rising age. Autoantibodies, SSA/SSB, occur in the majority, but a large minority, 39 %, were SSA/SSB negative. SLE-sSS shares many features with pSS such as a striking female predominance, older age at onset and neuropathy. It is a novel observation, with possible therapeutic implications, that an inflammatory state with higher levels of pro-inflammatory cytokines occurred in SLE-sSS than in SLE-nonsSS.

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Figures legends:

Fig 1: Age stratified frequencies (%) of SLE-sSS in defined age spans

Fig 2: Sjögren associated autoantibodies in SLE-sSS and SLE-nonsSS patients, stratified for age

Fig 2A Occurrence of SSA-Ro52 positivity in patients with SLE-sSS (black) and SLE-nonsSS (grey), stratified by age. Fig 2B Occurrence of SSA-Ro60 positivity in patients with SLE-sSS (black) and SLE-nonsSS (grey), stratified by age. Fig 2C Occurrence of SSA-Ro52 positivity in patients with SLE-sSS (black) and SLE-nonsSS (grey), stratified by age.

Fig 3: Subjective and objective ocular symptoms in patients with SLE-sSS and SLE-nonsSS and in controls.

Fig 3A Subjective ocular symptoms in controls, SLE-nonsSS and SLE-sSS as defined by a validated questionnaire according to item 1 in the American-European Consensus Criteria (AECC)(15). 3B Objective ocular symptoms in controls, SLE-nonsSS and SLE-sSS, measured according to item 3 in AECC by Schirmer's test where a positive value is given to an amount of <5mm of tears collected during 5 min from either one or both eyes.

Fig 4: Subjective and objective oral symptoms in patients with SLE-sSS and SLE-nonsSS and in controls.

Subjective oral symptoms in controls, SLE-nonsSS and SLE-sSS as defined by a validated questionnaire according to item 2 in the American-European Consensus Criteria (AECC)(15). Objective oral symptoms in controls, SLE-nonsSS and SLE-sSS as measured according to item 5 in AECC(15) by whole unstimulated flow (WUSF) over 15 minutes, where a positive value is given to an amount of < 1.5ml of saliva collected during 15 min.

Legend supplementary Figure 1

Supplementary Fig 1A: **Subjective ocular symptoms and their association with positivity for SSA-Ro52, SSA-Ro60 and SSB-La.**

Supplementary Fig 1A: SSA-/SSB- =The absence of antibodies targeting SSA/Ro52, SSA/Ro60 and SSB/La, Triple SSA+/SSB+ = presence of antibodies targeting all three antigens: SSA/Ro52, SSA/Ro60 and SSB/La.

Supplementary Fig 1B: Subjective oral symptoms and their association with positivity of Ro52, Ro60 and La.

SSA-/SSB- = The absence of antibodies targeting SSA/Ro52, SSA/Ro60 and SSB/La, Triple SSA+/SSB+ = presence of antibodies targeting all three antigens: SSA/Ro52, SSA/Ro60 and SSB/La.

Frequency of SLE-sSS among 504 SLE patients, stratified by age

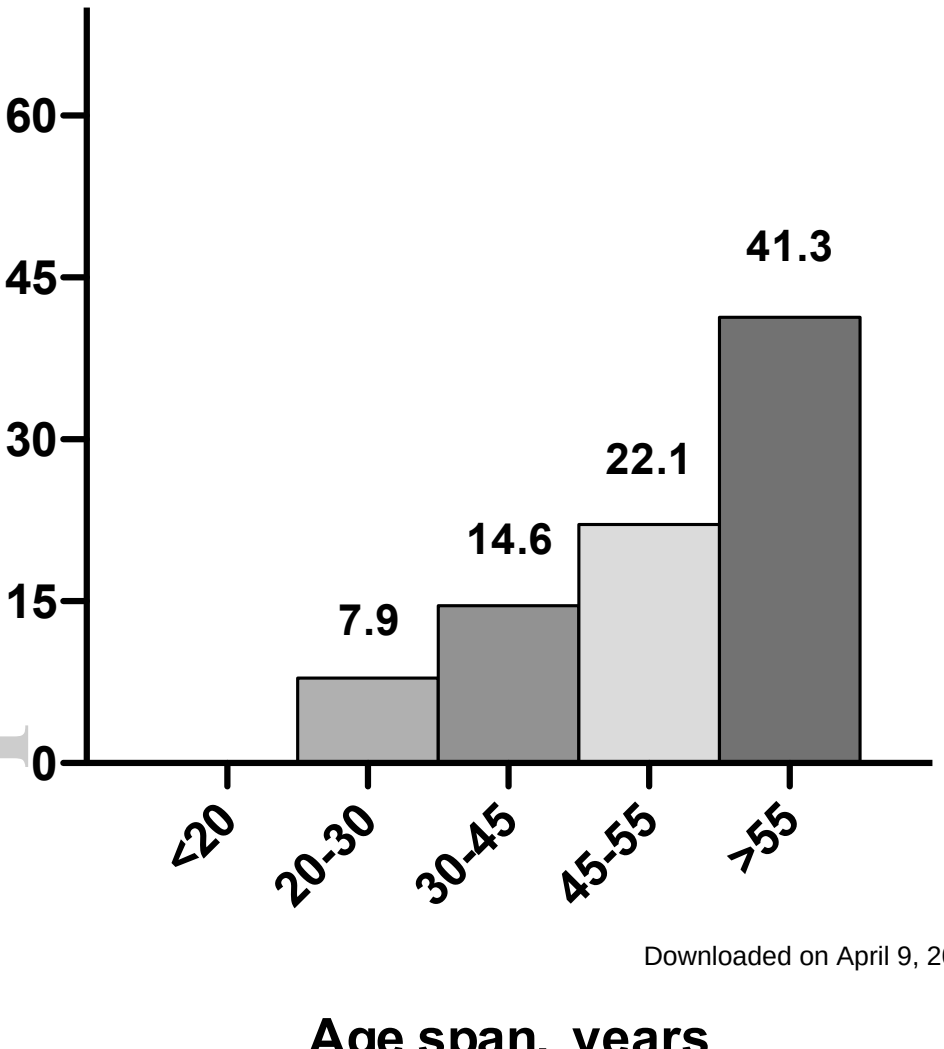
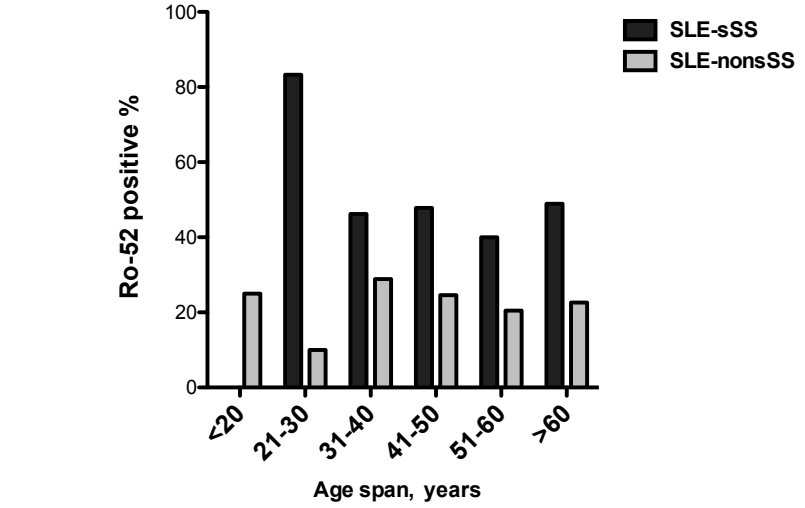


Fig 1: Age stratified frequencies (%) of SLE-sSS in defined age spans

A



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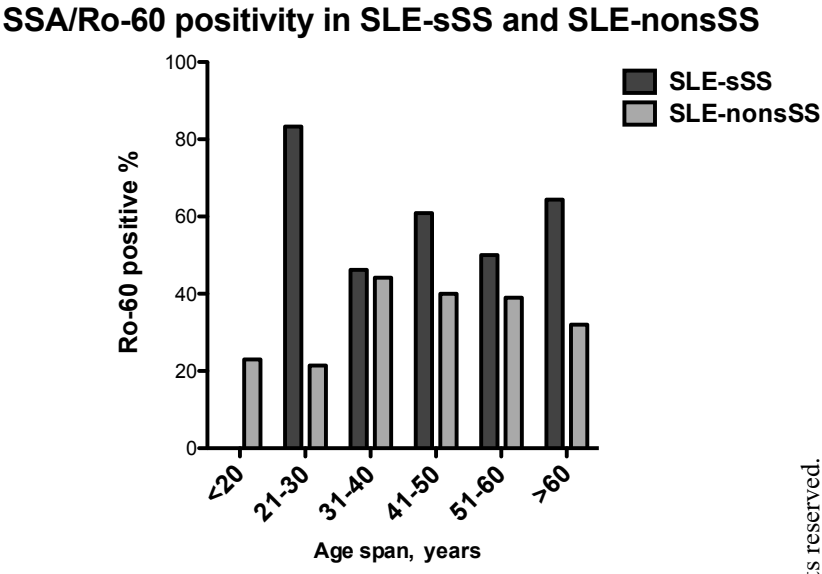

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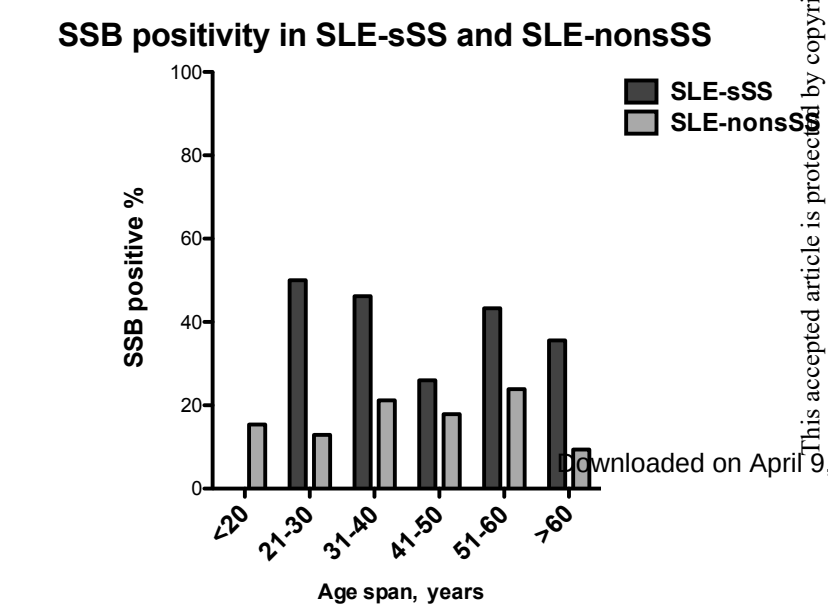

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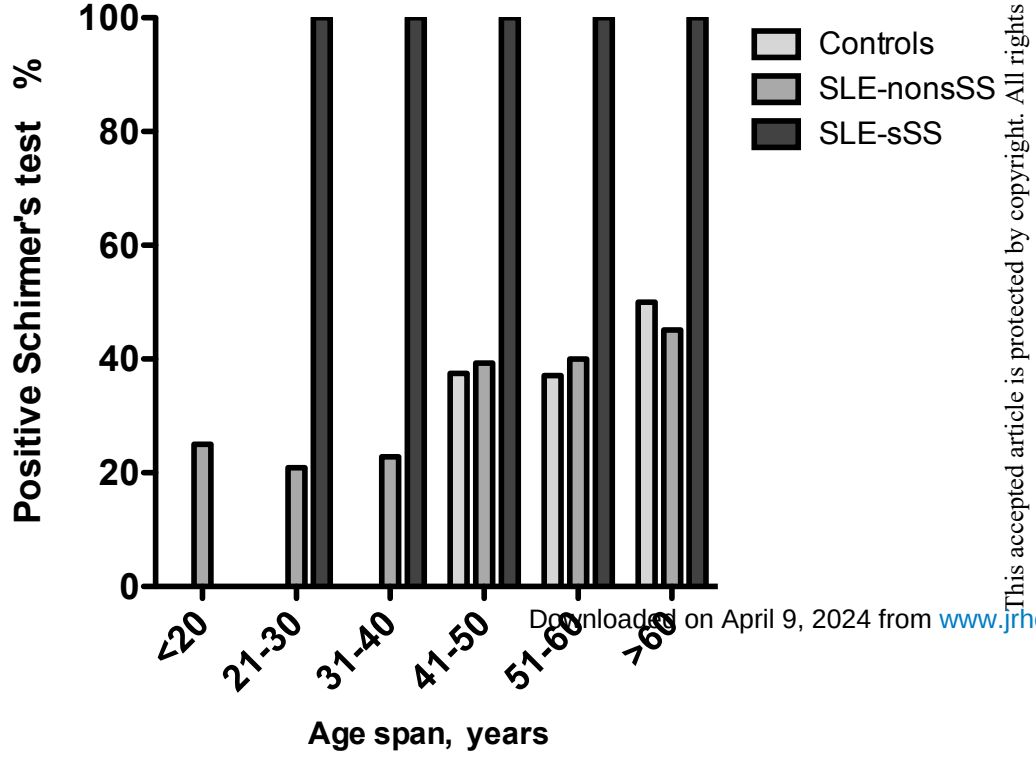
Ocular symptoms in Controls, SLE-nonsSS and SLE-sSS

A

Positive Schirmer's test in Controls, SLE-nonsSS and SLE-sSS

B



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Oral symptoms in Controls, SLE-nonsSS and SLE-sSS

A

WUSF in controls, SLE-nonsSS & SLE-sSS

B

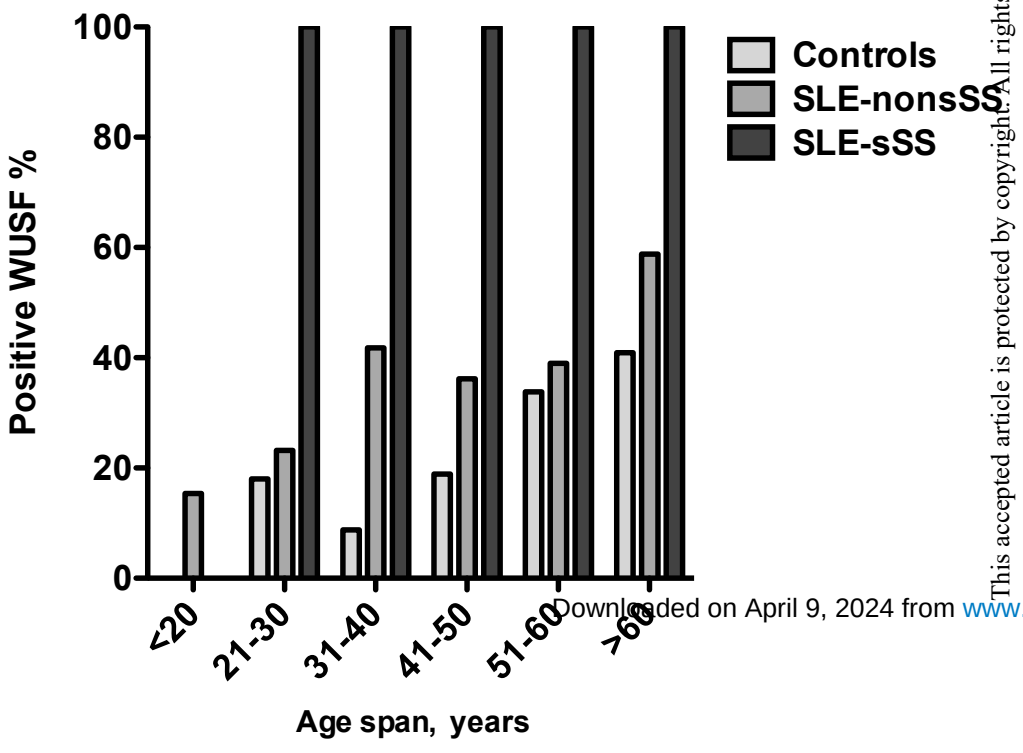

Fig 4: Subjective and objective oral symptoms in patients with SLE-sSS and SLE-nonsSS and in controls.

Subjective oral symptoms in controls, SLE-nonsSS and SLE-sSS as defined by a validated questionnaire according to item 2 in the American-European Consensus Criteria (AECC)(15). Objective oral symptoms in controls, SLE-nonsSS and SLE-sSS as measured according to item 5 in AECC(15) by whole unstimulated flow (WUSF) over 15 minutes, where a positive value is given to an amount of < 1.5ml of saliva collected during 15 min.

Table 1

Basic characteristics of SLE patients, controls and SLE-sSS versus SLE-nonsSS

	SLE	Controls	p-value	SLE-sSS	SLE-nonsSS	p-value
Number (%)	**504 (100)**	**319 (100)**		**117(23.2)**	**387 (76.7)**	
Basic characteristics (mean +/- SD or Number (%))						
Age, years	46.0+/-15.2	47.4+/-14.6	0.20	54.6+/-13.6	43.4+/-14.7	<0.0001
Age at SLE onset	33.9+/-15.5	nd	nd	40.4+/-15.6	31.9+/-14.9	<0.0001
Disease duration, years	12.1+/-12.3	nd	nd	14.2+/-12.8	11.5+/-12.1	0.16
Number of SLE criteria*	5.7+/-1.4	nd	nd	5.7+/-1.5	5.7+/-1.4	0.40
Gender (female)	435(86.3)	296(91.9)	**0.01**	112(95.7)	323(83.4)	**0.0007**
Smoking current	96(19.0)	47(14.6)	0.22	20(17.1)	76(19.6)	0.58
Smoking ever	258(51.2)	151(46.9)	0.22	62(53)	196(50.6)	0.65
Primary SS	108(21.6)	nd	nd	25(21.9)	83(21.6)	0.99
Autoantibodies (positivity)						
anti-dsDNA	190(38.7)	5(1.6)	**<0.0001**	36(31.3)	154(41)	**0.06**
anti-SSA-Ro52	140(27.9)	3(0.9)	**<0.0001**	56(47.9)	84(21.8)	**<0.0001**
anti-SSA-Ro60	206(41.3)	5(1.6)	**<0.0001**	69(59)	137(35.9)	**<0.0001**
anti-SSB-La	113(22.6)	10(3.1)	**<0.0001**	44(37.6)	69(18)	**<0.0001**
anti-Sm	94(18.8)	1(0.3)	**<0.0001**	19(16.2)	75(19.5)	0.42
anti-SmRNP	130(26.2)	0(0)	**<0.0001**	29(25)	101(26.5)	0.74
anti-RNP 68	51(10.2)	0(0)	**<0.0001**	11(9.4)	40(10.4)	0.74
anti-RNP A	129(26.0)	10(3.1)	**<0.0001**	30(25.6)	99(26.1)	0.91
aCL IgG	108(25.5)	0(0)	**<0.0001**	24(24.7)	84(25.8)	0.83
aCL IgM	26(6.0)	2(0.6)	**<0.0001**	8(8.3)	18(5.4)	0.32
aβ_2GPI IgG	110(26.4)	0(0)	**<0.0001**	24(25.3)	86(26.7)	0.77
aβ_2GPI IgM	33(7.6)	3(0.9)	**<0.0001**	8(8.3)	25(7.4)	0.82
RF IgG	52/339(15.3)	10/261(3.8)	**<0.0001**	17/80(21.2)	35/259(13.5)	0.09

RF IgM	88/364(24.2)	14/283(4.9)	**<0.0001**	32/83(38.6)	56/281(19.9)	0.0005
RF IgA	109/341(32.0)	12/282(12.4)	**<0.0001**	34/74(45.9)	75/267(28.0)	0.004
Other laboratory analyses						
IgA g/L	2.90+/-1.46	2.30+/-1.06	**<0.0001**	3.19+/-1.80	2.82+/-1.32	0.38
IgG g/L	13.63+/-5.54	10.89+/-2.14	**<0.0001**	14.85+/-5.99	13.25+/-5.35	**0.009**
IgM g/L	1.24+/-1.26	1.26+/-0.69	**0.0002**	1.23+/-1.09	1.24+/-1.31	0.89
C3 g/L	0.88+/-0.26	1.06+/-0.21	**<0.0001**	0.90+/-0.26	0.87+/-0.26	0.33
C4 g/L	0.15+/-0.08	0.21+/-0.06	**<0.0001**	0.16+/-0.08	0.15+/-0.07	0.16
Lupus anti-coagulant	104(20.7)	0(0)	**<0.0001**	20(17.1)	84(21.8)	0.27
Cytokines median (IQR)#						
TNF-α□ pg/mL	4.5(3.1-6.2)	2.3(2.0-2.8)	**<0.0001**	4.9(3.6-7.1)	4.4(3.0-6.0)	**0.008**
IL-6 pg/mL	1.2(0.7-2.2)	0.5 (0.4-0.7)	**<0.0001**	1.5(0.8-3.0)	1.1(0.6-2.0)	**0.009**
MCP-4 pg/mL	78 (53.6-123.8)	55.8 (40.8-81.1)	**<0.0001**	94.9 (66.9-131.3)	74.7 (52.4-120.0)	**0.019**
MIP-1β pg/mL	72.7(50.8-108.1)	43.7 (33.4-56.4)	**<0.0001**	81.1 (54.8-123.6)	68.9(50.3-105.1)	**0.020**
IL-12/23p40 pg/mL	180.6 (122.9-286.3)	131.0 (99.5-179.3)	**<0.0001**	211.3 (141.4-363.8)	177.1 (119.6-274.5)	**0.031**
IP-10 pg/mL	744.4 (457.5-1497.4)	352.13 (258.9-478.9)	**<0.0001**	808.0 (536.4-1911.7)	726.4 (440.8-1471.0)	**0.036**
Lupus manifestations (ever) Number (%)*						
Malar rash	246(48.8)	0(0)	**<0.0001**	55(47.0)	191(49.4)	0.65
Discoid lesions	88(17.5)	0(0)	**<0.0001**	17(14.5)	71(18.3)	0.34
Photosensitivity	318(63.1)	57(17.7)	**<0.0001**	82(70)	236(61)	0.07
Oral ulcers	168(33.5)	11(3.4)	**<0.0001**	41(35)	127(33)	0.67
Arthritis	406(80.7)	13(4.0)	**<0.0001**	93(79.5)	313(81.1)	0.70
Pleuritis	185(36.8)	2(1)	**<0.0001**	50(42.7)	135(35)	0.12
Pericarditis	88(17.5)	0(0)	**<0.0001**	24(20.5)	64(16.6)	0.32

Nephritis	202(40.2)	1(0.3)	**<0.0001**	37(31.9)	165(42.6)	0.03
Psychosis	10(2)	2(1)	0.11	1(1)	9(2.3)	0.46
Seizures	49(9.7)	5(1.6)	**<0.0001**	11(9.4)	38(9.8)	0.89
Leucopenia	242(48)	3(1)	**<0.0001**	67(57.3)	175(45.2)	**0.02**
Lymphopenia	273(54.2)	2(1)	**<0.0001**	68(58.1)	205(53)	0.32
Thrombocytopenia	100(19.8)	2(1)	**<0.0001**	24(20.5)	76(19.6)	0.83
Raynaud's phenomenon	195(38.7)	10(3.1)	**<0.0001**	51(43.6)	144(37.2)	0.21
Vasculitis UNS [ε]	57(11.4)	0(0)	**<0.0001**	9(7.8)	48(12.6)	0.15
Interstitial lung disease	24(4.8)	0(0)	**<0.0001**	7(6.0)	17(4.4)	0.47
Peripheral neuropathy	47(9.4)	6(1.9)	**<0.0001**	18(15.4)	29(7.5)	**0.01**
Disease activity and damage indices						
SLICC/ACR DI >1	314(62.9)	nd	nd	84(73.0)	230(59.9)	**0.01**
SLAM >6 [ε]	298(59.5)	nd	nd	78(67.2)	220(57.1)	**0.05**
SLEDAI >2	356(71.2)	nd	nd	82(70.7)	274(71.4)	0.88

SD= standard deviation, primary SS=fulfills criteria for primary Sjögren's syndrome with the exception that patients are diagnosed with SLE, SSA/B = Sjögren's syndrome A/B, Sm=Smith, RNP= ribonucleoprotein, aCL= anti-cardiolipin, Ig=immunoglobulin, β_2GPI=anti $\beta_2$glycoprotein-I, C=complement factor, RF=rheumatoid factor, IQR= interquartile range, [#]= Cytokines were determined in 431 SLE patients and 315 controls, TNF= tumor necrosis factor, IL=interleukin, MCP=monocyte chemoattractant protein, MIP= macrophage inflammatory protein, IP=interferon gamma inducible protein, SLICC/ACR DI= Systemic Lupus International Collaborating Clinics /ACR Damage index, SLAM= Systemic Lupus Activity Measure, SLEDAI= SLE Disease Activity Index, nd= not determined, * SLE criteria were determined according to ACR 1982 classification criteria

Table 2.

Frequency of organ manifestations in all SLE patients versus in SSA/SSB positive SLE patients

Lupus manifestations	Missing Data No.	SLE cohort N_{total}=504 (100%)	anti-Ro52+ N_{Ro52+}=140 (27.9%)	p-value	anti-Ro60+ N_{Ro60}=206 (41.3%)	p-value	anti-La/SSB+ $N_{La/SSB}$=113 (22.6%)	p-value
Secondary Sjögren's syndrome	0	117 (23.2)	56/117(47.9)	**<0.0001**	69/117(59.0)	**<0.0001**	44/117(37.6)	**<0.0001**
Malar rash	0	246(48.8)	58/137(41.4)	**0.04**	103/201(50.0)	0.67	54/103(47.8)	0.78
Discoid lesions	0	88(17.5)	27/137(19.3)	0.52	46/201(22.3)	**0.02**	25/103(22.1)	0.14
Photosensitivity	0	318(63.1)	94/137(67.1)	0.26	142/201(68.9)	**0.02**	78/103(69.0)	0.14
Oral ulcers	2	168(33.5)	50/137(35.7)	0.46	68/201(33.0)	0.87	40/103(35.4)	0.58
Arthritis	1	406(80.7)	109/137(77.9)	0.33	161/201(78.2)	0.26	80/103(70.8)	**0.002**
Pleuritis	1	185(36.8)	53/137(37.9)	0.75	73/201(35.4)	0.61	48/103(42.5)	0.14
Pericarditis	1	88(17.5)	27/137(19.3)	0.48	33/201(16.0)	0.47	18/103(15.9)	0.63
Nephritis	1	202(40.2)	47/136(33.8)	0.07	72/200(35.1)	**0.05**	29/102(25.9)	**0.0005**
Psychosis	0	10(2)	2/137(1.4)	0.57	5/201(2.4)	0.57	1/103(0.9)	0.33
Seizures	0	49(9.7)	7/137(5.0)	**0.02**	15/201(7.3)	0.11	7/103(6.2)	0.14
Leucopenia	0	242(48.0)	77/137(55.0)	**0.04**	117/201(56.8)	**0.0006**	71/103(62.8)	**0.0003**
Lymphopenia	0	273(54.2)	85/137(60.7)	**0.05**	121/201(58.7)	0.06	70/103(61.9)	**0.05**
Trombocytopenia	0	100(19.8)	25/137(17.9)	0.46	42/201(20.4)	0.79	23/103(20.4)	0.90

Raynaud's phenomenon	0	195(38.7)	55/137(39.3)	0.87	82/201(39.8)	0.60	41/103(36.3)	0.57
Vasculitis UNS*	6	57(11.4)	18/135(13.0)	0.50	31/198(15.3)	**0.03**	16/102(14.3)	0.29
Interstitial lung disease	0	24(4.8)	10/137(7.1)	0.12	10/201(4.9)	0.96	6/103(5.3)	0.76
Peripheral neuropathy	3	47(9.4)	15/136(10.8)	0.45	26/199(12.7)	**0.02**	13/102(11.6)	0.32

*= All clinical vasculitis