Smoking associates with distinct clinical phenotypes in patients with systemic lupus erythematosus: a nationwide Danish cross-sectional study

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ABSTRACT

Objectives SLE displays large clinical heterogeneity that beyond genetic factors may be determined by environmental exposures. In this Danish nationwide study, we aimed to determine if clinical subsets of SLE were associated with smoking history.

Methods At each of six participating centres, incident or prevalent inpatients and outpatients with SLE were consecutively included. Manifestations forming the basis of SLE classification were registered in an electronic chart system. Patients also provided questionnaire-based data on environmental exposures, including smoking history. Hierarchical cluster analysis was conducted to determine and characterise subsets of patients with similar traits of disease manifestations. Levels of smoking exposure by pack-years were correlated to the identified SLE subsets, as well as discrete SLE manifestations.

Results The cohort consisted of 485 patients (88% women and 92% Caucasian) with SLE of which 51% were ever smokers. Common disease manifestations comprised non-erosive arthritis (81%), malar rash (57%), lymphopenia (55%), photosensitivity (50%) and persistent proteinuria (41%). We identified three distinct phenotypic clusters characterised by their preponderance of (A) neurological, serosal and mucosal involvement; (B) renal, haematological and immunological disorders; and (C) acute and chronic skin manifestations. Cluster B was the youngest and had the lowest level of smoking exposure. Age-adjusted regression analyses showed that compared with never smokers a smoking history of >20 pack-years was associated with neurological disorder (OR=3.16), discoid rash (OR=2.22), photosensitivity (OR=2.19) and inversely with haematological disorder (OR=0.40), renal disorder (OR=0.40) and non-erosive arthritis (OR=0.45), p<0.05 for all.

Conclusions Our findings support that SLE presents in varying clinical phenotypes and suggest that they may have differentiated associations with smoking history.

INTRODUCTION

SLE is a systemic inflammatory autoimmune disease characterised by a broad spectrum of clinical and serological manifestations.[1–3] This is reflected by the currently accepted delineation of SLE,[4] but also by SLE having a complex and multifactorial aetiology.[1] Although several genetic and environmental risk factors for SLE have been identified, they cannot explain the full risk of SLE.[5 6] It is well accepted that SLE phenotypically can be divided into more homogenous subsets by cluster analyses that partition cases into clusters, which may then be linked to, for example, disease severity or treatment.[7–9] Consequently, investigating clustering of patients, and association with environmental risk factors might lead to a better understanding of the sources of SLE heterogeneity.[10 11] Indeed, few studies have investigated the role of environmental factors such as smoking in relation to SLE expression. Scrutinising the heterogeneity of SLE and defining distinct environmental

Key messages

What is already known about this subject?
► SLE displays large clinical and serological heterogeneity.
► Smoking is generally acknowledged to be a risk factor for SLE.

What does this study add?
► The identification of three distinct phenotypic clusters of patients with SLE, characterised by preponderance of neurological, renal and skin involvement, respectively.
► Smoking was found to be differentially associated with these phenotypic clusters.

How might this impact on clinical practice or future developments?
► Future ascertainment of risk factors in SLE should also address the clinical heterogeneity of SLE.

risk factors for SLE expression is imperative for understanding the course of SLE and improving disease prevention, management and intervention strategies.[12]

Epidemiological studies have shown that current smoking and smoking more than 10 pack-years are associated with an increased risk of SLE, which persists for up to 5 years after smoking cessation. In addition, smoking more than 20 pack-years doubles the risk of SLE among current smokers.[13] Possible explanations for the association between smoking and increased risk of SLE include modifying neutrophil extracellular traps and expression of inflammatory response-related genes.[14] Studies of patients with SLE investigating the effects of smoking on pathognomonic clinical features have found associations between smoking and the presence of anti-double stranded DNA (anti-dsDNA), as well as anti-Smith, anti-Ro and anti-La antibodies. Studies investigating the relationship between smoking and SLE phenotypes have suggested that smoking may be associated with cutaneous manifestations of SLE, such as photosensitivity and other active skin manifestations.[11 15]

Based on data collected in a setting of a collaborative, large-scale, nationwide study of genetics and environmental exposures in Danish patients with SLE, we want to determine whether subsets of patients or specific disease manifestations are associated with history of smoking.

METHODS
Study population and design
Patients fulfilled established classification criteria for SLE by the American College of Rheumatology (ACR)[16] and comprised patients attending the following centres from May 2018 to end January 2020: Copenhagen University Hospital at Rigshospitalet and Gentofte Hospital, Aarhus University Hospital, Odense University Hospital, Aalborg University Hospital and North Denmark Regional Hospital; all affiliated to the research network, SLEDAN, that aims to facilitate SLE research protocols and standardisation in Denmark.

Based on previous Danish experience,[17 18] a major goal of SLEDAN is to include the majority of prevalent and incident Danish patients with SLE. First, to identify risk factors of SLE and SLE phenotypes in SLE Gene-Environment Interaction Study (SLEGEIST) initiated in 2017.

Clinical data were entered into Danish Rheumatologic Database (DANBIO), a Danish nationwide clinical database, in which information regarding, for example, demographics, disease activity and medication for patients with inflammatory joint and connective tissue disorders has been accumulated since 2000.[19] DANBIO also offers access to the national infrastructure, Danish Rheumatologic Biobank, in which corresponding biosamples have been collected since 2015. SLEDAN has developed a specific module for DANBIO to register manifestations that form the basis of SLE classification as well as measures of disease activity and organ damage.

Information regarding lifestyle, reproductive and environmental factors was directly entered into DANBIO by web-based self-report.

Clinical definitions
Clinical and serological manifestations of SLE were collected in DANBIO during clinical visits and supplemented by chart review. Afterwards, data were extracted from DANBIO, quality controlled by internal validation and made available for analyses. SLE manifestations were all defined according to the ACR classification criteria,[16] except for urinary cellular cast microscopy and false-positive syphilis test since these were not routinely performed in our cohort. Autoantibodies were determined using routine methods, that is, ANAs were typically determined by indirect immunofluorescence methods and other autoantibodies by ELISA; titres above 1:160 and titres above 2 times the upper limit were considered positive, respectively. Lupus anticoagulant was determined by routine mixing tests of patient plasma with pooled control plasma.

Exposure definitions
By web-based survey at inclusion, patients were asked if they were current smokers, previous smokers or never smokers. If patients had a smoking history, they were asked to detail how many years they had been smoking, how much daily, and if so, when they had stopped smoking. The cumulated number of pack-years (mean number of cigarettes per day/20 multiplied by years of smoking) was determined at inclusion for all patients. Patients were stratified into four mutually exclusive groups according to accumulated pack-years in accordance with previous works[13] by using the following lower limits: 0, >0, >10, >20 pack-years. Never smokers were defined as having 0 pack-years.

Statistical analysis
Descriptive data were presented as medians, IQRs and ranges for continuous variables and as frequencies and percentages for categorical variables.

Hierarchical cluster analysis using Ward's linkage method and the Sørensen-Dice similarity measure were performed.[20] Clustering is thus based on a quantitative measure of similarity, such that symptoms in the same cluster are more similar to each other than to symptoms in another cluster.[21] The number of clusters was so defined that none of them included less than 100 patients. X^2, Kruskal-Wallis and Jonckheere-Terpstra tests were used when comparing groups of categorical, continuous and ordinal variables, respectively.

Associations between smoking and SLE disease manifestations were examined by logistic regression analyses adjusted for age at last visit and sex. Analyses were performed for all disease manifestations with the four strata of cumulative pack-years as an ordinal variable using never smokers as reference for trend analyses. Analyses were conducted using IBM SPSS statistics V.25.0.

Table 1 Characteristics of 485 patients with SLE included in the SLEDAN study cohort with registrations of demographics, clinical characteristics and smoking history

Age at last visit (years)	49 [38–60] (20–86)
Age at SLE diagnosis (years)	31 [23–42] (5–83)
SLE disease duration (years)	13 [6–23] (0–51)
Women, n (%)	427 (88)
Ethnicity, n (%)	
Caucasian	448 (92)
Asian	17 (4)
Middle East	13 (3)
African	7 (1)
Smoking history	
Ever smoker, n (%)	249 (51)
Pack-years (PY) among ever smokers	7.5 [1.5–20.0] (0.01–125)
0<PY≤10, n (%)	143 (30)
10<PY≤20, n (%)	49 (10)
20<PY, n (%)	57 (11)

Unless indicated otherwise, results are median [IQR] (range).

RESULTS

Over 21 months, 573 patients with SLE were enrolled in SLEGEIST by the six national centres. Detailed smoking history was obtained from 485 patients who form base of this study with. The 88 patients who were not included in the analyses of this work did not differ from the rest with respect to age, sex, ethnicity or cumulated clinical manifestations (data not shown). The study population consisted of 88% women and 92% were of Caucasian origin. The median age at diagnosis was 31 years and the median disease duration was 13 years (table 1).

At enrolment, 51% had a history of smoking, active or previous, with a median of 7.5 cumulated pack-years (table 1). Cumulated pack-years differed between men and women—median [IQR] (range) was, respectively, 4.5 [0–20] (0–125) and 0 [0–7.5] (0–72), p=0.003.

Classification-related manifestations

Cumulatively, common clinical manifestations comprised non-erosive arthritis (81%), malar rash (57%), lymphopenia (55%), photosensitivity (50%) and proteinuria (41%), as shown in table 2.

ANA occurred in 99% of the patients and was consequently excluded in subgroup analyses. Besides ANA, 88% had a history of abnormal immunoserology with anti-dsDNA antibodies (79%) being the most prominent finding.

A hierarchical cluster analysis was performed based on the manifestations for classification listed in table 3. This analysis revealed a cluster (A) of 185 (38%) patients characterised by a relatively high prevalence of neurological disease, oral ulcers, serositis and photosensitivity; a second cluster (B) of 159 (33%) patients who were characterised by renal, haematological and immunological disorders; and a third cluster (C) of 141 (29%) patients characterised by a high prevalence of photosensitivity, malar and discoid rash as well as a low prevalence of renal and neurological disorders. The three clusters differed with respect to age at diagnosis, age at last visit, ever smoking and pack-years of smoking, but not with respect to sex, Caucasian origin or disease duration (table 3).

Association between cumulative smoking and clinical manifestations

To further investigate any differences in smoking history between clinical subsets, we tested for associations between smoking and items for SLE classification (table 2). Compared with never smoking, a history of >20 pack-years was associated with discoid rash (OR 2.22, p=0.05) and photosensitivity (OR 2.19, p=0.02), whereas haematological disorder (OR 0.40, p=0.007), renal disorder (OR 3.16, p=0.025) and non-erosive arthritis (OR 0.45, p=0.02) were inversely associated. Inverse association between a history of >20 pack-years and haematological disorder seemed driven by lymphopenia (table 2). Smoking of >20 pack-years was also associated with neurological disorder (OR 3.16, p=0.01), but since patients with neurological disorder clustered with patients who had also a relatively high prevalence of photosensitivity and discoid rash, we also performed a regression analysis that adjusted for these cutaneous manifestations, which did not change the degree of the association (OR 3.09, p=0.018).

DISCUSSION

We found that the widely acknowledged phenotype variation among patients with SLE also applied for Danish patients of largely Caucasian ancestry. However, the variation was not random as we demonstrated clustering of respectively internal and external clinical manifestations among the individual patients with SLE. As for the whole group of patients with SLE, we were able to identify at least three phenotypical subsets that respectively comprised mainly neurological, serosal and mucosal involvement (A); renal, haematological and immunological involvement (B); and skin involvement (C). Genetic variation may only explain phenotype variation to some extent,[22] and since smoking has been considered a risk factor for SLE[23] this study investigated if smoking was associated with any subsets of SLE. We did show that the two subsets with the highest degree of mucocutaneous and neurological involvement had the highest proportion of patients reporting a history of smoking. The subset with the lowest proportion of ever smokers was distinctly characterised by having the highest occurrence of renal disorder and no occurrence of neurological disorder as defined in the revised ACR 1997 classification criteria.[16]

These observations prompted further investigation of the association between smoking exposure and discrete manifestations of SLE. We observed a positive association

Table 2 Cumulative prevalence of disease manifestations used for classification by the American College of Rheumatology and their association by age and sex-adjusted ORs to strata of cumulative pack-years of smoking in 485 patients with SLE

	Cumulative prevalence, n (%)	Never smokers, reference	Ever smokers, OR (95% CI)†			P value (trend)†
		0 pack-years, n=236 (49%)	>0–10 pack-years, n=143 (30%)	>10–20 pack-years, n=49 (10%)	>20 pack-years, n=57 (11%)	
Malar rash	278 (57)	1	1.21 (0.79 to 1.85)	0.56 (0.30 to 1.05)	0.93 (0.50 to 1.72)	0.17
Photosensitivity	244 (50)	1	1.03 (0.67 to 1.58)	1.31 (0.69 to 2.49)	**2.19 (1.12 to 4.26)***	0.13
Discoid rash	51 (11)	1	0.74 (0.33 to 1.68)	1.59 (0.63 to 4.03)	**2.22 (1.00 to 4.95)***	0.10
Oral ulcers	161 (33)	1	1.22 (0.79 to 1.88)	0.82 (0.41 to 1.61)	0.76 (0.38 to 1.50)	0.51
Non-erosive arthritis	391 (81)	1	0.95 (0.54 to 1.68)	**0.31 (0.15 to 0.61)****	**0.45 (0.21 to 0.94)***	**0.003**
Serositis	172 (35)	1	0.87 (0.56 to 1.35)	1.02 (0.54 to 0.94)	0.72 (0.38 to 1.36)	0.72
Pleuritis	141 (29)	1	0.78 (0.49 to 1.24)	1.01 (0.52 to 1.96)	0.64 (0.32 to 1.28)	0.49
Pericarditis	85 (18)	1	1.00 (0.57 to 1.74)	1.17 (0.54 to 2.55)	0.83 (0.37 to 1.85)	0.93
Renal disorder	198 (41)	1	0.85 (0.55 to 1.30)	0.52 (0.26 to 1.01)	**0.40 (0.20 to 0.81)***	**0.034**
Neurological disorder	43 (9)	1	0.96 (0.42 to 2.15)	1.88 (0.70 to 5.09)	**3.16 (1.26 to 7.96)***	0.063
Seizures	33 (7)	1	0.81 (0.32 to 2.07)	1.40 (0.44 to 4.48)	2.57 (0.94 to 7.04)	0.22
Psychosis	12 (2)	1	0.96 (0.22 to 4.10)	2.33 (0.43 to 12.6)	2.60 (0.43 to 15.8)	0.60
Haematological disorder	355 (73)	1	0.78 (0.48 to 1.27)	0.62 (0.31 to 1.24)	**0.40 (0.20 to 0.77)***	**0.047**
Haemolytic anaemia	51 (11)	1	**0.47 (0.22 to 0.99)***	0.57 (0.19 to 1.71)	0.67 (0.23 to 1.91)	0.21
Leucopenia	162 (33)	1	0.86 (0.56 to 1.34)	0.72 (0.36 to 1.41)	0.55 (0.27 to 1.15)	0.38
Lymphopenia	267 (55)	1	**0.64 (0.42 to 0.98)***	**0.37 (0.19 to 0.70)****	0.63 (0.34 to 1.18)	**0.010**
Thrombocytopenia	127 (26)	1	1.13 (0.71 to 1.80)	1.16 (0.58 to 2.31)	0.74 (0.35 to 1.58)	0.73
Immunological disorder	426 (88)	1	0.91 (0.45 to 1.86)	0.51 (0.22 to 1.21)	0.78 (0.33 to 1.82)	0.48
Anti-dsDNA antibodies	384 (79)	1	1.01 (0.58 to 1.74)	**0.40 (0.20 to 0.78)***	0.68 (0.33 to 1.38)	**0.29**
Anti-Smith antibodies	59 (12)	1	1.05 (0.54 to 2.02)	1.87 (0.79 to 4.40)	1.11 (0.40 to 3.09)	0.54
Antiphospholipid antibodies	186 (38)	1	**0.61 (0.40 to 0.94)***	0.99 (0.53 to 1.85)	0.93 (0.50 to 1.72)	0.81
ANA‡	479 (99)	NA				

Values in bold are considered significant.

*P<0.05; **P<0.005.

†Logistic regression adjusting for age and sex.

‡Composite ANA: positive in the presence of either anti-dsDNA, anti-Smith or ANA (immunofluorescence or equivalent assay) at any point in time.

dsDNA, double stranded DNA; NA, not applicable.

between the number of pack-years and cutaneous manifestation, that is, photosensitivity and discoid rash. Previous studies of associations between smoking and SLE disease manifestations have found strong associations between cutaneous manifestations and smoking.[24] In a study comprising mainly patients with cutaneous types of lupus erythematosus, smoking was distinctly associated with cutaneous manifestations and not SLE.[25] In a Canadian SLE cohort, ever smoking was also found to associate with discoid rash and photosensitivity[11] but smoking did not associate with cutaneous damage as suggested by others.[15]

In this cohort, we found an association between high exposure of pack-years and neurological disorder, which seemed equally driven by psychosis and seizures. Adjusted analyses showed that this association was independent of other manifestations associated to smoking and neurological disorder such as photosensitivity and discoid rash.

Serositis was most prevalent in the patient cluster characterised by a high prevalence of neurological disorder and smoking as also reported by others,[26 27] but was not by itself associated with smoking. Smoking has also been associated to neuropsychiatric entities of organ damage as well as with general progression of damage.[28] However, we did not address aspects of damage in this study but focused on the association between smoking and defining features of SLE as we expect the SLE phenotype to be of central importance in determining what types of damage that may accrue as exemplified by the increased risk of cerebral vascular events in patients with SLE with other neuropsychiatric manifestations.[29]

Since smoking by many is considered a risk factor for SLE[13 30] and nephritis being a prominent feature of SLE, it was intriguing in this study to find a negative association between smoking >10 pack-years and renal disorder

Table 3 Demographic and smoking characteristics for three case-based clusters of 485 patients with SLE. Clusters were derived by hierarchical cluster analysis of manifestations used for classification by the American College of Rheumatology

	Cluster A n=185 (38%)	Cluster B n=159 (33%)	Cluster C n=141 (29%)	P value*
Manifestations for classification				
Malar rash, n (%)	52 (28)	89 (56)	137 (97) ↑	NA
Photosensitivity, n (%)	111 (60) ↑	49 (31)	84 (60) ↑	NA
Discoid rash, n (%)	21 (11)	7 (4)	23 (16) ↑	NA
Oral ulcers, n (%)	88 (48) ↑	26 (16)	47 (33)	NA
Arthritis, n (%)	158 (85)	120 (76)	113 (80)	NA
Serositis, n (%)	117 (63) ↑	29 (18)	28 (20)	NA
Neurological, n (%)	39 (21) ↑	0 (0)	4 (3)	NA
Renal, n (%)	58 (31)	139 (87) ↑	2 (1)	NA
Haematological, n (%)	135 (73)	128 (81) ↑	92 (65)	NA
Immunological, n (%)	164 (89) ↑	152 (96) ↑	113 (80)	NA
Resulting characteristics				
Age at last visit (years)†	49 [40–61] (20–86)	45 [35–56] (20–79)	53 [39–62] (22–86)	**0.002**
Age at diagnosis (years)†	32 [24–44] (14–83)	28 [21–39] (4.8–69)	33 [24–46] (11–77)	**0.001**
Disease duration (years)†	14 [6–23] (1–51)	15 [6–24] (0–45)	16 [7–24] (2–51)	0.77
Women, n (%)	161 (87)	137 (86)	129 (91)	0.32
Caucasian, n (%)	176 (95)	142 (89)	130 (92)	0.13
Ever smoker, n (%)	100 (54) ↑	68 (43)	81 (57) ↑	**0.03**
Pack-years (PY), n (%)				**0.002‡**
None	85 (46)	91 (57)	60 (43)	
0<PY≤10	51 (28)	44 (28)	48 (34)	
10<PY≤20	21 (11)	14 (9)	14 (10)	
20<PY	28 (15)	10 (6)	19 (14)	

↑Indicates cluster with the highest prevalence of the corresponding feature, two arrows were designated when relative difference between groups was <10%.
Values in bold are considered significant.
*X^2 or Kruskal-Wallis test as appropriate.
†Median [IQR] (range).
‡B versus A+C, Jonckheere-Terpstra test.
NA, not applicable.

as defined by persistent proteinuria according to the ACR classification criteria. As the number of pack-years was associated with increasing age and male sex, we performed adjusted analyses that furthermore indicated a dose-dependent relationship between smoking exposure and history of proteinuria. Not many studies have directly addressed this association; in one study, cigarette consumption was not associated with lupus nephritis.[31] Active or recent smoking has in ANA-positive individuals been associated with plasma cytokine levels suggestive of a proinflammatory effect of smoking.[32] However, smoking may also have various immune-suppressive effects that are mediated by reduced neutrophil and antigen-presenting activity.[33] To this end, it is of interest that we also found that history of haematological disorder, including lymphopenia, and anti-dsDNA positivity was inversely related to an increasing number of pack-years.

Studies of the association of smoking with autoantibodies such as anti-dsDNA antibodies, a serological hallmark of SLE, are conflicting. Anti-dsDNA-positive SLE has been associated with current and heavy smoking,[13 34] whereas others have not found such association.[35 36] Varying frequencies of anti-dsDNA positivity in the studies mentioned (30%–80%) may compromise direct comparison. We did not find smoking to be associated with ever presence of anti-Smith antibodies in line with a previous study.[24] Nor did we find antiphospholipid antibodies to be associated with smoking exposure; this in contrast to others.[36]

Associations between smoking exposure and various autoantibody profiles are probably just as heterogeneous as our study has demonstrated for clinical manifestations. However, associations between autoantibody production and phenotypic differentiation could be mediated by

ineffective clearance of apoptotic and necrotic cells due to smoking.[37] Indeed, it has been shown that smoking causes dose-dependent cell death signalling: lower doses cause apoptosis, whereas higher doses induce necrosis.[38] Induction of apoptosis, stimulation of T cells and enhancing of phototoxic effect of smoking may thus play important pathogenic roles for manifestations of SLE. Another smoking-related mechanism that may induce overlapping clinical and serological phenotypes is post-translational modification of autoantigens.[34]

In our population of patients with SLE, there was a clear inverse relation between smoking and arthritis which is non-erosive as defined by the ACR classification criteria. Contrary to this, smoking in subjects that have shared epitope significantly increases the risk of anti-citrullinated antibody-positive rheumatoid arthritis,[39] which is characterised by increased risk of erosive joint involvement and extra-articular manifestations. These findings may thus suggest different pathophysiological mechanisms of arthritis in these two patient populations.

A strength of this study is the relatively high number of subjects with detailed clinical and exposure data registered in the DANBIO registry that reflects Danish routine practice of patients treated for inflammatory joint and connective tissue disease with high coverage.[19 40] This enables us to register and study common and less common disease manifestations on a national level. Limitations of this study include incomplete data for some of the enrolled patients, which however did not differ with respect to demography and clinical features compared with the patients studied. Limitations also include: potential regional and temporal variations regarding used assay systems, that data on damage were not included in this report and that definitions of organ involvements were restricted to the defining items used for SLE classification, for example, the definition of neurologic manifestations by seizures and psychoses only. Our cohort comprised 92% Caucasians and associations may therefore not be generalised to non-Caucasian populations which may differ with respect to clinical and serological manifestations.[41] Our study did not contain a validation cohort but did corroborate various previous findings of associations between smoking and SLE disease manifestations. Lack of smoking data before each specific disease manifestation might also weaken the association as may potential recall bias. However, to partly address the issue of temporality, we used accumulated smoking history and disease manifestations while adjusting for age. As for our findings of inverse clinical associations to smoking, these need to be replicated also taking into account the role of medications and treatment response since smoking has been shown to negatively impact treatment response in cutaneous[42] as well as systemic lupus.[24]

Although disease mechanisms may vary between discrete disease manifestations and are not yet fully understood, the differentiated clinical associations with smoking observed by others and us may suggest biological roles for smoking in the development of specific SLE phenotypes. They do also prompt interest for specific and functional studies of potential interactions between genetics and risk exposures to address pathoimmunological implications in patients with diverse disease manifestations and potentially allow for targeted therapies, including personalised medicine, to develop and being implemented.[43] Our findings support the conceptual feasibility of such approaches although causal inferences cannot be deduced from our study.

In conclusion, our findings of differentiated associations between smoking and various subsets of patients with SLE corroborate the notion of SLE being a clinically heterogeneous disease and suggest that this should be taken into account in future studies of SLE risk factors by stratification of clinical phenotypes.

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