Smoking associates with increased BAFF and decreased interferon-γ levels in patients with systemic lupus erythematosus

Warren David Raymond,1 Matthew Hamdorf,1 Michael Furfaro,1 Gro Oсти Eilertsen,2 Johannes Cornelis Nossent1,2

ABSTRACT

Objective In SLE, smoking increases the burden of cutaneous disease and organ damage, and leads to premature mortality. However, the effect of smoking on disease manifestations and cytokine levels of patients with SLE is unclear. This study compared characteristics of patients with SLE across smoking status, and determined the association of smoking with serum cytokine levels.

Method A cross-sectional study of patients with SLE (n=99) during a research visit in which smoking status was ascertained. Smoking status was compared across classification criteria (American College of Rheumatology Classification Criteria for SLE (ACR97), disease activity (SLE Disease Activity Index), autoantibody levels, accrued damage (Systemic Lupus International Collaborating Clinics/ACR Damage Index), and circulating concentrations of serum interferon-gamma (IFN-γ), interleukin (IL)-1β, IL-4, IL-6, IL-10, IL-12, IL-17, B cell-activating factor (BAFF), tumour necrosis factor-alpha, transforming growth factor beta 1 (TGF-β1), macrophage inflammatory protein 1 alpha (MIP-1α), MIP-1β and monocyte chemoattractant protein 1. Linear regression models determined the association between smoking and cytokine levels, adjusting for age and sex, clinical characteristics (model 1), and anti-inflammatory (IL-4, IL-10 and TGF-β1) and regulatory (IL-1β) cytokines (model 2).

Results Among patients with SLE (97.9% ANA+; mean 48.48 years old; 86.9% female; mean 10 years of disease duration), 35.4% (n=35 of 99) were smoking (an average of 7 cigarettes/day for 24 years). Smokers had increased odds of prevalent ACR97 malar rash (OR 3.40, 95% CI 1.23 to 9.34) and mucosal ulcers (OR 3.31, 95% CI 1.19 to 8.60), Raynaud’s phenomenon (OR 5.15, 95% CI 1.95 to 13.56) and increased non-steroidal anti-inflammatory drug use (OR 6.88, 95% CI 1.99 to 23.72). Smoking associated with 27% increased BAFF levels (95% CI 4.8% to 48%) and 42% decreased IFN-γ levels (95% CI –79% to −5%) in model 2.

Conclusion In patients with SLE, smoking independently associated with increased BAFF and decreased IFN-γ levels, and an increased frequency of arthritis, migraine and Raynaud’s phenomenon. Smoking cessation is advisable to reduce systemic inflammation, reduce disease activity and improve host defence.

INTRODUCTION

SLE is the prototypical autoimmune disease characterised by chronic, multisystem inflammation.1 Patients experience an unpredictable disease course, which leads to end-stage organ damage and premature mortality.1,2 Epidemiological evidence suggests that smoking contributes to the development of ANA and...
dsDNA autoantibody formation, which are central to the pathogenesis of SLE, particularly lupus nephritis (LN)\textsuperscript{3}, and smoking has been linked to an increased risk of developing a range of autoimmune diseases, including SLE.\textsuperscript{4,4} In patients with SLE, smoking is linked to cutaneous manifestations,\textsuperscript{7} damage accrual, including the earlier onset of end-stage renal disease in those with LN,\textsuperscript{8} and premature mortality.\textsuperscript{9,10} However, the association between smoking and SLE disease activity is relatively understudied.\textsuperscript{11}

Cigarette smoke exposes the epithelial cells of the larynx, bronchi and lung to more than 60 chemical carcinogens, which each has the potential to cause DNA damage.\textsuperscript{12} Furthermore, smoking has been shown to increase and decrease many proinflammatory and anti-inflammatory cytokines in the general population, with\textsuperscript{13} and without SARS-CoV-2 (COVID-19),\textsuperscript{14} and in a cohort with Sjogren’s syndrome.\textsuperscript{15} Yet, there are (very) limited data on the impact of smoking on serum cytokines in patients with SLE, especially in the context of medication use and organ damage.\textsuperscript{1} In SLE, cytokines such as B-cell activating factor (BAFF),\textsuperscript{16} transforming growth factor beta 1 (TGF-β1),\textsuperscript{17} and interferons (IFNs)\textsuperscript{18} are associated with disease activity and severity. Therefore, if smoking were to exacerbate abnormal levels of these cytokines, it would have implications on the treatment and management of SLE. Thus, in this study, we aimed to describe the impact of smoking with a range of clinical, serological and immunological characteristics in a cohort of patients with SLE; and to determine the association between smoking and cytokine levels adjusting for age, sex, medication use, disease activity and organ damage.

**METHOD**

This is a cross-sectional study of 99 patients with SLE fulfilling the American College of Rheumatology’s (ACR) classification criteria for SLE.\textsuperscript{19,20} Smoking was defined as the current consumption of either prefabricated or hand-rolled cigarettes at the research visit. Ex-smokers (median 10 years since quitting and a median 3.5 pack-year exposure) were counted as non-smoking. This was done to align with the stronger association of current smoking rather than ex-smoking or non-smoking behaviours with disease activity and severity in patients with SLE.\textsuperscript{21,22}

Clinical data including disease activity and laboratory results were collected at a research visit, where sera taken were stored within 2 hours at −70°C. Cytokine assays were performed for all participants at the same time. Serological, immunological and biochemical levels were measured in an accredited laboratory on samples taken at the time of research visit. Disease activity was recorded using the SLE Disease Activity Index-2K (SLEDAI-2K).\textsuperscript{23} We calculated Lupus Low Disease Activity State (LLDAS) scores over time,\textsuperscript{24} and presented these data as either >30%, >50%, >70% of their time spent in an LLDAS as per Sharma et al.\textsuperscript{25}

Damage accrual was captured prospectively for each participant with the Systemic Lupus International Collaborating Clinics/ACR Damage Index for SLE (SDI) during a median follow-up of 10 years from date of SLE diagnosis through to the research visit.\textsuperscript{26} Medication data were collected at the research visit, which documented the use of prednisone (oral or intravenous methylprednisone), cytostatic agents (azathioprine, cyclophosphamide or mycophenolate) or immunomodulators (hydroxchloroquine or rituximab).

Cytokines (IFN-gamma (IFN-γ), interleukin (IL)-1β (IL-1β), IL-4, IL-6, IL-10, IL-17A, BAFF, macrophage inflammatory protein 1 alpha (MIP-1α) (patients with SLE only), MIP 1 beta (MIP-1β) (patients with SLE only), monocyte chemoattractant protein 1 (MCP-1), tumour necrosis factor-alpha (TNF-α) and TGF-β1) were measured in stored (−70°C) serum samples by a quantitative sandwich immunoassay (Single Analyte ELISAarray kit; SuperArray Bioscience Corp, Frederick, Maryland, USA). All assays were run in duplicate and the results averaged. The manufacturer’s recommendations were followed throughout, and the same lot was used for each cytokine. For statistical purposes, values below the limit of detection (LOD) were replaced by the LOD value.

**Statistical methods**

Continuous clinical, serological, and immunological variables are described as a mean±SD or median and IQR depending on the data distribution. Continuous data were compared across smoking status with t-test, Mann-Whitney U test, one-way analysis of variance (ANOVA) or Kruskal-Wallis test. Categorical clinical, serological, and immunological variables are described as frequency (n) and proportion (%), and compared across smoking status with logistic regression (presented as an OR with 95% CIs or a Fisher’s exact test). Cytokine levels were described with a geometric mean and 95% CI after undergoing log-transformed to improve normality and then back-transformed with Euler’s number.

The associations between smoking and proinflammatory cytokines were determined using Spearman correlation coefficients (Rs) and age-adjusted and sex-adjusted linear regression models. Additional multiple regression modelling of the association between smoking and cytokine levels included clinical characteristics (age, sex, prednisone use, immunosuppressant use, SLEDAI score and SDI score) which are known to influence cytokine levels (model 1). Finally, we performed an ad-hoc multiple regression model which determined the association of smoking with serum BAFF, IFN-γ, IL-12, IL-17, TNF-α or MIP-1β, adjusted for model 1 plus anti-inflammatory cytokines (IL-4, IL-10, TGF-β1) and the regulatory cytokine (IL-1β). This was to determine whether the minimally adjusted association(s) between smoking and cytokine levels held after accounting for known lower TGF-β1 levels in patients with SLE,\textsuperscript{17} and to account for the
immunosuppressive effects of smoking on key regulatory and anti-inflammatory cytokines IL-1β, IL-4 and IL-10.27–30

RESULTS

Clinical characteristics of patients with SLE at the research visit compared across smoking status

At the research visit, patients with SLE (97.9% ANA+) were on average 48 years old, 86.9% female and had been followed up for an average of 10 years. At the research visit, 35.4% (n=35 of 99) were currently smoking (average of 9 cigarettes per day for 15 years), with cessation occurring on average 10 years prior to the research visit. Current smokers had a significantly higher pack-year smoking exposure than ex-smokers (9 vs 3.5 pack-years, p=0.003). Within current smokers, 57.1% (n=20 of 35) had a <10 pack-year smoking exposure. A total of 34.3% (n=12 of 35) had a 10–20 pack-year smoking exposure and 8.6% (n=3 of 35) had a ≥20 pack-year smoking exposure. Current and non-smokers had an equivalent burden of comorbid vascular, pulmonary, malignancy and metabolic pathology, as well as accrued damage (SDI >0) and total SDI scores. However, patients with any smoking exposure (current and/or ex-smokers) had higher accrued damage (69.0% vs 46.3%; OR 2.57, 95% CI 1.12 to 5.89; p=0.025) and malignancy (20.7% vs 4.9%; OR 5.09, 95% CI 1.07 to 24.13; p=0.041).

There was a higher cumulative prevalence of malar rash (OR 3.40, 95% CI 1.23 to 9.34; p=0.018), mucosal ulcers (OR 3.31, 95% CI 1.36 to 8.05; p=0.008), arthritis (OR 3.19, 95% CI 1.19 to 8.60; p=0.022) and Raynaud’s phenomenon (OR 5.15, 95% CI 1.95 to 13.56; p=0.001) in current smokers (online supplemental table 1). SLEDAI-2K scores were equivalent across smoking status, although current smokers had increased odds of migraine (OR 2.82, 95% CI 1.07 to 7.44; p=0.037), with similar representation of other SLEDAI features. Both patient (4 vs 2, p=0.003) and physician (3 vs 2, p=0.046) global disease activity (GDA) visual analogue scale (VAS) scores were however higher. The increased odds of Raynaud’s phenomenon in current smokers were higher in male patients (data not shown). Laboratory findings demonstrated that current and non-smokers had similar immunological disease activity, including ANA and anti-dsDNA seropositivity, hypocomplementemia and cytopenias. However, current smokers had higher levels of haemoglobin (13.06 vs 12.99, p=0.036), mean corpuscular volume (MCV) (91.91 vs 88.52, p=0.014) and B cells (14 vs 0.08, p=0.037), with lower ApoA 1 lipoprotein levels (1.48 vs 1.61, p=0.039). Smoking was not associated with anti-dsDNA titres (ELIA Rs −0.03, p=0.776), complement protein (C3) (Rs −0.07, p=0.480) or C4 (Rs 0.14, p=0.174) (online supplemental table 4).

The management of SLE required non-steroidal anti-inflammatories in 15.2% (n=15 of 99), prednisone in 50.5% (50 of 99), hydroxychloroquine in 58.6% (n=58 of 99) and immunosuppression in 36.4% (n=36 of 99). Medication requirement for SLE was similar across smoking status with the exception of additional non-steroidal anti-inflammatory drug (NSAID) requirement in smokers (OR 6.88, 95% CI 1.99 to 23.72; p=0.002). Medication requirement for comorbid conditions was equivalent across smoking status (online supplemental table 5).

Comparison of cytokine levels between patients with SLE and healthy controls

Compared with controls, patients with SLE had higher average levels of BAFF (1.74 vs 0.94 ng/mL, p<0.001) and MCP-1 (137.42 vs 98.22 pg/mL, p=0.028), while having lower levels of IL-1β (26.85 vs 67.86 pg/mL, p<0.001) and TGF-β1 (581.74 vs 733.39, p=0.018). Within patients with SLE, smokers had higher average levels of BAFF (2.01 vs 1.61 ng/mL, p=0.034), yet lower levels of IFN-γ (39.37 vs 82.98 pg/mL, p=0.002), TNF-α (35.41 vs 58.37 pg/mL, p=0.024) and MIP-1β (509.26 vs 625.65 pg/mL, p=0.022). Compared with controls, patients with SLE who smoked had higher levels of BAFF (2.01 vs 0.94 ng/mL, p<0.001) and MCP-1 (118.9 vs 98.22 pg/mL, p=0.031), and lower IL-1β (21.85 vs 67.86 pg/mL, p=0.006) but equivalent IFN-γ levels (39.37 vs 53.88, p=0.064) (table 2).

Association of smoking with cytokine levels

Smoking correlated with increased BAFF levels (Rs 0.20, p=0.043) leading to 27% and 28% increased BAFF levels after adjusting for age and sex (95% CI 3% to 57%; p=0.023) and clinical covariates (model 1) (95% CI 5% to 57%, p=0.015), respectively. Smoking inversely correlated with IFN-γ levels (Rs −0.32, p=0.001) and a 55% decrease IFN-γ in both the age-adjusted and sex-adjusted (95% CI −71% to −27%, p=0.001) and clinical covariate adjusted models (95% CI −72% to −28%, p=0.001). Smoking inversely associated with TNF-α and MIP-1β levels in the univariate, age-adjusted and sex-adjusted model, and model 1. Smoking inversely correlated with IL-4 (Rs −0.25, p=0.015), but was not in the adjusted models. Smoking associated with reduced levels of IL-1β in the age-adjusted and sex-adjusted model only. Smoking associated with decreased IL-12 and IL-17 in the age-adjusted and sex-adjusted model and model 1, but not model 2 (table 3).

Analysis further adjusting for anti-inflammatory or regulatory cytokines

With further adjustment for anti-inflammatory cytokines (IL-4, IL-10 and TGF-β1) and regulatory cytokine IL-1β (model 2), smoking increased BAFF levels by 27% (95% CI 6% to 48%; p=0.014) and decreased IFN-γ levels by 42% (95% CI −79% to −5%; p=0.026); however, smoking no longer associated with changes in IL-12, IL-17, TNF-α or MIP-1β (which were dependent on IL-4 levels) (table 3).
Table 1  Characteristics of patients with SLE at the research visit compared across smoking status

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Current smoker</th>
<th>Not currently smoking</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Participants, n</strong></td>
<td>99</td>
<td>35</td>
<td>64</td>
<td></td>
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<tr>
<td><strong>Age, mean±SD</strong></td>
<td>48.48±15.75</td>
<td>46.91±13.49</td>
<td>49.33±16.89</td>
<td>–</td>
<td>0.941</td>
</tr>
<tr>
<td><strong>Male, n (%)</strong></td>
<td>13 (13.1)</td>
<td>7 (20.0)</td>
<td>6 (9.4)</td>
<td>–</td>
<td>0.135</td>
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<tr>
<td><strong>Female, n (%)</strong></td>
<td>86 (86.9)</td>
<td>28 (80.0)</td>
<td>58 (90.6)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><strong>Years of follow-up, median (IQR)</strong></td>
<td>10.42 (5.08–17.67)</td>
<td>10.42 (5.08–16.75)</td>
<td>10.46 (4.92–17.67)</td>
<td>–</td>
<td>0.941</td>
</tr>
<tr>
<td><strong>Smoking details</strong></td>
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<tr>
<td><strong>Ex-smoker, n (%)</strong></td>
<td>25 (39.1)</td>
<td>–</td>
<td>25 (39.1)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><strong>Cigarettes per day, median (IQR)</strong></td>
<td>7 (3–10)</td>
<td>7 (3–10)</td>
<td>9 (4–10)</td>
<td>–</td>
<td>0.428</td>
</tr>
<tr>
<td><strong>Years of consistent daily smoking, median (IQR)</strong></td>
<td>20.0 (13.0–28.00)</td>
<td>24.0 (18.0–30.0)</td>
<td>15.0 (5.0–23.0)</td>
<td>–</td>
<td>0.064</td>
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<tr>
<td><strong>Pack-years, median (IQR)</strong></td>
<td>5.0 (3.0–12.25)</td>
<td>9.00 (3.75–14.00)</td>
<td>3.5 (2.0–5.0)</td>
<td>–</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Years since quit smoking, median (IQR)</strong></td>
<td>10 (6–18)</td>
<td>–</td>
<td>10 (6–18)</td>
<td>–</td>
<td></td>
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<tr>
<td><strong>Cumulative ACR97 classification criteria</strong></td>
<td></td>
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<tr>
<td><strong>Cumulative ACR97 items, median (IQR)</strong></td>
<td>5 (4–7)</td>
<td>6 (4–7)</td>
<td>5 (4–7)</td>
<td>–</td>
<td>0.651</td>
</tr>
<tr>
<td><strong>Positive ANA, n (%)</strong></td>
<td>97 (97.9)</td>
<td>35 (100.0)</td>
<td>62 (96.9)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><em><em>Mucosal-cutaneous features</em>, n (%)</em>*</td>
<td>95 (96.0)</td>
<td>34 (97.1)</td>
<td>61 (95.3)</td>
<td>1.67 (0.17 to 16.71)</td>
<td>0.662</td>
</tr>
<tr>
<td><strong>Malar rash, n (%)</strong></td>
<td>66 (67.3)</td>
<td>29 (82.9)</td>
<td>37 (58.7)</td>
<td>3.40 (1.23 to 9.34)</td>
<td>0.018</td>
</tr>
<tr>
<td><strong>Photosensitivity, n (%)</strong></td>
<td>59 (60.2)</td>
<td>23 (65.7)</td>
<td>36 (57.1)</td>
<td>1.44 (0.61 to 3.39)</td>
<td>0.407</td>
</tr>
<tr>
<td><strong>Discoid lupus, n (%)</strong></td>
<td>42 (42.9)</td>
<td>18 (51.4)</td>
<td>24 (38.1)</td>
<td>1.72 (0.75 to 3.97)</td>
<td>0.203</td>
</tr>
<tr>
<td><strong>Mucosal ulcers, n (%)</strong></td>
<td>31 (31.6)</td>
<td>17 (48.6)</td>
<td>14 (22.2)</td>
<td>3.31 (1.36 to 8.05)</td>
<td>0.008</td>
</tr>
<tr>
<td><strong>Disease activity at the research visit (SLEDAI)</strong></td>
<td></td>
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<tr>
<td><strong>Clinical disease activity, n (%)</strong></td>
<td>84 (84.8)</td>
<td>28 (28.0)</td>
<td>56 (56.5)</td>
<td>0.57 (0.19 to 1.74)</td>
<td>0.324</td>
</tr>
<tr>
<td><strong>Migraine, n (%)</strong></td>
<td>22 (22.2)</td>
<td>12 (12.3)</td>
<td>10 (10.6)</td>
<td>2.82 (1.07 to 7.44)</td>
<td>0.037</td>
</tr>
<tr>
<td><strong>Arthritis, n (%)</strong></td>
<td>21 (21.2)</td>
<td>12 (34.3)</td>
<td>9 (14.1)</td>
<td>3.19 (1.19 to 8.60)</td>
<td>0.022</td>
</tr>
<tr>
<td><strong>Clinical SLEDAI score, median (IQR)</strong></td>
<td>6 (2–12)</td>
<td>6 (2–14)</td>
<td>6 (2–10)</td>
<td>–</td>
<td>0.981</td>
</tr>
<tr>
<td><strong>Total SLEDAI score, median (IQR)</strong></td>
<td>8 (2–14)</td>
<td>8 (4–16)</td>
<td>8 (2–11)</td>
<td>–</td>
<td>0.998</td>
</tr>
<tr>
<td><strong>Patient GDA VAS, median (IQR)</strong></td>
<td>3 (1–5)</td>
<td>4 (3–6)</td>
<td>2 (1–5)</td>
<td>–</td>
<td>0.003</td>
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<tr>
<td><strong>Physician GDA VAS, median (IQR)</strong></td>
<td>2 (1–4)</td>
<td>3 (2–4)</td>
<td>2 (1–3)</td>
<td>–</td>
<td>0.046</td>
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<tr>
<td><strong>LDAS-50</strong></td>
<td>35 (37.2)</td>
<td>13 (37.1)</td>
<td>22 (37.3)</td>
<td>0.99 (0.42 to 2.36)</td>
<td>0.989</td>
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<tr>
<td><strong>LDAS-30</strong></td>
<td>62 (66.0)</td>
<td>21 (60.0)</td>
<td>41 (69.5)</td>
<td>0.66 (0.28 to 1.58)</td>
<td>0.349</td>
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<tr>
<td><strong>LDAS-70</strong></td>
<td>17 (18.1)</td>
<td>8 (22.9)</td>
<td>9 (15.3)</td>
<td>1.65 (0.57 to 4.78)</td>
<td>0.357</td>
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<td><strong>Secondary conditions</strong></td>
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<td></td>
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<tr>
<td><strong>Raynaud's phenomenon, n (%)</strong></td>
<td>25 (25.3)</td>
<td>16 (16.7)</td>
<td>9 (9.1)</td>
<td>5.15 (1.95 to 13.56)</td>
<td>0.001</td>
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<tr>
<td><strong>Medication requirement</strong></td>
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<td></td>
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<tr>
<td><strong>Prednisone, n (%)</strong></td>
<td>50 (50.5)</td>
<td>18 (51.4)</td>
<td>32 (50.0)</td>
<td>1.06 (0.46 to 2.41)</td>
<td>0.892</td>
</tr>
</tbody>
</table>

Continued
Biomarker studies

DISCUSSION

This cross-sectional cohort study demonstrates that in patients with SLE, current smoking associates with increased BAFF levels and decreased IFN-γ levels. This was on a background of current smokers having increased joint manifestations necessitating higher NSAID usage, migraine, Raynaud’s phenomenon and increased GDA scores. Current smokers had accrued more malar rash and mucosal ulcers over time (ACR97), but not skin damage (OR 3.82, p=0.281). Having ever smoked was associated with increased odds of organ damage and cancer development. However, current smokers and non-smokers were similar with respect to the remaining ACR97 classification criteria items and scores, cross-sectional SLEDAI-2K scores, damage accrual (SDI >0, 59.6%), comorbidity and other medication requirement.

The harmful effects of cigarette smoking are well established in the general population, and the more recent phenomenon of smoking e-cigarettes (vaping) is gradually being shown to equally so. 31 The 35.4% of patients with SLE smoking and the 25 (39.1%) ex-smokers aligned with Norwegian national survey data on smoking prevalence and cessation at the time of the study. 32 Compared with other studies of patients with SLE, smoking prevalence

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Overall</th>
<th>Current smoker</th>
<th>Not currently smoking</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisone dose (mg), median (IQR)</td>
<td>5.00 (5.00–7.50)</td>
<td>5.00 (5.00–7.50)</td>
<td>5.00 (5.00–10.00)</td>
<td>0.898</td>
<td></td>
</tr>
<tr>
<td>Non-steroidal anti-inflammatory, n (%)</td>
<td>15 (15.2)</td>
<td>11 (31.4)</td>
<td>4 (6.3)</td>
<td>6.88 (1.99 to 23.72)</td>
<td>0.002</td>
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<tr>
<td>Hydroxychloroquine, n (%)</td>
<td>58 (58.6)</td>
<td>19 (54.3)</td>
<td>39 (60.9)</td>
<td>0.76 (0.33 to 1.75)</td>
<td>0.521</td>
</tr>
<tr>
<td>Immunosuppressants, n (%)</td>
<td>36 (36.4)</td>
<td>13 (37.1)</td>
<td>23 (35.9)</td>
<td>1.05 (0.45 to 2.48)</td>
<td>0.905</td>
</tr>
</tbody>
</table>

**Comorbidity**

| Metabolic disorder, n (%) | 61 (61.2) | 20 (57.1) | 41 (68.3) | 0.62 (0.26 to 1.46) | 0.274 |
| Dyslipidaemia, n (%) | 49 (49.1) | 15 (42.9) | 34 (57.6) | 0.55 (0.24 to 1.28) | 0.168 |
| BMI ≥30, n (%) | 12 (12.9) | 3 (8.6) | 9 (15.5) | 0.51 (0.13 to 2.03) | 0.340 |
| Diabetes, n (%) | 3 (3.2) | 3 (8.6) | 0 (0.0) | – | – |
| Hypertension, n (%) | 49 (49.6) | 15 (42.9) | 34 (56.7) | 0.57 (0.25 to 1.33) | 0.196 |
| Cardiovascular disease, n (%) | 31 (31.1) | 12 (34.3) | 19 (29.7) | 1.24 (0.51 to 2.98) | 0.637 |
| Heart attack, n (%) | 13 (13.8) | 6 (17.1) | 7 (11.9) | 1.54 (0.47 to 5.01) | 0.476 |
| Thromboembolic disease, n (%) | 13 (13.8) | 4 (11.4) | 9 (15.3) | 0.72 (0.20 to 2.53) | 0.605 |
| Stroke, n (%) | 5 (5.3) | 2 (5.7) | 3 (5.1) | 1.13 (0.18 to 7.13) | 0.895 |
| Cancer, n (%) | 11 (11.7) | 1 (2.9) | 10 (16.9) | 0.14 (0.02 to 1.18) | 0.071 |
| Pulmonary diseases†, n (%) | 13 (13.1) | 6 (17.1) | 7 (10.9) | 1.69 (0.52 to 5.48) | 0.386 |
| Damage accrual (SDI >0), n (%) | 59 (59.6) | 24 (68.6) | 35 (54.7) | 1.81 (0.76 to 4.30) | 0.181 |
| Total SDI score, median (IQR) | 2.00 (1.00–3.00) | 1.00 (1.00–2.00) | 2.00 (2.00–4.00) | 0.140 |
| Skin damage, n (%) | 3 (3.0) | 2 (5.7) | 1 (1.6) | 3.82 (0.33 to 43.68) | 0.281 |
| Pulmonary damage, n (%) | 7 (7.1) | 4 (11.4) | 3 (4.7) | 2.62 (0.55 to 12.46) | 0.225 |
| Malignancy damage, n (%) | 14 (14.1) | 3 (8.6) | 11 (17.2) | 0.45 (0.12 to 1.74) | 0.249 |

Bolded findings are significantly different across current and those not currently smoking.

*Also includes alopecia.
†Pulmonary diseases include fibrotic and obstructive diseases (asthma n=6).

ACR97, American College of Rheumatology Classification Criteria for SLE; BMI, body mass index (kg/m²); GDA, global disease activity; LDAS-50, Low Disease Activity State-50; SDI, Systemic Lupus International Collaborating Clinics/ACR Damage Index; SLEDAI, SLE Disease Activity Index; VAS, visual analogue scale.
**Table 2** Cytokine levels in patients with SLE and 31 healthy controls (reference levels) at the research visit, compared across smoking status

<table>
<thead>
<tr>
<th>Participants, n</th>
<th>Healthy controls</th>
<th>SLE (99)</th>
<th>t-test</th>
<th>SLE smokers (35)</th>
<th>SLE non-smokers (64)</th>
<th>t-test</th>
<th>ANOVA*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Geometric mean (95% CI)</td>
<td>Geometric mean (95% CI)</td>
<td>P value</td>
<td>Geometric mean (95% CI)</td>
<td>Geometric mean (95% CI)</td>
<td>P value</td>
<td>P value</td>
</tr>
<tr>
<td>BAFF, ng/mL</td>
<td>0.94 (0.84 to 1.04)</td>
<td>1.74 (1.58 to 1.92)</td>
<td>&lt;0.001 *</td>
<td>2.01 (1.72 to 2.34)</td>
<td>1.61 (1.42 to 1.83)</td>
<td>0.034 #</td>
<td>&lt;0.001 %</td>
</tr>
<tr>
<td>IFN-γ, pg/mL</td>
<td>53.88 (35.81 to 81.06)</td>
<td>63.75 (50.51 to 80.47)</td>
<td>0.480</td>
<td>39.37 (27.80 to 55.74)</td>
<td>82.98 (61.96 to 111.13)</td>
<td>0.002 #</td>
<td>0.006 %</td>
</tr>
<tr>
<td>IL-1β, pg/mL</td>
<td>67.86 (40.08 to 114.90)</td>
<td>26.85 (22.08 to 32.63)</td>
<td>&lt;0.001 *</td>
<td>21.85 (16.80 to 28.43)</td>
<td>30.04 (23.02 to 39.22)</td>
<td>0.123</td>
<td>&lt;0.001 %</td>
</tr>
<tr>
<td>IL-4, pg/mL</td>
<td>10.98 (7.47 to 16.14)</td>
<td>11.65 (9.23 to 14.70)</td>
<td>0.801</td>
<td>8.99 (6.58 to 12.28)</td>
<td>13.43 (9.78 to 18.44)</td>
<td>0.102</td>
<td>0.237</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>19.12 (14.72 to 24.84)</td>
<td>20.84 (17.66 to 24.59)</td>
<td>0.605</td>
<td>18.23 (13.93 to 23.87)</td>
<td>22.42 (18.12 to 27.73)</td>
<td>0.239</td>
<td>0.416</td>
</tr>
<tr>
<td>IL-10, pg/mL</td>
<td>11.14 (7.82 to 15.86)</td>
<td>13.31 (10.28 to 17.24)</td>
<td>0.481</td>
<td>10.87 (7.56 to 15.65)</td>
<td>14.87 (10.47 to 21.11)</td>
<td>0.253</td>
<td>0.374</td>
</tr>
<tr>
<td>IL-12, pg/mL</td>
<td>38.92 (27.44 to 55.20)</td>
<td>31.51 (25.58 to 38.81)</td>
<td>0.318</td>
<td>24.18 (17.39 to 33.62)</td>
<td>36.41 (27.88 to 47.55)</td>
<td>0.062</td>
<td>0.099</td>
</tr>
<tr>
<td>IL-17, pg/mL</td>
<td>51.01 (37.26 to 69.82)</td>
<td>49.65 (41.20 to 59.82)</td>
<td>0.887</td>
<td>40.18 (30.82 to 52.38)</td>
<td>55.74 (43.42 to 71.55)</td>
<td>0.096</td>
<td>0.233</td>
</tr>
<tr>
<td>MCP-1, pg/mL</td>
<td>98.22 (76.47 to 126.15)</td>
<td>137.42 (118.36 to 159.54)</td>
<td>0.028 *</td>
<td>118.90 (93.40 to 151.37)</td>
<td>148.74 (122.84 to 180.10)</td>
<td>0.156</td>
<td>0.031 %</td>
</tr>
<tr>
<td>TNF-α, pg/mL</td>
<td>47.91 (33.68 to 68.15)</td>
<td>48.91 (39.58 to 60.45)</td>
<td>0.923</td>
<td>35.41 (26.10 to 48.03)</td>
<td>58.37 (44.20 to 77.07)</td>
<td>0.024 #</td>
<td>0.070</td>
</tr>
<tr>
<td>TGF-β1, pg/mL</td>
<td>733.39 (663.16 to 811.06)</td>
<td>581.74 (524.97 to 644.65)</td>
<td>0.018 *</td>
<td>509.26 (419.01 to 618.96)</td>
<td>625.65 (556.02 to 703.99)</td>
<td>0.057</td>
<td>0.007 %</td>
</tr>
<tr>
<td>MIP-1α, pg/mL</td>
<td>Not available</td>
<td>42.10 (31.65 to 55.99)</td>
<td>–</td>
<td>34.72 (21.44 to 56.22)</td>
<td>47.22 (32.54 to 68.50)</td>
<td>0.319</td>
<td>–</td>
</tr>
<tr>
<td>MIP-1β, pg/mL</td>
<td>Not available</td>
<td>228.65 (206.54 to 253.13)</td>
<td>–</td>
<td>195.24 (165.18 to 230.78)</td>
<td>249.30 (219.74 to 282.83)</td>
<td>0.022 #</td>
<td>–</td>
</tr>
</tbody>
</table>

Bolded findings are significantly different across either * SLE patients and healthy controls; # SLE patients who were currently smoking vs not currently smoking; and, % healthy controls, SLE non-smokers and SLE current smokers.

*One-way ANOVA compared cytokine levels between healthy controls, SLE smokers and SLE non-smokers.

ANOVA, analysis of variance; BAFF, B cell-activating factor; IFN-γ, interferon-gamma; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; MIP-1α, macrophage inflammatory protein 1 alpha; MIP-1β, macrophage inflammatory protein 1 beta; TGF-β1, transforming growth factor beta 1; TNF-α, tumour necrosis factor-alpha.
Table 3  Univariate and multivariate association between smoking and cytokine levels in patients with SLE

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th>Age and sex adjusted</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rs</td>
<td>P value</td>
<td>B (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Ln(BAFF)</td>
<td>0.20</td>
<td>0.043</td>
<td>0.24 (0.03 to 0.45)</td>
<td>0.023</td>
</tr>
<tr>
<td>Ln(IFN-γ)</td>
<td>−0.32</td>
<td>0.001</td>
<td>−0.79 (−1.25 to 0.32)</td>
<td>0.001</td>
</tr>
<tr>
<td>Ln(IL-1β)</td>
<td>−0.19</td>
<td>0.054</td>
<td>−0.42 (−0.82 to 0.02)</td>
<td>0.039</td>
</tr>
<tr>
<td>Ln(IL-4)</td>
<td>−0.25</td>
<td>0.015</td>
<td>−0.47 (0.98 to 0.02)</td>
<td>0.060</td>
</tr>
<tr>
<td>Ln(IL-6)</td>
<td>−0.15</td>
<td>0.142</td>
<td>−0.26 (−0.61 to 0.09)</td>
<td>0.088</td>
</tr>
<tr>
<td>Ln(IL-10)</td>
<td>−0.04</td>
<td>0.725</td>
<td>−0.42 (−0.96 to 0.12)</td>
<td>0.123</td>
</tr>
<tr>
<td>Ln(IL-12)</td>
<td>−0.19</td>
<td>0.055</td>
<td>−0.51 (−0.93 to 0.08)</td>
<td>0.021</td>
</tr>
<tr>
<td>Ln(IL-17)</td>
<td>−0.12</td>
<td>0.237</td>
<td>−0.43 (−0.80 to 0.06)</td>
<td>0.022</td>
</tr>
<tr>
<td>Ln(MCP-1)</td>
<td>−0.16</td>
<td>0.104</td>
<td>−0.24 (−0.55 to 0.07)</td>
<td>0.129</td>
</tr>
<tr>
<td>Ln(TNF-α)</td>
<td>−0.24</td>
<td>0.016</td>
<td>−0.55 (−0.99 to 0.11)</td>
<td>0.014</td>
</tr>
<tr>
<td>Ln(TGF-β1)</td>
<td>−0.17</td>
<td>0.1</td>
<td>−0.20 (−0.41 to 0.02)</td>
<td>0.076</td>
</tr>
<tr>
<td>Ln(MIP-1α)</td>
<td>−0.13</td>
<td>0.21</td>
<td>−0.28 (−0.90 to 0.35)</td>
<td>0.381</td>
</tr>
<tr>
<td>Ln(MIP-1β)</td>
<td>−0.27</td>
<td>0.007</td>
<td>−0.27 (−0.49 to 0.06)</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Model 1 adjusted for age, sex, prednisone use, immunosuppressant use, SLEDAI score, SDI score.
Model 2 adjusted for model 1 plus (regulatory cytokine) IL-1β* and anti-inflammatory cytokines (IL-4**, IL-10# and TGF-β%) (*, **, #, % indicate that this cytokine was dropped from the independent variables of the model).
Bolded findings: current smoking associated with changes (+/-) in the level of the cytokine of interest smoking.

BAFF, B cell-activating factor; IFN-γ, interferon-gamma; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; MIP-1α, macrophage inflammatory protein 1 alpha; MIP-1β, macrophage inflammatory protein 1 beta; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; SLEDAI, SLE Disease Activity Index; TGF-β1, transforming growth factor beta 1; TNF-α, tumour necrosis factor-alpha.
was equivalent to studies based in the USA (32% current smokers) and Denmark (51% ever-smoked), but higher than in Canada (14% current smokers) and Brazil (8% current smokers). 

Similarly, smoking intensity (cigarettes per day and pack-years) in our cohort was equivalent to those who reported it. 

Despite the Norwegian effort to promote smoking cessation, the prevalence of smoking is currently around 20%, and it is not expected to fall below 10% until 2029, which indicates that an opportunity still exists for clinicians to encourage smoking cessation in their patients.

Our cohort was similar to other studies about the impact of smoking on SLE, with respect to participants’ age (mean 48 years old), disease duration (an average of 10 years) and sex distribution. The higher odds of prevalent (ACR97) mucosal ulcers in our cohort add to the established link between current smoking and active mucocutaneous features in SLE.

In spite of the small numbers, we showed a signal of more skin damage in current smokers (OR 3.82; p>0.05), although similar for the over-representation of cumulative cutaneous features in smokers. Our findings differed to others who showed that having ever smoked or having had a >20 pack-year history increased the odds of discoid lesions and photosensitivity. The increased odds of mucosal rather than discoid lesions and photosensitivity may result from differences in the ultraviolet (UV) light exposure at each research setting, with Tromso (Northern Norway) having lower peak UV (~4) compared with the Canadian and Danish studies (~6UV index). We showed that having ever smoked rather than current smoking alone resulted in proportionally more damage accrual and malignancy. This aligned with Bourré-Tessier et al who reported that smoking associated with lung cancer in patients with SLE. Taken together, patients with SLE should be advised to cease smoking to reduce the odds of mucocutaneous disease flares, damage accrual and cancer development.

Similar to other cross-sectional studies, smoking exhibited little influence on total SLEDAI score, rather we showed increased odds of arthritis, migraine and vasculitis (Raynaud’s phenomenon). In contrast, other studies showed that smoking increased average SLEDAI scores, increased cutaneous-SLE disease activity, and either slowed or prevented the reduction of SLEDAI scores after treatment with belimumab. Herein, the increased odds of arthritis in patients with SLE who smoked contradicted the inverse association between a smoking history of >20 pack-years and non-erosive arthritis (OR=0.45) reported by Leffers et al. However, in the context of the lower IFN-γ levels shown herein, our data align with murine models, which showed that smoking can cause a collagen-induced arthritis.

Patients with SLE who smoked also had more migraine, headache and Raynaud’s phenomenon (disproportionately affecting men). This fits with the increased frequency of headache and vasculitis in active smokers, and the increased odds of neurological disorders in those with smoking history of >20 pack-years reported elsewhere. Patients with SLE who smoked also had increased patient and physician GDA scores (0–10 mm VAS). Yet, similar to Golder et al, we found that smoking status (current or ex-smoking) had no influence on LLDAS-30, LLDAS-50 or LLDAS-70 scores (all p>0.30).

Thus, in patients with SLE, we suspect that the harmful effects of smoking to health, systemic inflammation and disease activity are not entirely captured by the SLEDAI-2K, which may mean that the impact of smoking is potentially obscured in current treat-to-target indices.

For similar medication profiles, current smokers trended towards a reduction in the prevalence (OR 0.52, p=0.125) and severity of the typical cytopenias seen in SLE, and current smokers had increased levels of haemoglobin and MCV. The increased (27%) serum BAFF levels in patients with SLE who smoked confirmed the 8.7% and 24% increase seen in (ANA-/ANA+) smokers in the Nurse’s Health Study (n=1177), respectively. Smoking has been shown to increase serum BAFF levels in the absence of autoimmune disease. The overexpression of BAFF in SLE is hypothesised to be driven by localised inflammation or B cell activation resulting from inherent immune dysregulation in SLE. BAFF levels have also been associated with disease activity and organ damage in some but not all cohort studies. Yet, the failure of monoclonal anti-BAFF therapy to dampen disease activity suggests that aberrant BAFF production is a feature rather than a direct cause of lupus disease activity. Furthermore, smoking has been shown to reduce the efficacy of belimumab (BAFF inhibition) and antimalarial agents in the management of SLE. Taken together, the independent association between smoking and BAFF levels, on a background of increased physician GDA score arthritis and vasculopathies, suggests that smoking cessation should be a priority in the management of SLE to reduce systemic inflammation and disease activity, which would help to reduce serum BAFF levels.

Innate antiviral immune competence is a major function of many type I IFNs, which along with IL-12, IL-15 and IL-18, can induce type II IFN (IFN-γ) production. Dysregulation of type I IFN, and potentially type II IFN (IFN-γ), can lead to uncontrolled virus replication coupled with uncontrolled inflammation which causes tissue damage. IFN-γ in inflammatory and autoimmune pathologies has bidirectional immunoregulatory effects,
which often moderate disease severity, and maintains anti-
viral and microbial defences, among other features.32–36
We showed increased IFN-γ levels in non-smoking
patients with SLE compared with healthy controls (82.98
vs 53.88 pg/mL, p<0.001).18 37 However, patients with
SLE who smoked had IFN-γ levels similar to and trending
lower than those of healthy controls (39.37 vs 53.88 pg/
ml, p=0.064). The suppression of IFN-γ levels within
patients with SLE who smoked (model 2) aligns with the
literature about smoking-induced suppression of type I
and II IFN cytokines.38–42 With respect to IFNs, smoking
can cause phosphorylation-dependent downregulation
of IFN-1A receptor (IFN-1AR) by reactive oxygen species
(ROS),43 which, although unproven as yet, may result
from exposure to the major phenolic components of
cigarette tar, hydroquinone and catechol.27 Additionally,
the phosphorylation-dependent downregulation of IFN-
1AR by ROS was ameliorated by the antioxidant N-acetyl-
cysteine (NAC).44 Interestingly, in patients with SLE,
disease activity was shown to improve within 1 month
of administration of NAC (2.4–4.8 g/day),45 which suggests
that perhaps, NAC supplementation may be beneficial in
patients with SLE, especially for those struggling with
smoking cessation.46 Nicotine has been recently cited as
having beneficial proinflammatory and anti-inflammatory
effects47; and pertinent to SLE, nicotine was shown to
augment TGF-β levels and increase expression of FoxP3
in mice.48 However, in our study, TGF-β1 levels were lowest
in patients with SLE who smoked. Thus, while nicotine
replacement therapy has its use in smoking cessation,
its psychoactive properties (addictive), vasoconstrictive
properties (perhaps responsible for the increased odds of
headache and Raynaud’s phenomenon seen herein) and
the significantly reduced levels of TGF-β1 levels of current
smokers make its potential use as an immune modulator
unlikely.49 Ultimately, the ability of NAC supplementation
and nicotine to influence systemic and immune-mediated
inflammation requires further study in patients with SLE.
The clinical phenotype of patients with SLE who smoked
aligns with murine model data, in which, the absence
of IFN-γ had a significant reduction in serum immunoglob-
ulins, ANA and anti-dsDNA seropositivity, renal immune
deposits and proteinuria.38 Similarly, in murine models
of collagen-induced arthritis, IFN-γ was protective against
arthritis by inhibiting the development of Th17 cyto-
kines.46–47 Taken together, smoking in SLE depletes IFN-γ
to levels similar to or beyond those of healthy controls,
with the cost of more arthritis, migraine/headaches and
vasculitis, which necessitates increased NSAID use; and
while we lacked the data, we suspect that smoking would
have a higher prevalence of infection or viral reactiva-
tion as well. Future research could investigate the scope
for NAC supplementation to help prevent a rebounding
effect of type I and II (IFN-γ) IFNs to aberrant levels in
patients undergoing smoking cessation.18 48 Furthermore,
use of type I or II IFN inhibitors in patients with SLE who
smoke would likely be met with poorer treatment response
and further increase the development and severity of viral
infection or reactivation.49

Levels of IL-1β are reported to be higher in patients with
SLE relative to controls, especially with higher disease
activity,75 76; and lower levels of IL-1β have been shown
in well-managed,77 quiescent78 and now inversely associ-
ated with smoking in patients with SLE (age-adjusted and
sex-adjusted model). Our data and others indicate that in
patients with SLE, the by-products of smoking can reduce
the levels of conventionally proinflammatory (TNF-α,
IL-12, IL-17 and MIP-1β) and anti-inflammatory (IL-4)
cytokines.47 79 While the depletion of these cytokines may
not be significant enough to cause immune-mediated
pathology, in the context of a reduced regulatory capacity
of IL-1β, this may increase the susceptibility of patients
with SLE to infections, atherosclerosis, cardiovascular
disease and cancer development.94–77 Thus, we speculate
that smoking cessation may help normalise levels of IL-1β
in patients with SLE, which would help to inhibit unde-
sirable immune responses, possibly by increasing the
Treg functionality via Th2 cytokines, and give protection
against comorbidity.

The strength of this study is the availability of a large
range of disease characteristics in all the patients, the
inclusion of longitudinal organ damage data, and the
adjustment for a range of cytokines involved in regu-
larising or exacerbating SLE. Some of the limitations
of this study lie in the fact that our patients were all of
Northern European descent and were mostly in a state
of low disease activity, such that results cannot be extrap-
olated to cohorts with a different genetic or clinical
makeup. The smoking exposure was captured prospect-
ively since SLE diagnosis (with the question ‘Have you
smoked earlier or do you smoke daily?’) as well as at the
research visit, meaning that the details of ex-smokers’
cigarette consumption, including ‘cigarettes smoked per
day’, ‘years of continuous smoking’ and the ‘years since
quit smoking’ to determine pack-years, was defined retro-
spectively by self-report. We lacked complete data on
socioeconomic status and did not collect data on alcohol
consumption, although we suspect these exposures
would have minimal effect on the adjusted associations
with cytokine levels. While we demonstrated an independ-
ent association between smoking and IFN-γ, we did not
have data on other type I IFNs, thus we cannot determine
whether smoking directly influenced IFN-γ levels via type
I IFNs or other cytokines, such as IL-18, which can stim-
ulate the production of IFN-γ. Finally, our results were
based on clinical and serological findings and, therefore,
cannot confirm the cellular source or causation of effects
of smoking on BAFF, IFN-γ and IL-1β, for which further
experimental studies will be needed.

CONCLUSION
Smoking independently associated with increased serum
BAFF and decreased IFN-γ in patients with SLE. Smoking
status associated with a clinical phenotype, which
including mucocutaneous features, arthritis, migraine/ lupus headaches and secondary Raynaud’s phenomenon, and necessitated increased use of NSAIDs. Smoking cessation should be encouraged in SLE with the aim to reduce systemic inflammation (BAFF and physician GDA), arthritis, vasospasticity, cutaneous features and NSAID requirement, while improving medication efficacy (hydroxychloroquine) and innate immune competence (IFN-γ).

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Contributors All authors have contributed to the study design and writing of this manuscript. WDR is responsible (guarantor) of the overall content. WDR conducted the statistical analysis and assures the integrity and accuracy of the data presented in this study.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval Patients provided written informed consent for this study, which was approved by the regional ethics committee (REE: 2015/1/400).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplemental information.

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