Antibodies to *Chlamydia trachomatis* and reproductive health issues in women with SLE: a case–control study

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ABSTRACT

Background SLE is an autoimmune condition affecting predominantly women. Little is known regarding *Chlamydia trachomatis* infection in women with SLE, which may drive autoimmunity and contribute to obstetrical and vascular complications.

Methods This single-centre, case–control study set primary endpoint in the comparative seropositivity rate to *C. trachomatis* major outer membrane protein (MOMP) and chlamydial heat-shock protein-60 (cHSP60) in age-matched subjects. The secondary endpoints were obstetrical outcomes, cardiovascular events and results from screening procedures for cervical cancer.

Results Eighty-four women with SLE and 50 age-matched controls were included. Seropositivity to *C. trachomatis* did not differ significantly between groups (10% of cases positive for anti-MOMP vs 12% of controls; 43% of cases positive for anti-cHSP60 vs 32% of controls). Women with SLE were more often of non-Caucasian ethnicity and had lower educational level. They relied less frequently on oral contraception and resorted more frequently to elective pregnancy termination. Pre-eclampsia and ectopic pregnancy occurred only in SLE. Women with SLE also experienced more cardiovascular events. In SLE, antibodies to cHSP60 were associated with a history of pericarditis and abnormal screening tests for cervical cancer. Antibody titres to *C. trachomatis* were not associated with disease activity or SLE treatment, nor were there associations with other gynaecological, obstetrical or vascular outcomes.

Conclusion Prevalence of antibodies to *C. trachomatis* was not increased in women with SLE. No significant association was found between these antibodies and obstetrical or cardiovascular complications.

INTRODUCTION

SLE is a chronic inflammatory disorder characterised by multiorgan damage and the generation of a vast array of autoantibodies. SLE mainly affects women of reproductive age. The strong gender predilection suggests that sexual hormones are involved in disease pathogenesis and progression, but gender-specific genetic and environmental differences may also be implicated.[1–3] It has been postulated that the autoimmune processes underlying SLE may be triggered by certain pathogens in genetically susceptible individuals. One prime candidate is Epstein-Barr virus,[4] although results regarding its implication in SLE are conflicting. Information on the role of sexually transmitted pathogens in SLE is limited. *Chlamydia trachomatis* is a common sexually transmitted obligatory intracellular bacteria. Infection with *C. trachomatis* usually results in urethritis in men and cervicitis in women. In women, *C. trachomatis* infection is frequently clinically silent, and some develop endometritis, salpingitis or pelvic inflammatory disease, which may lead to ectopic pregnancy, miscarriage and tubal infertility.[5 6] About 10% of individuals in the general population have antibodies to *C. trachomatis*.[7–9] In Switzerland, *C. trachomatis* seroprevalence and *C. trachomatis* prevalence assessed by quantitative PCRs in urogenital samples are similar to those in other European countries, being respectively 10%[6] and 5%.[10] This prevalence of chlamydia DNA was slightly lower (about 2%) in young asymptomatic Swiss men.[11] *C. trachomatis* infection has been linked to reactive arthritis, an autoimmune entity mostly diagnosed in male subjects.[12] *C. trachomatis* produces microbial heat-shock proteins (HSP), such as chlamydial HSP60 (cHSP60), a stress molecule with a high degree of homology between species.[13–15] Thus, infection with *C. trachomatis* may trigger an immune response with generation of anti-cHSP60 antibodies, which could cross-react with human HSP60. Human HSP60 is expressed on endothelial cells. HSP60-reactive T cells as well as anti-HSP60 antibodies have been linked to atherosclerosis.[16] In SLE, the presence of antibodies to human HSP60 was associated with increased arterial vascular events, when associated with antiphospholipid antibodies.[17] Accelerated atherosclerosis is of particular concern in SLE. Based on the role of *C. trachomatis* in autoimmunity and vascular damage, we

hypothesised that infection with *C. trachomatis* may be more prevalent in women with SLE. A study in patients with reactive arthritis had indeed found an increased seroprevalence of antibodies to *C. trachomatis* in controls with SLE compared with healthy subjects, although this difference was not statistically significant.[18] Serology for *C. trachomatis*, both against chlamydial major outer membrane protein (MOMP) and cHSP60, has a limited sensitivity for acute infection.[19] It is however the most reliable tool to assess past infection. A positive serology tends to be associated with more severe infections (eg, pelvic inflammatory disease).[20] Our main objective was to investigate the prevalence of both antibodies to *C. trachomatis* MOMP and to the less specific cHSP60 in female patients with SLE, compared with age-matched controls. We also investigated pregnancy outcomes and cardiovascular events in both groups, as these are of particular concern in women with SLE[21 22] and because of their potential link with *C. trachomatis* infection.

METHODS

We conducted a case–control study between 15 December 2014 and 13 April 2015. All participants were recruited sequentially. Cases were female adults with SLE attending the outpatient clinic of Lausanne University Hospital. Controls were women attending the same outpatient clinic for allergy tests. Cases and controls were matched per decade of age, in order to obtain the same age distribution between groups. All participants provided a blood sample and completed a questionnaire on reproductive health outcomes. Additional information was gathered from hospital files and gynaecologists' records, when available. Inclusion criteria for cases were definite SLE according to the revised American College of Rheumatology (ACR)[23] and/or the Systemic Lupus International Collaborating Clinics criteria.[24] Cases were recruited into the Swiss SLE Cohort Study and assessed according to a detailed questionnaire on SLE features. Exclusion criteria for controls were evident autoimmunity or non-allergic inflammatory conditions. Exclusion criteria for both groups were active neoplasia or infection.

The questionnaire assessed demographical, gynaeco-obstetrical and cardiovascular variables. Data included age at menarche, age at first intercourse, means of contraception, number of sexual partners, number of pregnancies and pregnancy outcomes, occurrence of menopause and frequency of gynaecological follow-up. Additional data were extracted from the hospital medical file and obtained from gynaecologists in private practice through a telephone interview, and included details on screening procedures for cervical cancer (Pap test according to Bethesda classification, and where available colposcopy and biopsy results), on pregnancy outcomes (spontaneous fetal losses, extrauterine pregnancies, preterm births before the 32nd week of gestation, pre-eclampsia defined as hypertension >140/90 mm Hg and proteinuria >300 mg/24 hours since the 20th week of gestation,

and eclampsia defined as status epilepticus in a context of pre-eclampsia) and on thromboembolic events (significant ischaemic stroke translating in an acute neurological symptomatology, acute ST-elevation myocardial infarction (STEMI) or non-STEMI, unprovoked acute deep venous thrombosis and clinically significant acute pulmonary embolism; all cardiovascular events were documented by radiological findings or coronarography).

Serology against *C. trachomatis* was performed in all participants using ELISA, with IgG to *C. trachomatis* MOMP (*Chlamydia trachomatis*-IgG-pELISA; ref 497-TMB; Medac, Wedel, Germany) and HSP60 (cHSP60-IgG-pELISA; ref 435; Medac). The MOMP Medac assay is described as one of the most sensitive and specific commercially available, noticeably because it does not cross-react with *C. pneumoniae* MOMP and HSP60.[25] Cross-reactivity of *C. trachomatis* anti-HSP60 with human anti-HSP60 is unknown and was not assessed in the present study. Intra-assay variability was 3.4% (coefficient of variation for MOMP assay) and 4.3% (coefficient of variation for cHSP60 assay), as reported by the manufacturer.

Relative luminescence (defined according to the manufacturer's instruction) of 1.1 or more was considered as a positive result, and a relative luminescence of 0.9 or less was considered negative. Before analyses, we decided to consider borderline antibody titres 0.9–1.1 as negative.

Clinical information regarding lupus disease duration, activity according to Safety of Estrogens in Lupus Erythematosus National Assessment-Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) score,[26] and past and present treatment was were documented for SLE cases.

Distribution comparisons (Student's t-test) and bivariate analyses (χ^2 and Fisher's methods according to the number of cases) were used to assess differences between groups (group matching). P values under 0.05 were considered statistically significant. Missing values were excluded from analyses. Given the explorative nature of the study, we did not apply post-hoc procedures to correct for multiple comparisons.[27] Analyses were conducted using SPSS V.22 for Windows.

RESULTS
Baseline characteristics

Eighty-four women with SLE and 50 age-matched controls were included (figure 1). The mean age at inclusion was 44 years. Demographics in women with SLE and controls are compared in table 1. Women with SLE significantly differed from controls with regard to ethnic background, with 58% being Caucasians, compared with 88% in the control group. Educational level also tended to be lower in the SLE group, with 57% having attended college, professional school or university, compared with 72% in the control group.

There was no difference in age at menarche, age at first intercourse or number of sexual partners between groups. The use of oral contraceptives was significantly
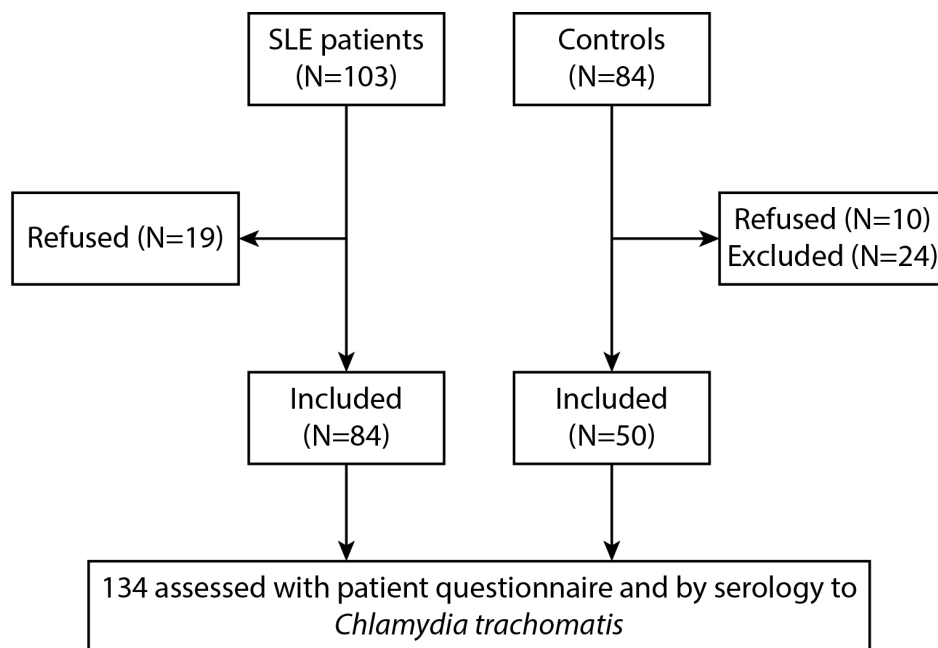
Figure 1 Study flow chart. Cases with SLE are on the left and age-matched controls are on the right. Controls excluded after screening had systemic autoimmunity (n=8), autoimmune endocrinopathy (n=6), chronic spontaneous urticaria (n=4), active neoplasia (n=3) and other unclear medical conditions (n=3; pulmonary transplant for cystic fibrosis, non-allergic asthma and pruritus of unknown origin).

lower in SLE and reported by 66% of women, compared with 82% of controls. On the other hand, only women with SLE had received medroxyprogesterone acetate injections. Other means of contraception did not differ between groups. Forty-one (49%) women with SLE declared having reached menopause, compared with 18 (36%) of controls (NS). In the SLE group, 79% had seen a gynaecologist in the 2 years preceding inclusion, compared with 90% of controls (NS).

Primary outcomes
Antibody titres to *C. trachomatis* MOMP and cHSP60 are shown in figure 2. As expected, there was a moderate correlation between MOMP and cHSP60 antibody titres (Spearman's r=0.29). The proportion of seropositives did not differ between groups, with 8 (10%) cases positive to MOMP compared with 6 (12%) controls, and 36 (43%) cases positive to cHSP60, compared with 16 (32%) controls (p=0.77 and p=0.27, respectively). The rate of seropositivity to *C. trachomatis* antigens did not correlate with age, socioeconomic background, ethnicity, early sexual activity, type of contraception used and number of sexual partners reported. Also, seropositivity to MOMP or cHSP60 was not associated with historical results for *C. trachomatis* vaginal swab testing, although only performed in 25% of patients and exceptionally positive. Finally, seropositivity was not associated with self-reported *C. trachomatis* infection (data not shown).

Secondary outcomes
Pregnancy rates did not differ between groups (71% of patients in the SLE group and 72% of controls).

Obstetrical outcomes are shown in table 2. There was no difference in the number of live births and spontaneous fetal losses. Extrauterine pregnancies were reported in three patients with SLE and did not occur in controls. Elective abortions were more frequent in SLE and reported by 48% of women with pregnancies, compared with 22% of controls. Pre-eclampsia, eclampsia and hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome occurred only in the SLE group and were reported in 10% of patients with pregnancies. No association was observed between elective abortion or any pregnancy complication (fetal losses, prematurity in subjects with SLE or in controls; extrauterine pregnancy, pre-eclampsia/eclampsia in subjects with SLE) and either anti-MOMP or anti-cHSP60 antibodies (data not shown).

Cardiovascular events are listed in table 3. Taken together, arterial and venous vascular events had occurred in 29% of women with SLE, compared with 4% of controls (p=0.001). This difference was mainly due to venous thromboembolism, which had only occurred in patients with SLE. Stroke and myocardial infarction were more frequent in SLE, although not statistically significant. Patients with arterial vascular events were older than those without, although this was not statistically significant (median age 51 years compared with 43; p=0.055). Within the SLE group, vascular events occurred in 15 of 41 (37%) of those without a history of positive testing for antiphospholipid antibodies, compared with 7 of 42 (16%) of those negative (p=0.047). This association was only seen with arterial vascular events, which occurred in 10 patients positive to antiphospholipid antibodies,

Table 1 Baseline characteristics of 84 women with SLE and 50 age-matched controls

	Cases	Controls	P values
Age, mean±SD, years	44±13	44±14	0.97
Ethnic origin, n (%)			0.001
Caucasian	49 (58)	44 (88)	
African	14 (17)	1 (2)	
Asian	11 (13)	1 (2)	
Other	10 (12)	4 (8)	
Higher education*, n (%)	48 (57)	36 (72)	0.099
Age at menarche, n (%), years			0.58
9–11	12 (14)	11 (22)	
12–13	44 (52)	22 (44)	
14–15	22 (26)	12 (24)	
≥16	6 (7)	5 (10)	
Having used contraceptive methods, n (%)	77 (92)	46 (92)	1.00
Oral contraception	55 (66)	41 (82)	0.048
Contraceptive implant	2 (2)	3 (6)	0.36
Medroxyprogesterone acetate injection	8 (10)	0 (0)	0.025
Diaphragm	4 (5)	5 (10)	0.29
Condom	34 (41)	27 (54)	0.15
Intrauterine device	23 (27)	13 (26)	1.00
Age at first sexual intercourse, n (%), years			0.36
<16	8 (10)	8 (16)	
16–20	46 (55)	31 (62)	
>20	26 (31)	10 (20)	
Never had sexual intercourse	4 (5)	1 (2)	
Number of sexual partners, n (%)			0.34
0–1	26 (31)	12 (24)	
2–4	33 (39)	17 (34)	
≥5	25 (30)	21 (42)	
Menopause at inclusion, n (%)	41 (49)	18 (36)	0.16
Last gynaecological visit <2 years ago, n (%)	66 (79)	45 (90)	0.10
Having a gynaecologist in private practice, n (%)	50/83 (60)	36/49 (74)	0.14

*Defined as continued education after obligatory school.

compared with 1 patient negative to antiphospholipid antibodies (p=0.003). No association was found between cardiovascular events (stroke, myocardial infarction, pulmonary embolism, deep venous thrombosis) and either anti-MOMP or anti-cHSP60 antibodies, in patients with SLE or in controls (data not shown). The results from screening procedures for cervical cancer were available in 80% of participants, with abnormal tests reported in 30% of screened subjects with SLE and 27% of controls (NS). Findings were atypical squamous cells of undetermined significance (ASC-US) in 8 patients, low-grade squamous intraepithelial lesions/cervical intraepithelial neoplasia-grade 1 (LSIS/CIN-1) in 15, high-grade squamous intraepithelial lesions/cervical intraepithelial neoplasia-grade 2-3 (HSIL/CIN2-3) in 7, and 1 had an unspecified abnormal result.

Association of serological results within the SLE group

Disease characteristics and treatment modalities of patients with SLE are shown in table 4. Subjects with SLE had a median disease duration of 8 years and fulfilled a median number of 6 ACR classification criteria for SLE. Most patients with lupus suffered from photosensitive rash, arthritis and haematological disorder. Forty-three (52%) had lupus nephritis, with four patients under permanent dialysis or one having received a kidney graft. The median SELENA-SLEDAI score was 5, consistent with moderately active SLE in most patients. Seventy-one per cent of patients were taking antimalarials, and approximately half were taking low-dose corticosteroids and/or immunosuppressants during the month preceding assessment. The most frequently used immunosuppressant was mycophenolate mofetil (45% of subjects under
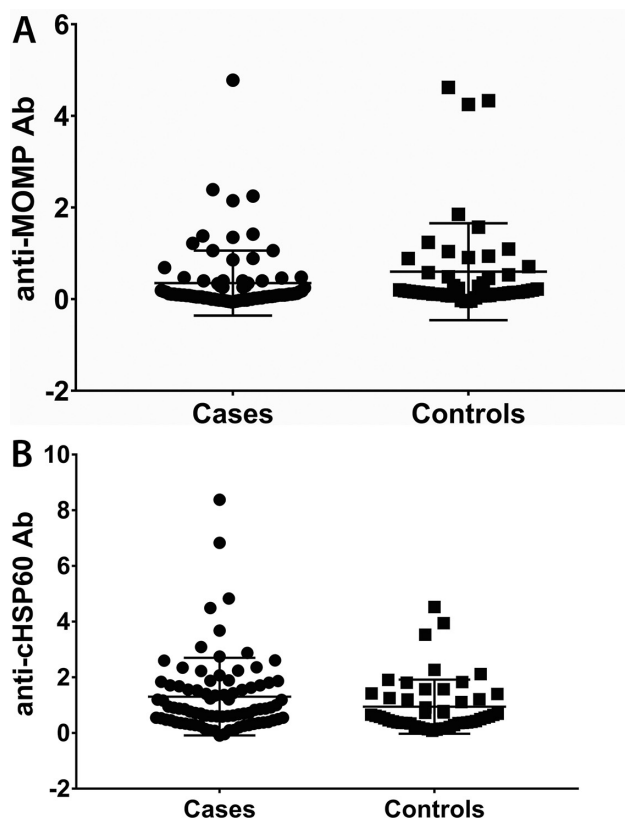
Figure 2 Antibody (Ab) titres to *Chlamydia trachomatis* major outer membrane protein (A) and chlamydial heat-shock protein-60 (cHSP60) (B) in 84 women with SLE (cases) and 50 age-matched controls.

Table 3 Cardiovascular events in 84 women with SLE and 50 age-matched controls

History of cardiovascular event, Subjects, n (%)	Cases	Controls	P values
Arterial events*	11 (13)	2 (4)	0.13
Stroke	9 (11)	1 (2)	0.09
Myocardial infarction	4 (5)	1 (2)	0.65
Venous event†	15 (18)	0 (0)	0.001
Deep vein thrombosis	10 (12)	0 (0)	0.013
Pulmonary embolism	7 (8)	0 (0)	0.045
Arterial and/or venous events	22 (26)	2 (4)	0.002

*2 patients with SLE suffered from both stroke and myocardial infarction.
†4 patients with SLE had both venous and arterial vascular events.

immunosuppression). Twelve patients received biologics at the time of assessment (rituximab in 6, belimumab in 4, abatacept in 1 and tocilizumab in 1 patient). Seventy-five per cent of patients had been under immunosuppressant therapy at least once during the course of the disease.

Patient characteristics with regard to cHSP60 serology are shown in table 4. Those positive for cHSP60 antibodies had more often a history of pericarditis (42% in cHSP60-positive, compared with 19% in cHSP60-negative; p=0.03). As previously reported, there was no significant association between cHSP60 seropositivity and vascular events (17% of arterial events in cHSP60-positive, compared with 10% in cHSP60-negative). There was

no significant association of antibodies to cHSP60 with other disease features, including global disease activity and treatment modalities. No associations between anti-MOMP and clinical characteristics were found (data not shown).

There was a significant association between anti-cHSP60 and abnormal Pap smear results in patients with SLE (50% with abnormal results in patients with anti-cHSP60 antibodies, compared with 16% in patients without these; p=0.004). No such difference was found in controls, nor was seropositivity to MOMP associated with cervical dysplasia in either group.

DISCUSSION

In this single-centre study, prevalence of antibodies to chlamydial MOMP and cHSP60 did not significantly differ between women with SLE and age-matched controls. The seroprevalence of 10% specific antibodies to *C. trachomatis* we found is similar to the reported frequency of *C. trachomatis* exposure in the general Swiss[6] and European female population.[8 9] Only a minority of subjects had undergone vaginal swab tests for *C. trachomatis*, and serology did not correlate with these findings nor with self-reported *C. trachomatis* infection. When comparing

Table 2 Pregnancy outcomes in 60 women with SLE and 36 age-matched controls

Pregnancy outcomes Subjects, n (%)	Cases	Controls	P values
History of elective abortion	29 (48)	8 (22)	0.017
History of fetal loss	23 (38)	11 (31)	0.51
≥3 fetal losses	3/23 (8)	1/11 (9)	1.00
History of ectopic pregnancy	3/53 (6)	0 (0)	0.28
Live births	52 (87)	31 (86)	1.00
Number of live births, median (IQR)	2 (1–3)	2 (2–3)	0.77
History of pre-eclampsia or eclampsia, n (%)	5/48 (10)	0/29 (0)	0.15
History of preterm birth, n (%)	5/48 (10)	1/29 (3)	0.40

Table 4 Disease features of 84 patients with SLE according to cHSP60 serology

	All cases (N=84)	Anti-cHSP60-positive (n=36)	Anti-cHSP60-negative (n=48)	P values
Age at assessment, mean±SD (range), years	44.2±13.4 (22–81)	41.9±11.6	45.9±14.5	0.18
Disease duration, median (IQR), years	8 (3–15)	9.5 (5–15)	7 (2–14)	0.31
Number of ACR classification criteria fulfilled, median (IQR)*	6 (5–7)	6 (5–8)	6 (5–7)	0.98
Nasopharyngeal ulcers, n (%)	26/83 (31)	15 (42)	11 (23)	0.097
Pericarditis, n (%)	24/83 (29)	15 (42)	9 (19)	0.03
Antiphospholipid antibodies, n (%)	41/83 (49)	22 (61)	19 (40)	0.077
Disease activity at assessment				
SELENA-SLEDAI, median (IQR)	5 (2–10)	6 (2–9)	4.5 (2–12)	0.68
Treatment				
Systemic corticosteroids during the past month, n (%)	40/77 (52)	20 (59)	20 (47)	0.36
High-dose steroids (prednisone >10 mg/day), n (%)	3/40 (8)	1 (5)	2 (10)	1.00
Antimalarial therapy during the past month, n (%)	55/77 (71)	26 (77)	29 (67)	0.45
Immunosuppressant during the past month, n (%)†	42/77 (55)	18 (53)	24 (56)	0.82
Immunosuppressant ever, n (%)	58 (75)	27 (79)	31 (66)	0.22
Damage				
Any thrombotic or ischaemic event, n (%)	22 (26)	11 (31)	11 (23)	0.46
Dialysis or renal transplantation, n (%)	5 (6)	4 (11)	1 (2)	0.16

cHSP60=chlamydial heat-shock-protein-6; SD=SD deviation; IQR=IQR range;
*No significant association found for photosensitivity, malar rash, discoid rash, arthritis, pleuritic, renal disorder, haematological disorder, seizures, psychosis, ANA, antidouble-stranded DNA antibodies and anti-Smith antibodies.
†Mycophenolate mofetil (n=19), azathioprine (n=9), rituximab (n=6), belimumab (n=4), methotrexate (n=4), calcineurin inhibitor (n=2), abatacept (n=1) and tocilizumab (n=1), without significant differences between groups.
ACR, American College of Rheumatology; cHSP60, chlamydial heat-shock protein-6; SELENA-SLEDAI, Safety of Estrogens in Lupus Erythematosus - National Assessment-Systemic Lupus Erythematosus Disease Activity Index.

known risk factors for sexually transmitted diseases such as early onset of sexual activity and increased number of sexual partners, we did not find a significant association with serology results nor differences between SLE and controls. Aside from these classic risk factors, contraceptive methods differed between SLE and controls, with lower use of combined oral contraceptive in women with SLE and higher use of medroxyprogesterone acetate injections (likely secondary to SLE diagnosis). A higher rate of elective abortion in patients with SLE may reflect a lower socioeconomic status and reduced access to effective contraception. Cases and controls differed in terms of ethnicity, with more patients with SLE being of African or Asian descent. In addition, fewer patients with SLE had achieved higher professional education compared with controls. This may reflect the increased prevalence of SLE in ethnic minorities and lower socioeconomic status.[28 29] Only women with SLE did experience pre-eclampsia, which is a well-known risk in this population.[30] Ectopic pregnancies were reported by three patients, all in the SLE group. However, the rate of live births was the same in SLE and controls. No association between pregnancy outcomes and seropositivity to *C. trachomatis* was found. These results contrast with previous observations suggesting a relationship between miscarriage and *C. trachomatis* infection.[6 31] Cardiovascular events were significantly more frequent in women with SLE compared with

age-matched controls. Indeed, accelerated atherosclerosis and thrombotic events linked to antiphospholipid antibodies are feared complications of SLE. Most of our patients with lupus had long-standing disease and half had severe disease with renal involvement. While vascular events were more frequent in patients with SLE with antiphospholipid antibodies, we did not find an association with antibodies to MOMP or cHSP60.

Very few studies have assessed exposure to *C. trachomatis* in SLE. One study on reactive arthritis had included 30 patients with SLE, which displayed a threefold increase in seroprevalence against *C. trachomatis* compared with healthy controls.[18] A study assessed *C. trachomatis* by vaginal swab test and did not find an increased prevalence in patients with SLE.[32] Another study investigated the potential relationship between coronary calcifications as a marker of atherosclerotic burden and PCR for *C. pneumoniae* and *C. trachomatis* in the peripheral blood monocytes of patients with SLE, without finding a clear association.[33] Most patients with SLE included in our study had disease features beyond articular and cutaneous involvement. A majority had received immunosuppressive treatment. Most patients had long-lasting and well-controlled disease at assessment. Antibodies to cHSP60 were slightly more prevalent in SLE and were associated with a history of lupus pericarditis. Others have found that a history of lupus pericarditis might define a

subgroup of patients with more aggressive disease.[34] We did not find an association of anti-cHSP60 antibodies with other features of severe SLE, although more patients in this group had end-stage renal disease. A possible explanation for an association of antibodies to cHSP60 with pericarditis could be the tendency of *Chlamydia* species to induce serositis, as in the Fitz-Hugh-Curtis syndrome or in some reported cases of pericarditis and myocarditis.[35] However, no association between anti-MOMP antibodies and pericarditis was found. Another finding with regard to anti-cHSP60 antibodies was their association with abnormalities on screening tests for cervical cancer. One could argue that human papilloma virus (HPV) usually underlying cervical dysplasia might be transmitted together with *C. trachomatis*, as coinfection frequently occurs and facilitates oncogenesis,[36] a finding reflected by the presence of antibodies to cHSP60. On the other hand, no association between cervical dysplasia and antibodies to *C. trachomatis* MOMP was found that would support this hypothesis. More studies relying on PCR techniques are needed to assess the role of HPV and *C. trachomatis* coinfection in SLE. Upregulation of HSP60 expression has been reported in women with cervical cancer.[37] One could argue that upregulation of HSP60 by dysplastic cells in the cervix might trigger cross-reactive antibodies, given the high grade of homology with cHSP60. We did however not assess whether antibodies to cHSP60 cross-react with human HSP60, which was beyond the scope of this study. It is finally possible that due to the multiplicity of statistical tests performed, the association of anti-cHSP60 antibodies with pericarditis and cervical dysplasia in the lupus subset is due to chance. We did not find an increase in cervical dysplasia in patients with SLE compared with controls. However, larger population-based studies have shown that cervical dysplasia is an important issue in SLE and that the risk increases in women with long-standing immunosuppression.[38]

Although this study has limitations, such as its cross-sectional design and the limited sample size, its strengths lie in the detailed assessment of participants and the use of two reliable and complementary serological tests for *C. trachomatis*. We considered the possibility that infection with *C. trachomatis* might drive autoimmunity in SLE, but the results show an equal distribution of antibodies to *C. trachomatis* in age-matched controls, which speaks against a prominent role of this pathogen in lupus and associated damage. Studies relying on PCR techniques to detect ongoing infection with *C. trachomatis* in patients with early lupus are needed to confirm these results. Our study results also support known issues in women with SLE, which are increased risk of adverse obstetrical outcomes and cardiovascular events.

REFERENCES

1. Lateef A, Petri M, Lateef A. Hormone replacement and contraceptive therapy in autoimmune diseases. *J Autoimmun* 2012;38:J170–J176.
2. Sánchez-Guerrero J, Uribe AG, Jiménez-Santana L, *et al*. A trial of contraceptive methods in women with systemic lupus erythematosus. *N Engl J Med* 2005;353:2539–49.
3. Rojas-Villarraga A, Torres-Gonzalez JV, Ruiz-Sternberg ÁM. Safety of hormonal replacement therapy and oral contraceptives in systemic lupus erythematosus: a systematic review and meta-analysis. *PLoS One* 2014;9
4. Ulff-Møller CJ, Nielsen NM, Rostgaard K, *et al*. Epstein-Barr virus-associated infectious mononucleosis and risk of systemic lupus erythematosus. *Rheumatology* 2010;49:1706–12.
5. Batteiger BE, Xu F, Johnson RE, *et al*. Protective immunity to chlamydia trachomatis genital infection: evidence from human studies. *J Infect Dis* 2010;201:178–89.
6. Baud D, Goy G, Jaton K, *et al*. Role of Chlamydia trachomatis in miscarriage. *Emerg Infect Dis* 2011;17:1630–5.
7. Lewis D, Newton DC, Guy RJ, *et al*. The prevalence of chlamydia trachomatis infection in Australia: a systematic review and meta-analysis. *BMC Infect Dis* 2012;12:113.
8. van Aar F, de Moraes M, Morré SA, *et al*. Chlamydia trachomatis IgG seroprevalence in the general population of the Netherlands in 1996 and in 2007: differential changes by gender and age. *Sex Transm Infect* 2014;90:434–40.
9. Baud D, Thomas V, Arafa A, *et al*. Waddlia chondrophila, a potential agent of human fetal death. *Emerg Infect Dis* 2007;13:1239–43.
10. Bally F, Quach A, Greub G, *et al*. Opportunistic testing for urogenital infection with Chlamydia trachomatis in south-western Switzerland, 2012: a feasibility study. *Euro Surveill* 2015;20:21051.
11. Baud D, Jaton K, Bertelli C, *et al*. Low prevalence of chlamydia trachomatis infection in asymptomatic young Swiss men. *BMC Infect Dis* 2008;8:45.
12. Zeidler H, Hudson AP. New insights into chlamydia and arthritis. promise of a cure? *Ann Rheum Dis* 2014;73:637–44.
13. Linhares IM, Witkin SS. Immunopathogenic consequences of Chlamydia trachomatis 60 kDa heat shock protein expression in the female reproductive tract. *Cell Stress Chaperones* 2010;15:467–73.
14. Eggert-Kruse W, Scholz S, Kirschfink M, *et al*. Recurrent miscarriages, innate immunity, and autoimmune reaction to chlamydial 60-kDa heat shock protein-is there an association? Fertil Steril 2014;101:1675–80.

15. Swanborg RH, Boros DL, Whittum-Hudson JA, *et al*. Molecular mimicry and horror autotoxicus: do chlamydial infections elicit autoimmunity? *Expert Rev Mol Med* 2006;8:1–23.
16. Wick G, Jakic B, Buszko M, *et al*. The role of heat shock proteins in atherosclerosis. *Nat Rev Cardiol* 2014;11:516–29.
17. Dieudé M, Correa JA, Neville C, *et al*. Association of autoantibodies to heat-shock protein 60 with arterial vascular events in patients with antiphospholipid antibodies. *Arthritis Rheum* 2011;63:2416–24.
18. Keat AC, Thomas BJ, Taylor-Robinson D, *et al*. Evidence of Chlamydia trachomatis infection in sexually acquired reactive arthritis. *Ann Rheum Dis* 1980;39:431–7.
19. Bas S, Muzzin P, Vischer TL. Chlamydia trachomatis serology: diagnostic value of outer membrane protein 2 compared with that of other antigens. *J Clin Microbiol* 2001;39:4082–5.
20. Ness RB, Soper DE, Richter HE, *et al*. Chlamydia antibodies, chlamydia heat shock protein, and adverse sequelae after pelvic inflammatory disease: the PID Evaluation and Clinical Health (PEACH) Study. *Sex Transm Dis* 2008;35:129–35.
21. Singh AG, Chowdhary VR. Pregnancy-related issues in women with systemic lupus erythematosus. *Int J Rheum Dis* 2015;18:172-81.
22. Ruiz-Irastorza G, Crowther M, Branch W, *et al*. Antiphospholipid syndrome. *Lancet* 2010;376:1498–509.
23. Hochberg MC. Updating the American college of rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1725;1997;40(9.
24. Petri M, Orbai A-M, Alarcón GS, *et al*. Derivation and validation of the systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. *Arthritis & Rheumatism* 2012;64:2677–86.
25. Baud D, Regan L, Greub G. Comparison of five commercial serological tests for the detection of anti-Chlamydia trachomatis antibodies. *European Journal of Clinical Microbiology & Infectious Diseases* 2010;29:669–75.
26. Bombardier C, Gladman DD, Urowitz MB, *et al*. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992;35:630–40.
27. Streiner DL, Norman GR. Correction for multiple testing: is there a resolution? *Chest* 2011;140:16–18.
28. Rees F, Doherty M, Grainge M, *et al*. The incidence and prevalence of systemic lupus erythematosus in the UK. *Ann Rheum Dis* 2014:1999–2012.
29. Feldman CH, Hiraki LT, Liu J, *et al*. Epidemiology and sociodemographics of systemic lupus erythematosus and lupus nephritis among US adults with Medicaid coverage, 2000-2004. *Arthritis & Rheumatism* 2013;65:753–63.
30. Eudy AM, Jayasundara M, Haroun T, *et al*. Reasons for cesarean and medically indicated deliveries in pregnancies in women with systemic lupus erythematosus. *Lupus* 2017;961203317720525.
31. Hornung S, THUONG BC, Gyger J, *et al*. Role of Chlamydia trachomatis and emerging Chlamydia-related bacteria in ectopic pregnancy in Vietnam. *Epidemiol Infect* 2015;143:2635–8.
32. CostaPinto L, Olavarria VG, Grassi MFR, *et al*. Prevalence of Chlamydia trachomatis endocervical infection in systemic lupus erythematosus patients and evaluation of the risk for HPV-induced lesions. *Rheumatol Int* 2013;33:631–6.
33. Kitumnuaypong T, Scalzi LV, Nalbant S. Jr. Is there a role for Chlamydia pneumoniae infection in systemic lupus erythematosus and in the associated atherosclerotic cardiovascular disease? Clinical and experimental rheumatology 2004;22:339–42.
34. Ryu S, Fu W, Petri MA. Associates and predictors of pleurisy or pericarditis in SLE. *Lupus Sci Med* 2017;4:e000221.
35. Fairley CK, Ryan M, Wall PG, *et al*. The organisms reported to cause myocarditis and pericarditis in England and Wales. *J Infect* 1996;32:223–5.
36. Zhu H, Shen Z, Luo H, *et al*. Chlamydia trachomatis infection-associated risk of cervical cancer. *Medicine* 2016;95:e3077.
37. Hwang YJ, Lee SP, Kim SY, *et al*. Expression of heat shock protein 60 kDa is upregulated in cervical cancer. *Yonsei Med J* 2009;50:399–406.
38. Wadstrom H, Arkema EV, Sjowall C, *et al*. Cervical neoplasia in systemic lupus erythematosus: a nationwide study. *Rheumatology* 2017;56:613–9.