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EXTENDED REPORT

Cigarette smoking and the risk of systemic lupus erythematosus, overall and by anti-double stranded DNA antibody subtype, in the Nurses' Health Study cohorts

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ABSTRACT

Objectives Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease, subtyped according to clinical manifestations and autoantibodies. Evidence concerning cigarette smoking and SLE risk has been conflicting. We investigated smoking and SLE risk, overall and by anti-double stranded DNA (dsDNA) presence, in two prospective cohort studies.

Methods The Nurses' Health Study (NHS) enrolled 121 701 US female nurses in 1976; Nurses' Health Study II (NHSII) enrolled 116 430 in 1989. Lifestyle, environmental and medical data were collected through biennial questionnaires. Incident SLE was confirmed by medical record review. Cox regression models estimated HRs of SLE, overall and by dsDNA subtype, in association with time-varying smoking status and cumulative smoking pack-years through the 2-year cycle prior to diagnosis, controlling for potential confounders.

Results Among 286 SLE cases identified (159 in NHS (1978–2012) and 127 in NHSII (1991–2013)), mean age was 49.2 (10.3) years and 42% were dsDNA+ at SLE diagnosis. At baseline, 45% of women had ever smoked, 51% of whom currently smoked. Compared with never smokers, current smokers had increased dsDNA+ SLE risk (HR 1.86 (1.14–3.04)), whereas past smokers did not (HR 1.31 (0.85–2.00)). Women who smoked >10 pack-years (vs never) had an elevated dsDNA+ SLE risk (HR 1.60(95% CI 1.04 to 2.45)) compared with never smokers. No associations were observed between smoking status or pack-years and overall SLE or dsDNA– SLE.

Conclusion Strong and specific associations of current smoking and >10 pack-years of smoking with dsDNA+ SLE were observed. This novel finding suggests smoking is involved in dsDNA+ SLE pathogenesis.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease with subtypes defined by autoantibodies and clinical manifestations. Anti-double stranded DNA (dsDNA) antibodies are specific for SLE, involved in lupus nephritis pathogenesis and disease activity biomarkers.[1–4] Patients with the dsDNA positive (dsDNA+) subtype have increased risk for a more aggressive disease course, particularly with lupus nephritis and vasculitis.

SLE pathogenesis involves both genetic and environmental factors.[5] Past studies suggest smoking is related to increased SLE risk, although results are conflicting, with two prior null prospective cohort studies.[6–10] In a SLE case-only study, current smokers were more likely than never smokers to have dsDNA antibodies (OR 4.0 (95% CI 1.6 to 10.4)).[11]

We investigated smoking and risk of developing SLE and SLE subtypes according to dsDNA status among women. We hypothesised that current smokers compared with never smokers would have an increased risk of overall and dsDNA+ SLE. We evaluated smoking and other SLE-related antibody subtypes characterised by anti-Ro and/or anti-La (Ro/La), or anti-Smith (Sm) antibodies. To our knowledge, no prior study has prospectively investigated smoking and risk of incident SLE stratified by autoantibody status.

PATIENTS AND METHODS

Study population

The Nurses' Health Study (NHS) and Nurses' Health Study II (NHSII) are prospective cohorts of registered female nurses who completed a baseline and biennial questionnaires on risk factors, lifestyle, health practices and diagnoses. In 1976, NHS enrolled 121 700 nurses aged 30–55 years from 11 US states. In 1989, NHSII enrolled 116 670 nurses aged 25–42 years from 14 states. Both cohorts are predominantly White (>90%), with >90% response rates to follow-up questionnaires and 5.0% of person-time lost to follow-up.[12] Deaths are reported by family members and ascertained via National Death Index searches, with cause of death validated by medical record review.

We excluded participants who reported prevalent SLE or other connective tissue diseases (CTD) and those without smoking information on baseline questionnaires. After exclusions, 117 157 NHS participants and 113 527 NHSII participants were included.

Identification of incident SLE

SLE self-reports were confirmed by CTD screening questionnaire and medical record review by two independent rheumatologists.[13 14] SLE cases fulfilled at least four American College of Rheumatology 1997 SLE classification criteria on medical record review.[15 16] Anti-dsDNA, Sm,

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Ro, La status at SLE diagnosis was determined by medical record review.

The primary outcome was SLE, overall and by dsDNA status (including dsDNA+ or dsDNA− (dsDNA negative) SLE). As secondary outcomes, we stratified by other SLE-related antibody subtypes including: (1) dsDNA and/or Sm positive (dsDNA+/Sm+) versus dsDNA and Sm negative (dsDNA− and Sm−) SLE, (2) Ro and/or La positive (Ro+/La+) versus Ro and La negative (Ro− and La−) SLE, and (3) positivity for any SLE-related antibody (dsDNA+/Sm+/Ro+/La+ SLE) versus none of these. Too few SLE cases had only anti-Ro, La, Sm or ribonucleoprotein (RNP) at diagnosis for separate analyses.

Smoking exposure

At baseline, participants reported smoking status (never/past/current) and age of smoking initiation. Current smokers provided number of cigarettes smoked per day, whereas past smokers reported age at quitting smoking and cigarettes/day before quitting. On subsequent questionnaires, participants reported smoking status and smoking intensity (1–4, 5–14, 15–24, 25–34 or 35–44 cigarettes/day). Smoking pack-years were derived by multiplying packs per day (20 cigarettes per pack) with years smoked. All smoking variables were time varying, updated every 2 years, as smokers often stop and restart.

Assessment of covariates

Sociodemographic data included age, race/ethnicity and US Census tract-based median household income as a measure of area socioeconomic status. Updated body mass index (BMI) was reported and caloric intake was calculated from a semiquantitative food frequency questionnaire.[17] Alcohol consumption was categorised as never, >0 to <5 g/day, ≥5 g/day as in a previous analysis.[18] Reproductive covariates, including oral contraceptive use, menarche onset age, menopausal status and postmenopausal hormone use, were examined as potential confounders.[13] Missing covariate data were carried forward one cycle and if missing beyond one cycle, we included a missing data variable category.

Statistical analysis

In our primary analyses, we assessed the association between time-varying smoking status and SLE risk, overall and by dsDNA subtypes, through the 2-year cycle prior to SLE diagnosis. Person-years of follow-up accrued from return of baseline questionnaire until the 2-year cycle prior to SLE diagnosis, end of follow-up, death or date of censor, whichever came first. Participants were censored for self-reported CTD (SLE, rheumatoid arthritis (RA), scleroderma, Sjögren's syndrome, mixed CTD or inflammatory myositis) not confirmed as SLE. We carried forward the last observation up to two questionnaire cycles for missing smoking status or duration.

We examined baseline characteristics across smoking status categories by cohort. We determined cut-points for categories of continuous exposure variables non-parametrically with restricted cubic splines.[19] We used Cox proportional hazards models to assess the HRs and 95% CI for smoking status and overall SLE, dsDNA+ and dsDNA− SLE in separate models, controlling for time-varying covariates. We constructed three models for each endpoint: (1) age and questionnaire period adjusted; (2) additional adjustment for alcohol; and (3) additional adjustment for race, socioeconomic status and reproductive factors. Based on the generalised Wald test for a joint hypothesis on all covariate–time interactions in the models, the proportional hazards assumption was not violated. NHS and NHSII data were pooled. In a sensitivity analysis to evaluate the robustness of pooling the data, HR estimates from the two cohorts were meta-analysed using a fixed effects model.

We conducted several secondary analyses. First, we investigated cumulative smoking in pack-years and risk of SLE and dsDNA subtypes. Second, we cross-classified smoking status and pack-years and examined SLE risk overall and by dsDNA. Third, we separately evaluated the associations of smoking intensity (collapsed to >0 to <15 or ≥15 cigarettes/day) and duration (≥20 years or <20 years) with SLE risk. Fourth, we conducted a 'lagged analysis' in which the exposure window ended two questionnaire cycles (at least 4 years) prior to the outcome window, as SLE may develop insidiously prediagnosis and illness could change smoking behaviour. Fifth, we examined smoking cessation. Lastly, we investigated the association between time-varying smoking and SLE with other autoantibody subtypes.

Data analyses were performed using SAS V.9.3 (SAS Institute). The Partners' HealthCare Institutional Review Board approved all aspects of this study.

RESULTS

Among 230 672 women with 5.6 million person-years of follow-up, we identified 286 incident SLE cases: 159 SLE cases in NHS and 127 in NHSII. Average annual SLE incidence rates in each cohort were 4.9 per 100 000 person-years for NHS and 5.3 per 100 000 person-years for NHSII, as expected for predominantly White women aged ≥25 years at cohort entry. At baseline, 45% of women in both cohorts were ever smokers, of whom 51% were current smokers. Table 1 displays age-adjusted baseline characteristics of study participants categorised by smoking status. Age, race, caloric intake, BMI, postmenopausal status, postmenopausal hormone use and early menarche were similar across smoking categories within each cohort. Alcohol consumption was higher among smokers than non-smokers. Most current smokers had smoked >10 pack-years, although women in NHS were heavier smokers than those in NHSII.

The presenting manifestations at SLE diagnosis, overall and by dsDNA subtype, are shown in table 2. Of the 286 incident SLE cases, 42% were dsDNA+ at diagnosis. Mean age at SLE diagnosis was 49.2 years (SD 10.3). There were more non-Whites in the dsDNA+ (12.6%) versus dsDNA− (6.1%) subgroup. Among women with dsDNA+ SLE, there were lower rates of arthritis (65.3% vs 79.4%), higher rates of haematological involvement (65.3% vs 53.3%) and similar rates of renal involvement (16.5% vs 16.4%) compared with dsDNA− SLE in records reviewed around the time of SLE diagnosis.

Among SLE cases, the largest proportion of past and current smokers smoked 15–24 cigarettes/day (34.4% and 37.5%). Mean smoking duration among SLE cases was greater for current than past smokers (26.4 (SD 8.9) vs 16.1 (SD 10.8) years). Among SLE cases, mean time since quitting among past smokers was 16.8 (SD 12.8) years. The mean age at SLE diagnosis was similar between dsDNA+ SLE (51.0 (SD 10.0) years) compared with dsDNA− SLE (50.9 (SD 11.3) years), yielding a nearly identical interval between age at smoking initiation among SLE ever-smokers (18.4 (SD 3.7) years) and age at SLE diagnosis for dsDNA+ and dsDNA− SLE cases.

No significant risk was observed among past or current smokers (vs never smokers) for SLE overall or dsDNA− SLE risk (table 3). However, current smoking was associated with a strongly increased risk of dsDNA+ SLE after age and sex adjustment (HR 1.77 (95% CI 1.09 to 2.88)) and additional

Table 1 Baseline age-standardised characteristics of participants in the Nurses' Health Study (NHS) in 1976 and Nurses' Health Study II (NHSII) in 1989 categorised by smoking status

	NHS (n=117 145)			NHSII (n=113 527)		
Characteristics	**Never**	**Past**	**Current**	**Never**	**Past**	**Current**
Number of participants (%)	51 655 (44.1)	26 889 (23.0)	38 601 (33.0)	74 166 (65.3)	24 152 (21.3)	15 209 (13.4)
Mean age in years (SD)*	42.4 (7.4)	42.6 (7.1)	42.4 (7.1)	34.0 (4.7)	35.2 (4.5)	34.8 (4.6)
White race (%)	92	94	94	91	94	93
Median income ≥$60 000 (%)†	46	53	49	43	50	40
Mean calorie intake (kcal/day, SD)	1588 (502)	1553 (488)	1546 (510)	1799 (547)	1783 (542)	1753 (559)
Mean body mass index (kg/m^2, SD)	24.1 (4.3)	23.9 (4.3)	23.2 (3.9)	24.1 (5.1)	24.1 (5.0)	24.1 (5.0)
Smoking in pack-year categories						
0 (%)	100	0	0	100	0	0
>0 to ≤10 (%)	0	58	20	0	69	36
>10 (%)	0	42	80	0	31	64
Oral contraceptive use, ever (%)	45	49	49	81	89	89
Postmenopausal (%)	31	30	34	6	6	8
Any postmenopausal hormone use (%)	13	14	15	3	3	4
Early menarche (≤10 years) (%)	6	6	6	8	8	9
Alcohol use in categories (g/day) (%)‡						
None	33	19	19	43	28	28
>0 to <5	27	27	24	42	43	40
≥5	19	34	32	15	28	32

Means (SD) or percentages, age standardised to distribution of study population.
*Not age standardised.
†Zip code-level median household income from the US Census.
‡Cumulative average daily alcohol consumption.
g/day, grams per day; kcal/day, kilocalories per day.

adjustment for alcohol use (HR 1.91 (95% CI 1.17 to 3.12)). This risk remained significant in the multivariable (MV) model (HR 1.86 (95% CI 1.14 to 3.04)). Meta-analysing HRs from the two cohorts produced similar results for current versus never smoking (MV-adjusted HR for dsDNA+ SLE 1.81 (95% CI 1.10 to 2.96), Q value=0.01 with p=0.94, Tau^2=0), and no association with overall SLE or dsDNA− SLE. In a 'lagged' analysis allowing 4 years before SLE diagnosis, the risk of dsDNA+ SLE was potentially even more elevated among current versus never smokers (MV-adjusted HR 1.93 (95% CI 1.17 to 3.18)).

In secondary analyses, we examined smoking in pack-years (table 4). Based on the results of the restricted cubic splines, we defined pack-years using an ordinal variable (0 pack-years, >0 to ≤10 pack-years, >10 pack-years). Although no significant association for smoking in pack-years and risk of overall SLE or dsDNA− SLE was demonstrated, women who smoked >10 pack-years had a significantly elevated risk of dsDNA+ SLE (HR 1.60 (95% CI 1.04 to 2.45), p trend 0.04) compared with never smokers. In an analysis cross-classifying smoking status with pack-years, current smokers who smoked >10 pack-years had a potential 67% increased risk of dsDNA+ SLE (HR 1.67 (95% CI 0.98 to 2.85), p trend 0.07 across pack-year categories), but no increased risk of SLE overall (HR 1.05 (95% CI 0.72 to 1.51), p trend 0.81). No association was demonstrated between increased pack-years and all SLE or dsDNA+ SLE among past smokers.

Among current smokers, increasing smoking intensity (≥15 vs >0 to <15 cigarettes/day) was not associated with increased dsDNA+ SLE risk after MV adjustment (p=0.38). However, among current smokers, increasing smoking duration was related to increased dsDNA+ SLE risk (MV HR 1.85 (95% CI 1.09 to 3.13)) for those continuing to smoke for ≥20 years compared with never smokers. No association was demonstrated for increasing smoking duration and overall or dsDNA− SLE, or among past smokers.

Among past smokers, no association between time since quitting and risk of SLE or dsDNA− SLE was found. However, after quitting smoking for >5 years, the risk of dsDNA+ SLE was no longer significantly elevated (HR 1.11 (95% CI 0.69 to 1.79) vs never smokers), demonstrating a significant threshold in risk reduction at >5 years (figure 1).

Current smoking, but not past smoking (compared with never smoking), was associated with a significantly increased risk of dsDNA+/Sm+ SLE (HR 1.87 (95% CI 1.14 to 3.06)) and dsDNA+/Sm+/Ro+/La+ SLE (HR 1.84 (95% CI 1.15 to 2.93)). However, no association was demonstrated between current or past smoking (vs never smoking) and other SLE subtypes identified by autoantibody profiles (table 5).

Table 2 Characteristics of participants at SLE diagnosis in Nurses' Health Study and Nurses' Health Study II by anti-double stranded DNA (dsDNA) antibody status

Characteristics at SLE diagnosis	**Overall SLE (n=286)**	**dsDNA+ SLE (n=121)**	**dsDNA− SLE (n=165)**
Mean age at diagnosis, years (SD)	49.2 (10.3)	49.9 (9.6)	48.7 (10.8)
White race (%)	91.6	88.4	93.9
Antinuclear antibody positive (%)	97.6	98.4	97.0
Arthritis (%)	73.4	65.3	79.4
Haematological involvement (%)	58.4	65.3	53.3
Renal involvement (%)	16.4	16.5	16.4
Mean number of ACR SLE criteria met (SD)	4.9 (1.1)	5.2 (1.2)	4.7 (0.9)
Diagnosed by ACR member rheumatologist (%)	79.0	76.0	81.2

ACR, American College of Rheumatology; dsDNA+, double stranded DNA positive; dsDNA−, double stranded DNA negative; SLE, systemic lupus erythematosus.

Table 3 Association between cigarette smoking status and risk of incident SLE among participants in Nurses' Health Study and Nurses' Health Study II, overall and by anti-double stranded DNA (dsDNA) antibody status

	Cigarette smoking status		
	Never	Past	Current
Overall SLE			
Cases/person-years	148/3 074 178	90/1 759 984	48/808 162
Age-adjusted HR (95% CI)*	1.00 (ref)	1.12 (0.86 to 1.47)	1.07 (0.77 to 1.50)
Alcohol-adjusted HR (95% CI)†	1.00 (ref)	1.22 (0.93 to 1.60)	1.17 (0.8 to 1.65)
Multivariable-adjusted HR (95% CI)‡	1.00 (ref)	1.18 (0.89 to 1.55)	1.14 (0.81 to 1.61)
dsDNA+ SLE			
Cases/person-years	56/3 073 263	39/1 759 395	26/807 828
Age-adjusted HR (95% CI)*	1.00 (ref)	1.29 (0.85 to 1.95)	**1.77 (1.09 to 2.88)**
Alcohol-adjusted HR (95% CI)†	1.00 (ref)	1.37 (0.89 to 2.09)	**1.91 (1.17 to 3.12)**
Multivariable-adjusted HR (95% CI)‡	1.00 (ref)	1.31 (0.85 to 2.00)	**1.86 (1.14 to 3.04)**
dsDNA– SLE			
Cases/person-years	92/3 073 468	51/1 759 406	22/807 827
Age-adjusted HR (95% CI)*	1.00 (ref)	1.02 (0.72 to 1.45)	0.72 (0.44 to 1.16)
Alcohol-adjusted HR (95% CI)†	1.00 (ref)	1.13 (0.79 to 1.61)	0.79 (0.49 to 1.29)
Multivariable-adjusted HR (95% CI)‡	1.00 (ref)	1.09 (0.76 to 1.56)	0.76 (0.47 to 1.24)

p for heterogeneity between the cohorts >0.05 for all analyses.
*Adjusted for age (months), questionnaire cycle, cohort.
†Additionally adjusted for alcohol intake (never, >0 to <5 g/day, ≥5 g/day).
‡Additionally adjusted for race (White vs non-White), body mass index in WHO categories (18.5 to <25, 25 to <30, ≥30), zip code-level median household income from US Census (≥60 000 vs <60 000), oral contraceptive use (ever/never), age at menarche (≤10 vs >10 years), menopausal status and postmenopause hormone (PMH) use (premenopausal, postmenopausal/never used PMH, postmenopausal/ever used PMH).
Bold numbers meet statistical significance threshold of p<0.05.
dsDNA+, double stranded DNA positive; dsDNA–, double stranded DNA negative; SLE, systemic lupus erythematosus; WHO, World Health Organization.

DISCUSSION

In these large prospective cohorts of women followed for many years prior to SLE onset, we found a strong and specific association between smoking and dsDNA+ SLE. While no association was seen between smoking and risk of overall SLE, dsDNA+ SLE risk was increased nearly twofold among current smokers and by 60% among women who smoked >10 pack-years, compared with never smokers. Risks of dsDNA+/Sm+ and dsDNA+/Sm+/Ro+/La+ SLE were similarly elevated among current smokers. Among current smokers, dsDNA+ SLE risk was nearly doubled after smoking ≥20 years and we found a significant reduction in dsDNA+ SLE risk after quitting smoking

Table 4 Association between cigarette smoking in pack-years and risk of incident SLE among participants in Nurses' Health Study and Nurses' Health Study II, overall and by anti-double stranded DNA (dsDNA) antibody status

	Pack-years			
	Never smoker	>0 to ≤10	>10	p Trend
Overall SLE				
Cases/person-years	148/3 074 178	52/1 032 876	86/1 535 233	
Age-adjusted HR (95% CI)*	1.00 (ref)	1.03 (0.75 to 1.41)	1.16 (0.88 to 1.54)	0.28
Alcohol-adjusted HR (95% CI)†	1.00 (ref)	1.11 (0.81 to 1.54)	1.27 (0.96 to 1.68)	0.10
Multivariable-adjusted HR (95% CI)‡	1.00 (ref)	1.09 (0.79 to 1.51)	1.22 (0.92 to 1.61)	0.18
dsDNA+ SLE				
Cases/person-years	56/3 073 263	24/1 032 491	41/1 534 731	
Age-adjusted HR (95% CI)*	1.00 (ref)	1.27 (0.78 to 2.05)	**1.57 (1.04 to 2.39)**	**0.04**
Alcohol-adjusted HR (95% CI)†	1.00 (ref)	1.35 (0.83 to 2.20)	**1.68 (1.10 to 2.58)**	**0.02**
Multivariable-adjusted HR (95% CI)‡	1.00 (ref)	1.32 (0.81 to 2.16)	**1.60 (1.04 to 2.45)**	**0.04**
dsDNA– SLE				
Cases/person-years	92/3 073 468	28/1 032 494	45/1 534 739	
Age-adjusted HR (95% CI)*	1.00 (ref)	0.88 (0.57 to 1.35)	0.93 (0.64 to 1.35)	0.75
Alcohol-adjusted HR (95% CI)†	1.00 (ref)	0.97 (0.63 to 1.49)	1.03 (0.70 to 1.50)	0.87
Multivariable-adjusted HR (95% CI)‡	1.00 (ref)	0.94 (0.61 to 1.46)	0.98 (0.67 to 1.44)	0.96

p for heterogeneity between the cohorts >0.05 for all analyses.
*Adjusted for age (months), questionnaire cycle, cohort.
†Additionally adjusted for alcohol intake (never, >0 to <5 g/day, ≥5 g/day).
‡Additionally adjusted for race (White vs non-White), body mass index in WHO categories (18.5 to <25, 25 to <30, ≥30), zip code-level median household income from US Census (≥60 000 vs <60 000), oral contraceptive use (ever/never), age at menarche (≤10 vs >10 years), menopausal status and postmenopause hormone (PMH) use (premenopausal, postmenopausal/never used PMH, postmenopausal/ever used PMH).
Bold numbers meet statistical significance threshold of p<0.05
dsDNA+, double stranded DNA positive; dsDNA–, double stranded DNA negative; SLE, systemic lupus erythematosus; WHO, World Health Organization.

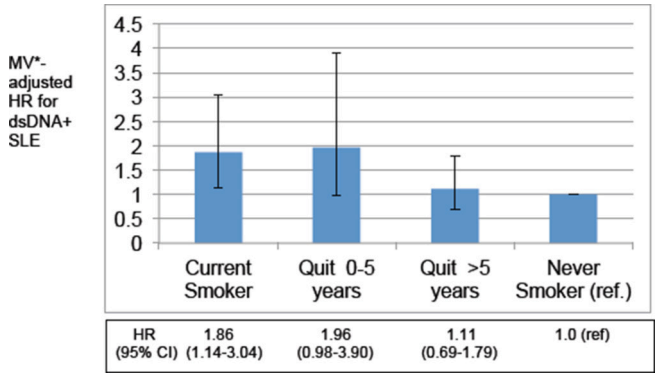

Figure 1 Association of smoking cessation and risk of anti-double stranded DNA positive (dsDNA+) SLE among participants in Nurses' Health Study and Nurses' Health Study II. p for heterogeneity between the cohorts >0.05 for all analyses. *Adjusted for age (months), questionnaire cycle, cohort, alcohol intake (never, >0 to <5 g/day, ≥5 g/day), race (White vs. non-White), body mass index in WHO categories (18.5 to <25, 25 to <30, ≥30) , zip code-level median household income from U.S. census (≥60,000 versus <60,000), oral contraceptive use (ever/never), age at menarche (≤10 vs. >10 years), menopausal status and post-menopause hormone (PMH) use (pre-menopausal, post-menopausal/never used PMH, post-menopausal/ever used PMH). CI, confidence interval; HR, hazard ratio; MV, multivariable; SLE, systemic lupus erythematosus.

for >5 years. Thus, we found positive short-term risk using time-varying updated smoking status and long-term risk using cumulative cigarette smoking in pack-years over up to 37 years. This is the largest and longest prospective study to investigate SLE risk using repeated measures of smoking exposure. These studies newly describe a specific association between current smoking and the subtype of SLE characterised by dsDNA antibodies.

Our findings are consistent with and extend prior studies. Although epidemiologic studies of smoking and SLE risk have been somewhat conflicting,[8 20 21] our earlier meta-analysis of seven case–control and two cohort studies demonstrated elevated SLE risk among current smokers (OR 1.5, 95% CI 1.09 to 2.08) compared with non-smokers, but not past smokers (OR 0.98, 95% CI 0.75 to 1.27).[22] Since then, additional case–control studies have demonstrated an elevated SLE risk among smokers compared with never smokers.[6–8 23] Prior studies included heterogeneous groups with varying race/ethnicities—with no association for smoking and SLE among black women,[10] a significantly increased risk among predominantly Hispanic smokers[20] and varied risks among Asian subgroups.[6 8]

Several case–control studies have reported dose–response relationships for SLE risk with increasing pack-years.[8 21 24] Two past prospective cohort studies, the NHS (1996) and the Black Women's Health Study (BWHS, 2003), did not demonstrate significant associations between smoking and SLE risk.[9 10] Both cohorts were limited at the time by small sample size, one-time baseline assessment of exposure in BWHS and short exposure duration.

In a recent case–control study, current smoking was associated with presence of ≥1 SLE-related autoantibody (OR 1.53 (95% CI 1.04 to 2.24)) and an increased rate of anti-RNP A positivity among patients with SLE, whereas former smoking was associated with increased risk of anti-Ro positivity among unaffected first-degree relatives.[25] Although our study was underpowered to evaluate the risk of all SLE-related antibody subtypes individually, our results demonstrate a strong association between current smoking and dsDNA+/Sm+ and dsDNA+/Sm+/Ro+/La+ SLE subtypes. Anti-dsDNA+ SLE may also be a more homogeneous and severe phenotype than dsDNA− SLE, possibly explaining the stronger association with smoking.

Epidemiologic evidence suggests that tobacco smoke exposure is associated with other autoimmune diseases such as RA, Graves' disease and primary biliary cirrhosis.[26–30] Notably, our findings parallel RA studies demonstrating an association between smoking and increased risk of seropositive RA (with rheumatoid factor and/or anti-cyclic citrullinated peptide antibodies), but not seronegative RA.[29 31] We have previously demonstrated increased risk of seropositive RA among both current (relative risk (RR) 1.58 (1.21–2.06)) and past smokers (RR 1.60 (1.27–2.02)), and with ≥10 pack-years of smoking, as well as with increased smoking duration and intensity compared with never smokers.[29] However, whereas RA risk remained elevated until 20 years after smoking cessation,[29] here we find dsDNA+ SLE risk was reduced after >5 years of smoking cessation.

Our results suggest a biological role for smoking in the development of dsDNA+ SLE, although the mechanistic basis is not yet understood. Exposures to toxic components from cigarette smoke (eg, tars, nicotine, carbon monoxide, polycyclic aromatic hydrocarbons and free radicals) induce oxidative stress, damage endogenous proteins and DNA, and lead to genetic mutations and gene activation.[32] Toxic smoke components also induce epigenetic changes, resulting in altered gene expression affecting immunity[33 34] and production of proinflammatory cytokines including tumour necrosis factor-α and interleukin-6.[35 36] Smoking also stimulates surface expression of CD95 on B and CD4+ T cells, potentially leading to ineffective clearing of apoptotic neutrophils and dsDNA autoantibody production.[37 39] Reactive oxygen species from tobacco damage DNA, forming immunogenic DNA adducts, which may result in dsDNA antibody production.[26 27] As in many tobacco-induced complex diseases, genetic background likely plays a role in whether a smoker will develop dsDNA antibodies and SLE. In a past case–control study, the cytochrome P450 1A1 rs4646903 and glutathione S-transferase M1 deletion genotypes, both involved in detoxification pathways, were associated with greatly increased SLE risk among smokers (OR 17.5 (95% CI 3.20 to 95.9)).[40] Our study was not designed to investigate disease mechanisms, and future research investigating gene–environment interactions and epigenetic modifications is warranted.

A major strength of the current study is the use of two large cohorts with over 5.6 million person-years of prospective follow-up. Detailed exposure data updated every 2 years allowed for evaluation of smoking status, cumulative smoking in pack-years, duration, intensity and time since quitting, enhancing precision and reducing the likelihood of misclassification of exposure, within-subject variation and recall biases. Autoantibody status was assessed at SLE diagnosis, minimising the possibility that SLE-specific antibodies may have normalised after drug treatment. Furthermore, our 'lagged' analysis demonstrated a potentially greater risk of current smoking for incident dsDNA+ SLE, suggesting that smokers may quit in the years immediately preceding SLE diagnosis. Our stringent method for SLE classification along with identification of SLE-associated antibodies increased the likelihood that identified cases were truly SLE.

Given our stringent definition of SLE, we may have excluded possible SLE cases upon medical record review that later may have become more clinically apparent. As we assessed dsDNA, Sm, Ro, La seropositivity at SLE diagnosis, cases that later developed these antibodies may have been misclassified as being negative. However, given that SLE-related antibodies become

Table 5 Association between cigarette smoking status and risk of incident systemic lupus erythematosus (SLE) among participants in Nurses' Health Study and Nurses' Health Study II, overall and by SLE autoantibody subtypes

	Cigarette smoking status		
	Never	Past	Current
dsDNA+/Sm+ SLE			
Cases/person-years	56/3 073 179	40/1 759 315	26/807 775
Age-adjusted HR (95% CI)*	1.00 (ref)	1.31 (0.87 to 1.98)	**1.77 (1.09 to 2.88)**
Multivariable-adjusted HR (95% CI)†	1.00 (ref)	1.33 (0.87 to 2.03)	**1.87 (1.14 to 3.06)**
dsDNA– and Sm– SLE			
Cases/person-years	92/3 073 375	50/1 759 273	22/807 765
Age-adjusted HR (95% CI)*	1.00 (ref)	1.00 (0.71 to 1.43)	0.72 (0.44 to 1.16)
Multivariable-adjusted HR (95% CI)†	1.00 (ref)	1.07 (0.75 to 1.54)	0.76 (0.47 to 1.24)
Ro+/La+ SLE			
Cases/person-years	19/3 072 720	15/1 759 053	3/807 523
Age-adjusted HR (95% CI)*	1.00 (ref)	1.37 (0.68 to 2.75)	0.85 (0.25 to 2.94)
Multivariable-adjusted HR (95% CI)†	1.00 (ref)	1.42 (0.69 to 2.91)	0.85 (0.25 to 2.94)
Ro– and La– SLE			
Cases/person-years	129/3 073 859	75/1 759 629	45/808 032
Age-adjusted HR (95% CI)*	1.00 (ref)	1.08 (0.81 to 1.45)	1.09 (0.77 to 1.55)
Multivariable-adjusted HR (95% CI)†	1.00 (ref)	1.14 (0.85 to 1.54)	1.18 (0.82 to 1.68)
dsDNA+/Sm+/Ro+/La+ SLE			
Cases/person-years	63/3 073 307	48/1 759 419	28/807 833
Age-adjusted HR (95% CI)*	1.00 (ref)	1.39 (0.94 to 2.03)	**1.75 (1.10 to 2.78)**
Multivariable-adjusted HR (95% CI)†	1.00 (ref)	1.41 (0.95 to 2.09)	**1.84 (1.15 to 2.93)**
dsDNA– and Sm– and Ro– and La– SLE			
Cases/person-years	85/3 073 202	42/1 759 200	20/807 715
Age-adjusted HR (95% CI)*	1.00 (ref)	0.92 (0.63 to 1.35)	0.67 (0.41 to 1.11)
Multivariable-adjusted HR (95% CI)†	1.00 (ref)	0.99 (0.67 to 1.45)	0.72 (0.43 to 1.20)

p for heterogeneity between the cohorts >0.05 for all analyses.
*Adjusted for age (months), questionnaire cycle, cohort.
†Additionally adjusted for alcohol intake (never, >0 to <5 g/day, ≥5 g/day), race (White vs non-White), body mass index in WHO categories (18.5 to <25, 25 to <30, ≥30) , zip code-level median household income from US Census (≥60 000 vs <60 000), oral contraceptive use (ever/never), age at menarche (≤10 vs >10 years), menopausal status and postmenopause hormone (PMH) use (premenopausal, postmenopausal/never used PMH, postmenopausal/ever used PMH).
Bold numbers meet statistical significance threshold of p<0.05
dsDNA, anti-double stranded DNA antibodies; dsDNA+/Sm+, dsDNA and/or Sm positive; dsDNA+/Sm+/Ro+/La+, dsDNA and/or Sm and/or Ro and/or La positive; dsDNA–/Sm–/Ro–/La–, dsDNA and Sm and Ro and La negative; dsDNA– and Sm–, dsDNA and Sm negative; La, anti-La antibodies; Ro, anti-Ro antibodies; Ro+/La+, Ro and/or La positive; Ro– and La–, Ro and La negative; Sm, anti-Smith antibodies; WHO, World Health Organization.

positive years before diagnosis,[41] this misclassification was likely uncommon. Furthermore, as NHS/NHSII enrolled women between the ages of 25 and 55, our study may not have captured early-onset SLE. Additionally, given that the NHS cohorts include mostly healthy, White US women working in advanced nursing professions, there is a potential lack of generalisability to younger women, men and non-Whites. It is not known whether the association between smoking and dsDNA+ SLE may vary by sex, age or race/ethnicity.[20]

This study demonstrates a strong and specific association between current smoking and risk of dsDNA+ SLE, a severe subtype of SLE. Current smoking and smoking >10 pack-years were associated with increased risk of dsDNA+ SLE, and SLE subtypes characterised by dsDNA+/Sm+ or dsDNA+/Sm+/Ro+/La+. Further studies may be able to assess the association between smoking and SLE with individual autoantibodies, although this may be challenging as they are highly intercorrelated. Smoking cessation was shown to reduce dsDNA+ SLE risk to that of non-smokers after 5 years, suggesting that dsDNA+ SLE risk is modifiable. These findings have implications for SLE prevention efforts using personalised strategies for risk stratification and modification. They also demonstrate the importance of studying specific SLE subtypes and provide insight into potential mechanisms of disease pathogenesis warranting further research.

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Contributors MB and KHC designed the study, analysed the data and wrote the first draft. SM and BL contributed to the data analysis. MB, SKT, SM, DK, JAS, EWK and KHC were involved in data collection. All authors were involved in drafting the article and revising it critically for important intellectual content, and all authors approved the final version to be published. MB and KHC had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Cigarette smoking and the risk of systemic lupus erythematosus, overall and by anti-double stranded DNA antibody subtype, in the Nurses' Health Study cohorts

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