Elevated B lymphocyte stimulator levels are associated with increased damage in an Irish systemic lupus erythematosus cohort

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Abstract

Objective. The overall aim of this study is to identify clinical and serological features that are associated with B lymphocyte stimulator (BLyS) elevation in a homogeneous Caucasian SLE population and thereby identify patients who are most likely to benefit from BLyS blockade.

Methods. Patients with SLE (as per ACR criteria) were recruited. Clinical history, disease activity measures and laboratory measures of disease were recorded. BLyS levels were determined by ELISA.

Results. BLyS elevation was defined as being higher than the 95th percentile of BLyS levels measured in controls. Patients were divided into two groups: those with elevated BLyS levels (group 1, n = 23) and those with normal BLyS levels (group 2, n = 22). Elevated BLyS levels were significantly associated with patients of younger age and shorter disease duration. In keeping with previous reports, patients with elevated BLyS levels had more active disease (SLEDAI 5.1 vs 0.86, P < 0.001); however, our analysis also demonstrates that BLyS elevation was significantly associated with increased organ damage at 5-year follow-up [Systemic Lupus International Collaborating Clinics/ACR Damage Index (SLICC/ACR DI) 0.53 vs 0.13, P = 0.012]. Furthermore, the presence of Sm autoantibody significantly predicted elevated BLyS levels in a Caucasian population. BLyS levels were significantly higher in those with musculoskeletal involvement, malar rash, renal disease and evidence of immunological activity.

Conclusion. BLyS blockade may be most beneficial if introduced early in the course of disease in young Caucasian patients presenting with renal, musculoskeletal and skin disease in an effort to reduce long-term damage.

Key words: systemic lupus erythematosus, B lymphocyte stimulator, age, damage, disease activity.

Introduction

SLE is a systemic autoimmune condition associated with increased morbidity and mortality [1]. High disease activity, as well as persistent disease activity, has been shown to correlate with organ damage and mortality in SLE [2, 3]. Increasing age and longer duration of disease are linked with higher damage scores [4, 5] in part due to the significant side effect profile of corticosteroid use [6]. In SLE, high damage scores (defined as non-reversible change, not related to active inflammation, occurring since the onset of lupus and present for at least 6 months) also predict mortality [6]. Thus the goal for treatment of patients with SLE involves minimizing exposure to potentially hazardous medications while controlling disease activity in a timely fashion.

The introduction of anti-BLyS therapy holds much promise for patient care with two phase 3 trials—BLISS-52 and BLISS-76—showing that anti-BLyS therapy in conjunction with standard SLE therapy significantly improves SLE Responder Index (SRI) at week 52 when compared...
with standard therapy alone [7, 8]. Despite this, many patients remain unresponsive to BLyS pathway intervention, with further studies highlighting the inconsistencies in the relationship between increased BLyS levels and disease activity [9–12]. As variations in both disease activity and disease course in SLE have been documented between groups from different ethnic backgrounds, the inconsistencies noted may well be accounted for by the differences in the populations studied. Therefore there is a pressing need to define both the optimal timing of anti-BLyS therapy and the patients most likely to benefit from BLyS pathway blockade by correlating serum BLyS levels with disease activity indices in a genetically homogeneous population. Thus the primary objective of this study is to investigate differences between BLyS expression in a homogeneous Irish Caucasian population and thereby identify patient characteristics/demographics and disease phenotypes that would most likely benefit from BLyS pathway blockade.

Patients and methods

Study population

The study was approved by the medical ethics committees of both Beaumont and St James hospitals. Participants provided written informed consent. Forty-five patients who met at least four of the ACR classification criteria for SLE were included. Twenty healthy age- and sex-matched controls were also recruited.

Data collection

Patients were included only if they could confirm that they were of Irish descent for three generations. Basic demographic data, the presence of ACR criteria and medication use were recorded. Disease activity was calculated using the Safety of Estrogens in Lupus Erythematosus National Assessment version of the SLE Disease Activity Index (SELENA-SLEDAI). Peripheral blood was obtained from each participant and serum was isolated and stored at −80 °C until use. Organ damage was measured using the Systemic Lupus International Collaborating Clinics/ACR Damage Index (SLICC/ACR DI) score. Initial damage indices were recorded at the time of serum collection with the follow-up damage score being calculated retrospectively through review of the medical records, an approach that has been validated previously [13]. Any patients with incomplete medical records at the 5-year follow-up period were also excluded.

Determination of plasma BLyS levels and lupus autoantibody status

Serum BLyS levels were determined by ELISA according to the recommendations of the manufacturer (R&D Systems). All samples were analysed in duplicate. ELISAs were used to evaluate sera for antibodies to dsDNA, Sm, RNP, Ro, La and cardiolipin (Axis Shield/Diastat Diagnostics).

Statistical analysis

Categorical variables were analysed using Fisher’s exact test. Normally distributed continuous variables were analysed using an unpaired t-test. The Mann–Whitney test was used in instances of non-normality. Spearman’s correlation was used to assess the relationship between serum BLyS levels, disease activity and dsDNA titres. Analyses were performed using GraphPad Prism version 5.04.

Results

Demographic and baseline clinical characteristics of the patients

Forty-five Caucasian patients were enrolled. Baseline patient demographics, disease characteristics and ACR classification criteria are summarized in Table 1. Mean duration of SLE was 7.1 years with a mean baseline SELENA-SLEDAI score of 3.3 (median 2, range 0–14) and a mean baseline damage score (SLICC/ACR DI) of 0.69 (range 0–4).

Elevated BLyS levels in SLE patients presenting with arthritis, malar rash, renal disease and immunological involvement

The mean BLyS level in our control population was 541 pg/ml (range 388–681 pg/ml), whereas the baseline level for SLE patients was 813 pg/ml (range 248–2868 pg/ml), the enhanced BLyS levels observed in SLE patients.

Table 1 Demographic characteristics, ACR criteria, disease activity, damage indices and medication use in SLE patients (n = 45)

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male 5 (11), Female 40 (89)</td>
</tr>
<tr>
<td>Age (years), mean (S.D.)</td>
<td>38.64 (11.1)</td>
</tr>
<tr>
<td>Age at diagnosis (years), mean (S.D.)</td>
<td>31.49 (11)</td>
</tr>
<tr>
<td>Duration of SLE (years), mean (S.D.)</td>
<td>7.1 (5.23)</td>
</tr>
<tr>
<td>Components of SLE diagnostic criteria (ever)</td>
<td></td>
</tr>
<tr>
<td>ANA positive</td>
<td>45 (100)</td>
</tr>
<tr>
<td>Malar rash</td>
<td>38 (84)</td>
</tr>
<tr>
<td>Discoid</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>31 (69)</td>
</tr>
<tr>
<td>Oral ulcers</td>
<td>20 (44)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>37 (82)</td>
</tr>
<tr>
<td>Seroartis</td>
<td>18 (40)</td>
</tr>
<tr>
<td>Renal disease</td>
<td>16 (35)</td>
</tr>
<tr>
<td>CNS disease</td>
<td>9 (20)</td>
</tr>
<tr>
<td>Haematological</td>
<td>34 (76)</td>
</tr>
<tr>
<td>Immunological</td>
<td>29 (64)</td>
</tr>
<tr>
<td>Disease activity measures</td>
<td></td>
</tr>
<tr>
<td>SELENA-SLEDAI score, mean (S.D.)</td>
<td>3 (3.1)</td>
</tr>
<tr>
<td>SLICC/ACR DI at clinic review, mean (S.D.)</td>
<td>0.69 (1.1)</td>
</tr>
<tr>
<td>SLICC/ACR DI at 5 years, mean (S.D.)</td>
<td>1.01 (1.2)</td>
</tr>
<tr>
<td>Prednisone use (current)</td>
<td>20 (44)</td>
</tr>
<tr>
<td>Prednisone dosage (mg/day), median (range)</td>
<td>0 (0–20)</td>
</tr>
<tr>
<td>Immunosuppressive drug use (current)</td>
<td></td>
</tr>
<tr>
<td>HCQ</td>
<td>38 (84)</td>
</tr>
<tr>
<td>AZA</td>
<td>11 (24)</td>
</tr>
<tr>
<td>MMF</td>
<td>4 (9)</td>
</tr>
<tr>
<td>CYC</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

Except where indicated otherwise, values are n (%).
being statistically significant \((P = 0.003)\). BLyS levels were compared for each of the individual ACR diagnostic criteria. BLyS levels were significantly higher in patients with a history of malar rash \((945 \text{ vs } 549 \text{ pg/ml}, P = 0.04)\), arthritis \((950 \text{ vs } 572 \text{ pg/ml}, P = 0.04)\), immunological involvement (defined as abnormal titre dsDNA or anti-Sm or positive aPL) \((1041 \text{ vs } 646 \text{ pg/ml}, P = 0.005)\) and renal disease (biopsy proven) \((1127 \text{ vs } 748 \text{ pg/ml}, P = 0.009)\) (Fig. 1).

Elevated BLyS levels associate with a younger age at diagnosis, shorter disease duration and increased disease activity and damage

Elevated BLyS levels were defined as values higher than the 95th percentile for BLyS levels in the healthy control population \((n = 20, >680 \text{ pg/ml})\). Our patients segregated into two distinct patient groups: those with levels close to those observed for our normal healthy control population.

**Fig. 1** The role of BLyS in Irish SLE patients as per clinical characteristics and disease duration.

(A) BLyS levels in age-matched healthy controls and SLE patients with normal and elevated BLyS. (B) BLyS levels are higher in patients with malar rash, arthritis, renal disease and immunological involvement. (C) Age, disease duration and age at diagnosis in SLE patients with elevated and normal BLyS levels.
(<680 pg/ml) and those with elevated levels of BLyS (>680 pg/ml). Twenty-three SLE patients (51%) fell above the cut-off and were therefore classified as having elevated BLyS levels, with the remaining 22 patients having normal BLyS levels.

SLE patients with elevated plasma BLyS levels were found to be significantly younger at the time of the study visit (32.97 ± 9.23 years, \( P = 0.0019 \)) with a shorter disease duration (4.96 ± 2.5 years, \( P = 0.0125 \)) and were younger at the time of their initial diagnosis (27.96 ± 35.18 years, \( P = 0.037 \)) (Fig. 1).

Patients with elevated BLyS levels had a mean SLEDAI score of 5.1 while patients with normal BLyS levels had a mean SLEDAI of 0.86 (\( P < 0.001 \)). In the overall patient group, BLyS levels showed significant correlation with disease activity as measured by SLEDAI (Spearman’s \( r = 0.682, \ P < 0.001 \)). Patients with elevated BLyS at the time of study enrolment accrued significantly more damage over the subsequent 5-year period, with a mean increase in damage score of 0.53 compared with 0.13 for patients with normal BLyS levels (\( P = 0.012 \)). Significantly, 11 patients with elevated BLyS levels accrued new damage (48%) while only 3 patients (14%) with normal BLyS levels suffered new damage. The odds ratio (OR) was 5.8 (95% CI 1.4, 25.2) (\( P = 0.023 \) by Fisher’s exact test). The change in SLICC/ACR DI correlated significantly with plasma BLyS levels (Spearman’s \( r = 0.399, \ P = 0.007 \)).

Given the concern that medication can effect circulating BLyS levels, we next analysed medication usage between groups. When patients with elevated BLyS and normal BLyS were analysed, no difference with regard to mean steroid dose was observed between groups (4 vs 2.5 mg, \( P = 0.34 \)). In addition, BLyS levels were not significantly different when patients were grouped into those taking steroids and those not on steroids, in keeping with the published literature detailing that while high doses of corticosteroid diminish BLyS levels, stable low doses have little or no effect on circulating BLyS levels [11]. Interestingly, however, mean BLyS levels were significantly higher in those patients requiring additional immunosuppression (i.e., AZA/CYC/MMF) to control their disease compared with those patients requiring Plaquenil alone for disease control, an effect that was independent of steroid dose (1136 vs 757 mg/dl, \( P < 0.01 \)).

Association of elevated BLyS levels in SLE with anti-Sm and anti-dsDNA and low complement

Lupus-associated autoantibody profiles were determined for all SLE patients. When the presence of each autoantibody specificity was assessed categorically, anti-Sm was significantly associated with elevated BLyS levels [OR 13.4 (95% CI 0.69, 258.3)] (\( P = 0.049 \) by Fisher’s exact test). Anti-dsDNA positivity was also significantly associated with BLyS elevation [OR 6.1 (95% CI 1.7, 22.1)] (\( P = 0.007 \) by Fisher’s exact test). In the overall patient group, anti-dsDNA levels correlated significantly with plasma BLyS level (Spearman’s \( r = 0.35, \ P = 0.007 \)).

Laboratory tests included full blood count, ESR, serum creatinine level and complement (C) 3 and 4 levels. Low C4 was significantly associated with elevated BLyS levels, present in 57% of patients with BLyS elevation vs 14% of patients with normal BLyS levels [OR 8 (95% CI 1.42, 45.1)] (\( P = 0.016 \) by Fisher’s exact test). No significant differences were found between the two groups with regard to haemoglobin levels, absolute white cell count, lymphocyte count, platelet count, serum creatinine or ESR.

Discussion

The results of our study demonstrate the importance of investigating the role of BLyS in genetically homogeneous SLE patient populations. Specifically, we highlight that elevated BLyS levels in Caucasian SLE patients are associated with increased damage accrual at 5-year follow-up. Furthermore, our results indicate that increased BLyS levels in Caucasian SLE patients are associated with shorter disease duration and easily identifiable clinical phenotypes, thus aiding physicians in identifying patients most likely to benefit from BLyS pathway blockade.

Given the significant association between BLyS levels and disease activity [12], as well as high disease activity and mortality in SLE [2, 3], timely control of disease is important. In keeping with previous reports, patients with elevated BLyS levels in our study had more active disease. However, our data suggest that the optimal timing for intervention with anti-BLyS therapy may be earlier than currently indicated for Caucasian patients. Patients with shorter disease duration have higher levels of BLyS. This highlights this group as a patient population who may be most likely to benefit from the opportune introduction of anti-BLyS therapy and indicates that the initiation of anti-BLyS therapy earlier in the course of disease in Caucasian patients may lead to improved outcomes for patients, especially as disease activity has been demonstrated to be highest earlier in the disease course.

Damage scores in SLE are associated with increasing age and longer duration of disease [4, 5]. Furthermore, recent work from Petri et al. [14] highlights the important role of corticosteroids in potentiating damage. Any strategy that improves disease activity without reliance on corticosteroids is likely to positively influence patient outcomes. In our study, elevated BLyS levels were associated with increased damage at 5 years. Of note, patients in our study on additional immunosuppression also had higher BLyS levels. This indicates that conventional immunosuppressive regimes may not be as effective at controlling BLyS-mediated disease, further supporting the use of targeted BLyS therapy in appropriate patient populations. Pepper et al. [15] have previously demonstrated the effectiveness of a B cell depletion regimen at diagnosis in reducing the use of oral steroids without adversely affecting patients’ renal outcomes in SLE. Post hoc analysis of the BLISS studies have also demonstrated that those patients who received placebo rather than anti-BLyS therapy...
received a significantly higher cumulative steroid dose over the follow-up period [16].

Thus, while high-dose steroids have been demonstrated to reduce BLYS levels [11], the use of anti-BLYS therapy early in the disease course to reduce the cumulative steroid dose has not been assessed to date, but such a strategy may be worth considering, particularly in younger patients, as we have demonstrated higher BLYS in this patient population as well as increased damage. Most previous studies have shown consistent correlations between BLYS levels and anti-dsDNA levels [9, 11, 12]. This observation was also seen in our study with higher dsDNA levels in patients with elevated BLYS. However, the correlation between disease activity in our group and BLYS expression was more marked than previously observed. Of note, the patients and controls in our study were from a highly conserved Caucasian genetic background and this may explain the higher number of patients who appear to have BLYS-mediated disease. Other studies involving African American patients with SLE have shown a lack of association between disease activity and elevated BLYS levels, suggesting that patients from different ancestral backgrounds may respond differently to anti-BLYS therapy [17]. In addition to anti-dsDNA positivity in our cohort, anti-Sm antibody was significantly associated with BLYS elevation. These patient populations were among the most likely groups to benefit from anti-BLYS therapy in post hoc analysis of the BLISS studies, suggesting that anti-BLYS therapy may be most beneficial in patients with elevated BLYS and therefore any disease phenotypes/characteristics that can help identify markers of BLYS elevation are to be welcomed [16]. In this regard, the BLISS trials showed significant improvements in both musculoskeletal and mucocutaneous organ domains in SLE patients [18]. In our study, patients with skin and joint involvement also had higher BLYS levels than those without these clinical characteristics, suggesting that their response to treatment reflected a subgroup of patients with elevated BLYS levels. This association may potentially allow identification of clinical phenotypes associated with BLYS elevation that may be more likely to respond to anti-BLYS therapy.

The numbers in this pilot study of BLYS in a highly homogeneous patient population were small due to the strict inclusion criteria, which involved all participants having to confirm they were of Irish descent for three generations prior to study enrolment. Further validation of these clinical and immunological associations are warranted in larger cohorts of genetically homogeneous populations followed for a longer time period, in particular to examine the role of serial BLYS measurements to predict ongoing damage accrual. Nonetheless, this study does highlight that increased BLYS levels in Caucasian SLE patient populations are associated with increased damage accrual at 5-year follow-up. In conclusion, our study suggests that early anti-BLYS therapy may be most beneficial in younger Caucasian patients with short disease duration in an effort to prevent long-term damage.

**Rheumatology key messages**

- BLYS promotes damage in SLE.
- BLYS levels are higher earlier in the disease course in Irish SLE patients.
- SLE patients with renal disease, malar rash, arthritis and immunological activity have higher BLYS levels.

**Acknowledgements**

The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

**Funding:** This work was supported by the Science Foundation Ireland (grant 08/IN.1/B2091), the Health Research Board Ireland (grant RP 2001 26) and the Royal College of Physicians in Ireland.

**Disclosure statement:** G.K. has received honoraria for attending an advisory board regarding belimumab from GSK. All other authors have declared no conflicts of interest.

**References**


