Systemic lupus erythematosus in a multiethnic cohort (LUMINA): XXVIII. Factors predictive of thrombotic events


Objective. To determine the relationship between the presence of antiphospholipid (aPL) antibodies, hydroxychloroquine use and the occurrence of thrombotic events in patients with systemic lupus erythematosus (SLE).

Methods: Four hundred and forty-two SLE patients from the LUMINA (Lupus in Minorities: Nature vs Nurture) cohort, a multiethnic (Hispanics from Texas, n = 99 and Puerto Rico, n = 36; African Americans, n = 172; and Caucasians, n = 135) cohort, were studied by generalized estimating equation (GEE) to determine the relationship between antiphospholipid (aPL) antibodies (measured as IgG and IgM aPL antibodies and/or the lupus anticoagulant) at enrolment or historically prior to enrolment, hydroxychloroquine use (ever) and the occurrence of thrombotic (central and/or peripheral, arterial and/or venous) events after adjusting for known and possible confounders [socioeconomic–demographic features, smoking, disease activity and damage, serum cholesterol levels, anti-oxidized low-density lipoprotein IgG and IgM antibodies, and high-sensitivity (hs) C-reactive protein]. Postanalysis correlation between aPL and anticardiolipin (aCL) assays was attempted by performing aCL assays on random samples of patients whose aPL status was known.

Results. A number of clinical variables were significant in the univariable analyses; however, in the multivariable GEE analyses, only smoking [odds ratio (OR) 2.777, 95% confidence interval (CI) 1.317–5.852] and disease activity as measured by the SLAM (Systemic Lupus Activity Measure) (OR 1.099; 95% CI 1.053–1.147) were significant. In particular, hydroxychloroquine use, which appeared to be protective against thrombotic events in the univariable analyses, was not retained in the multivariable analyses. aPL antibodies were not significant in either analysis. Few additional aPL-positive patients emerged from the validation study.

Conclusions. Smoking and disease activity emerged as important determinants in the occurrence of thrombotic events in our patients. Comprehensive treatment strategies should be directed to both smoking cessation and control of disease activity in patients with SLE.

Key words: Systemic lupus erythematosus, Thrombosis, Hydroxychloroquine, Antiphospholipid antibodies, LUMINA.
agent [14–16], exerting interference in the release of arachidonic acid from platelets [17, 18], blocking platelet aggregation and adhesion, and attenuating the size of thrombi [19]; furthermore, in mice it has been shown to reverse the thrombogenic properties of aPL antibodies [20].

In this study we attempted to explore the relationship between the presence of aPL antibodies and the use of hydroxychloroquine in the risk of thrombotic phenomena in a large multiethnic cohort of SLE patients, after adjusting for other known confounders.

Patients and methods

Study patients

The LUMINA (Lupus in Minorities: Nature versus Nurture) cohort consists of SLE patients with disease duration of ≤5 yr enrolled in a longitudinal study of outcome from three geographical areas and four institutions in the USA. The Institutional Review Board of each participating centre approved the LUMINA study, and written informed consent was obtained from each subject according to the Declaration of Helsinki. In the present study, 442 LUMINA patients (99 Hispanics from Texas, 36 Hispanics from the Island of Puerto Rico, 172 African Americans and 135 Caucasians), in whom aPL status at enrolment into the LUMINA cohort was known, were included in these analyses. The constitution of this cohort, the variables obtained prior to, at and after enrolment in the cohort (T0), and the frequency and nature of the study visits have been described in detail previously [21–23].

Briefly, patients are eligible to participate if they meet four of the American College of Rheumatology (ACR) criteria for the classification of SLE [24, 25], have disease duration of ≤5yr at T0 and live within the catchment areas of the participating institutions (University of Texas Health Science Center at Houston, TX; University of Alabama at Birmingham, AL; University of Texas Medical Branch at Galveston, TX, and University of Puerto Rico, San Juan, Puerto Rico) and are of Hispanic (Mexican American or Central American from Texas, and Puerto Rican from the Island of Puerto Rico), African American or Caucasian ethnicity. Information obtained at T0 and follow-up study visits includes socioeconomic–demographic and clinical data (encompassing medical history, current and previous treatment, laboratory and immunological data) from the time of diagnosis (the date patients met ACR criteria) onward. Disease activity according to the Systemic Lupus Activity Measure (SLAM) [26, 27] and damage caused by the disease or its treatments according to the Systemic Lupus International Collaborating Clinics (SLICC)/American College of Rheumatology Damage Index (SDI) [28] were also recorded at all visits except for patients with disease duration of less than 6 months; in these cases, the damage index could not have been obtained at T0. The SDI scores are presented without adjusting for the thrombotic events that may have been captured in this index.

Study visits

Follow-up visits were planned every 6 months for the first year and annually thereafter. Patients were encouraged to maintain contact with study coordinators and to notify them of any significant intervening life event.

Variables

Recorded thrombotic events were defined as either arterial or venous occurring after T0. Vascular arterial events included myocardial infarction, angina, stroke, intermittent claudication and/or peripheral arterial thrombosis. Venous thrombosis was visceral and/or peripheral. These thrombotic events were recorded during regularly scheduled study visits according to information provided by the patients and the data available in the medical records reviewed for the visit. No specific imaging or other ancillary studies were performed to confirm or rule out the prior occurrence of a thrombotic event. However, these studies had been performed at the time of the thrombotic event, if clinically indicated. These events were the dependent variable in generalized estimating equation (GEE) analyses.

Independent variables were all from the baseline visit (unless noted below), and included the presence of aPL antibodies (IgG and/or IgM) and/or the lupus anticoagulant (LAC), age, gender, ethnicity, smoking, disease duration, azathioprine, hydroxychloroquine and glucocorticoid use, the presence of mucocutaneous, joint, serosal, neuropsychiatric manifestations (as defined in the Supplementary data, available at Rheumatology Online), SLAM and SDI scores (as determinants of disease activity and damage, respectively), anti-oxidized low-density lipoprotein IgG and IgM (anti-oxLDL) antibodies [for the purpose of this study, abnormality was defined as greater than the mean (plus 1 standard deviation) of values for 50 unselected healthy individuals] by enzyme-linked immunosorbent assay (ELISA) (Speciality Laboratories, Santa Monica, CA, USA) [29], C-reactive protein [CRP; measured as high-sensitivity CRP (hs-CRP) by immunometric assay (Immulite 2000 Diagnostic Procedures Corporation, Los Angeles, CA, USA)] and serum cholesterol level.

The presence of disease manifestations, damage and activity indices reflect those recorded at the visit closest to the time of the thrombotic event. Given that precise exposure data, including dose and duration, were not available prior to T0 for hydroxychloroquine, glucocorticoid and azathioprine, their use was defined as that occurring prior to the recorded thrombotic event (from T0) regardless of the dose or exposure duration.

aPL antibodies were assayed from sera collected at T0 and yearly thereafter by commercially available ELISA (Louisville APL Diagnostics, Doraville, GA, USA). Patients with moderate titles, at T0 or subsequently, were considered to be aPL-positive (>13 GPL or MPL units). Patients in whom aPL (IgM or IgG) antibodies or the LAC were present between the time of diagnosis and T0 were also considered aPL-positive. LAC was determined by the Staclot assay [30, 31]. In addition, sera from 34 patients with positive IgG or IgM aPL antibodies and 34 patients with negative IgG or IgM aPL antibodies were randomly chosen for the assessment of IgG and IgM anti-cardiolipin antibodies using a commercially available ELISA (Zeus Scientific, Raritan, NJ, USA).

Statistical analyses

Univariable and multivariable analyses were performed using the GEE. GEE was chosen over other multivariable models, such as logistic or Cox hazards multivariable regressions, as if was felt that patients may be in and out of risk of developing a thrombotic event; in addition, GEE allows the maximal utilization of longitudinal data (regardless of the number of visits or the interval at which they occur). Variables with P ≤ 0.10 in the univariable analyses were entered into a stepwise GEE regression. The data are reported as odds ratios (OR) with their corresponding 95% confidence intervals (CI). A comparison of aPL status in those patients in whom aCL antibodies had been obtained was also performed.

Results

Of 442 LUMINA patients in whom aPL status was known, 46 were identified as having 51 recorded thrombotic events over
1446 visits followed over a mean (s.d.) of 88.4 (23.7) months (range 3.9–98.5 months).

Univariable analyses

The relationship between the different socioeconomic-demographic and clinical variables and the occurrence of thrombotic events is shown in Table 1. Of all the variables examined, smoking use, disease activity, disease damage, the presence of mucocutaneous and serosal manifestations, and glucocorticoid use were found to be associated with the occurrence of thrombotic events, while azathioprine use was marginally significant. In contrast, hydroxychloroquine use was found to be protective against these events. aPL antibodies were not associated with the occurrence of thrombotic events. All other variables examined, including age, gender, ethnicity, hs-CRP, cholesterol level, anti-oxidized IgG and IgM antibodies and other disease manifestations, were not associated, either positively or negatively, with the occurrence of thrombotic events.

Multivariable analysis

In multivariable analyses (Table 2), the occurrence of thrombotic events was associated with smoking (OR 2.777, 95% CI 1.317–5.852, P = 0.0073) and with disease activity (OR 1.099, 95% CI 1.053–1.147, P < 0.0001).

aPL and aCL assays

When comparing the limited number of samples in which aPL and aCL status was determined, a sensitivity between 46 and 50%, specificity between 96 and 98% and overall accuracy between 82 and 85% were found (data not shown). Given that very few aPL-positive patients were added with the aCL assays, these data did not substantially change the overall univariable and multivariable analyses presented (data not shown).

Discussion

We have examined by GEE analyses the factors that independently contribute to the occurrence of thrombotic events (arterial and/or venous; peripheral and/or central) in the LUMINA cohort. None of the socioeconomic-demographic features examined, including age, gender and ethnicity, were found to independently contribute to the occurrence of thrombotic events, with the exception of smoking (OR 2.777). Other variables that were either significant (P < 0.05) in the univariable analyses (damage, serosal and mucocutaneous manifestations and hydroxychloroquine use) or borderline significant (azathioprine use) were not retained in the multivariable GEE model.

We found no independent effect of either hydroxychloroquine or glucocorticoids except having previously shown that both are either protective against or contribute to damage accrual in SLE [32–34]. It is possible that the effect of hydroxychloroquine has been somewhat attenuated by smoking, as has been described previously [35, 36].

The very strong association found between disease activity and thromboses probably reflects the effect of ongoing inflammation in the vascular system. Furthermore, smoking and disease activity have been found to be associated [37]. Disease activity should be controlled if its direct and indirect detrimental consequences are to be prevented, given that thrombotic events may herald the occurrence of initial damage in lupus patients [38].

The association between smoking and the occurrence of thrombotic events was expected [33]. Smoking has been demonstrated to promote clotting abnormalities; in the lupus patient, this effect could be even more efficient (and thus deleterious) than in healthy individuals.

Contrary to what we had hypothesized based on the published literature [2, 9, 39–41], an association between aPL antibodies (each one individually or all taken together) was not found in either the univariable or the multivariable analyses even when a second assay was performed in a selected number of patient samples (not many aPL-positive patients were missed initially). Another possible explanation for our negative findings resides in the fact that aPL status was not examined around the time the thrombotic event occurred; likewise, aPL status was not assessed in a systematic manner at diagnosis (except for the LUMINA incident cases); rather, we used historical data which had never been examined by GEE analyses among LUMINA patients by generalized estimating equation analyses.

Table 1. Relationship between socioeconomic and clinical variables and thrombosis by univariable analyses among LUMINA patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>1.010</td>
<td>0.089–1.030</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (% women)</td>
<td>0.756</td>
<td>0.279–2.048</td>
<td>NS</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic, Texas</td>
<td>0.997</td>
<td>0.509–1.952</td>
<td>NS</td>
</tr>
<tr>
<td>Hispanic, Puerto Rico</td>
<td>1.419</td>
<td>0.436–4.613</td>
<td>NS</td>
</tr>
<tr>
<td>African–American</td>
<td>1.382</td>
<td>0.715–2.672</td>
<td>NS</td>
</tr>
<tr>
<td>Caucasian</td>
<td>0.692</td>
<td>0.328–1.460</td>
<td>0.0112</td>
</tr>
<tr>
<td>Smoking</td>
<td>2.642</td>
<td>1.248–5.593</td>
<td>0.0112</td>
</tr>
<tr>
<td>Disease duration (months): mean (s.d.)</td>
<td>0.980</td>
<td>0.955–1.005</td>
<td>NS</td>
</tr>
<tr>
<td>Disease manifestations (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucocutaneous</td>
<td>2.482</td>
<td>1.199–5.136</td>
<td>0.0143</td>
</tr>
<tr>
<td>Articular</td>
<td>1.554</td>
<td>0.664–3.639</td>
<td>NS</td>
</tr>
<tr>
<td>Serosal</td>
<td>3.423</td>
<td>1.938–6.047</td>
<td>0.0001</td>
</tr>
<tr>
<td>Renal</td>
<td>1.564</td>
<td>0.872–2.803</td>
<td>NS</td>
</tr>
<tr>
<td>Neuropsychiatric</td>
<td>1.394</td>
<td>0.725–2.680</td>
<td>NS</td>
</tr>
<tr>
<td>SLAM score: mean (s.d.)</td>
<td>1.112</td>
<td>1.068–1.158</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SDI score: mean (s.d.)</td>
<td>1.271</td>
<td>1.134–1.425</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cholesterol: mean (s.d.)</td>
<td>1.002</td>
<td>0.996–1.009</td>
<td>NS</td>
</tr>
<tr>
<td>High sensitivity-CRP: mean (s.d.)</td>
<td>1.004</td>
<td>0.999–1.010</td>
<td>NS</td>
</tr>
<tr>
<td>aPL positivity (%)</td>
<td>0.747</td>
<td>0.408–1.368</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-oxLDLe IgG: mean (s.d.)</td>
<td>0.999</td>
<td>0.997–1.003</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-oxLDLe IgM: mean (s.d.)</td>
<td>1.001</td>
<td>0.998–1.005</td>
<td>NS</td>
</tr>
<tr>
<td>Hydroxychloroquine use (%)</td>
<td>0.536</td>
<td>0.304–0.946</td>
<td>0.0314</td>
</tr>
<tr>
<td>Glucocorticoid use (%)</td>
<td>2.016</td>
<td>1.044–3.891</td>
<td>0.0368</td>
</tr>
<tr>
<td>Azathioprine use (%)</td>
<td>2.070</td>
<td>0.968–4.422</td>
<td>0.0606</td>
</tr>
</tbody>
</table>

Only P values <0.10 are shown.

Systemic Lupus Activity Measure.

Systemic Lupus International Collaborating Clinics Damage Index.

Antiphospholipid antibodies IgM and IgG and the lupus anti-coagulant.

Anti-oxidized IgG and IgM low-density lipoprotein antibodies. NS, not significant.

Table 2. Variables predictive of thrombotic events among LUMINA patients by generalized estimating equation analyses

<table>
<thead>
<tr>
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<th>95% CI</th>
<th>P</th>
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<tbody>
<tr>
<td>Smoking</td>
<td>2.777</td>
<td>1.317–5.852</td>
<td>0.0073</td>
</tr>
<tr>
<td>SLAM score</td>
<td>1.099</td>
<td>1.053–1.147</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Odds ratio; confidence interval.

Only variables with P ≤ 0.05 are shown.
with arterial events [2, 40], which may in part explain our negative findings; in fact, in separate analyses, LAC was found to be associated with venous thrombotic events [42]. Furthermore, venous and arterial thrombotic events do not have the same exact pathogenic mechanism; this may explain the differences between this study and the others mentioned.

In summary, our data demonstrate the roles of smoking and disease activity in the occurrence of thrombotic events in the lupus patient, while we failed to show the predisposing role of either LAC or other aPL antibodies. While rheumatologists are very efficient in the management of disease activity, we need to focus more on addressing the impact of smoking and enforcing effective strategies for discontinuing this pernicious habit in the lupus patient. The data presented favour addressing both.

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The authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at Rheumatology Online.

References

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