ANTI-RO FALSE-NEGATIVES DETECTION THROUGH B CELL SUBPOPULATIONS IN LUPUS NEPHRITIS

Purpose
The aim of the present study is to identify false-negatives for anti-Ro by analysing both 52 KDa and 60 KDa subunits separately, as well as to characterise if there are clinical or molecular differences in this subgroup of patients compared to anti-Ro negative cases.

Methods
A cross-sectional, observational study of patients diagnosed of SLE according to SLICC 2012 criteria was performed. In these patients a complete blood-test was made, and clinical data by personal interview was collected. INF1A, a cytokine involved in the pathogenesis of lupus nephritis, was measured by colorimetric methods. Biostatistical analysis was performed with R 3.3.2.

Results
We selected 69 SLE patients with negative results for anti-Ro (2.34±4.17 U/mL) out of 142 total SLE patients. A total of 51 patients were negative for both anti-Ro subunits and 18 cases presented positive results (up to 20 pg/mL) for at least one of them (See table 1).

The subgroup of patients that exhibit simultaneously high levels of anti-Ro52KDa and anti-Ro60KDa have higher clinical activity compared to negative anti-Ro cases (75% of active patients against 41.2% in anti-Ro negative patients). However, no differences in the accumulated damage evaluated by SLICC score between negative anti-Ro cases and patients with at least one positive subunit were observed.

We analyse serum levels of INF1A cytokine in the four groups of patients, and anti-Ro and subunits negative cases showed significant lower INF1A levels than the other patients (8.26±14.87 pg/mL and 26.62±40.71 pg/mL respectively; p=0.04). In addition, patients with high levels of anti-Ro52KDa subunit are those with the highest INF1A levels (anti-Ro 52+/anti-Ro60- 23.5±47.6 pg/mL of INF1A; anti-Ro 52+/anti-Ro60+ 36.4±37.9 pg/mL of INF1A).

Conclusion
In our anti-Ro seronegative patients, a 26% of false-negative cases were detected. These cases with high levels of almost one anti-Ro subunit showed significantly higher levels of INF1A in contrast to negative cases, supporting the fact that they are indeed a different group from the negative cases. Moreover, the high INF1A levels could be the reason of the observed differences in the clinical activity measured by SLE-DAI score in both groups.

Abstract PS1:8 Table 1

<table>
<thead>
<tr>
<th></th>
<th>NEGATIVES N=51</th>
<th>Anti-RO52KDa POSITIVES N=8</th>
<th>Anti-RO60KDa POSITIVES N=2</th>
<th>Anti-RO52KDa/ Anti-RO60KDa POSITIVES N=8</th>
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<tbody>
<tr>
<td>Anti-RO</td>
<td></td>
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<tr>
<td>[U/mL] Mean (DS)</td>
<td>1.92 (3.11)</td>
<td>1.65 (3.2)</td>
<td>0.5 (0.71)</td>
<td>6.15 (8.37)</td>
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<td>Anti-RO52KDa</td>
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<tr>
<td>[KDa pg/mL] Mean (DS)</td>
<td>1.26 (1.89)</td>
<td>147.24 (74.25)</td>
<td>1.05 (0.89)</td>
<td>196.82 (50.06)</td>
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<tr>
<td>Anti-RO60KDa</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>[KDa pg/mL] Mean (DS)</td>
<td>1.73 (2.71)</td>
<td>6.3 (7.01)</td>
<td>120.96 (111.78)</td>
<td>145.22 (76.69)</td>
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</table>

B CELL SUBPOPULATIONS IN LUPUS NEPHRITIS PATIENTS: CORRELATIONS WITH DISEASE ONSET AND OUTCOMES

Purpose
The relationship between B cells subsets distribution, clinical and laboratory parameters, therapeutic response and prognosis in lupus nephritis (LN) is still underestimated. The
EFFECTS OF BELIMUMAB TREATMENT ON B CELL HYPERACTIVITY AND TYPE-I INTERFERON EXPRESSION IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Purpose Belimumab, a monoclonal anti-BAFF antibody, has been approved for patients with active systemic lupus erythematosus (SLE) despite standard care of care immunosuppressive treatment (ST). However, the interference of belimumab with pathogenetic pathways of SLE is not fully understood. B cell hyperactivity and overexpression of type-I interferons (IFN) have been shown to be key elements in the pathogenesis of SLE. This study shows the effect of belimumab on biomarkers representing B cell hyperactivity and IFN expression in SLE patients.

Methods 20 SLE patients treated with belimumab (BT), 82 SLE patients with ST and 30 matched healthy controls (HC) were recruited. Siglec-1 expression on monocytes representing IFN signature, BCMA expression on different B cell subsets and the frequency of activated naive B cells (aNB) in PBMCs were analysed by FACS. Serum levels of BAFF plus soluble receptors sBCMA and sTACI were determined by ELISA.

Results Compared to ST, BCMA expression was reduced in BT on naive B (p<0.001) and memory B cells (p<0.05) but not on aNB, plasmablasts and plasma cells. In comparison to HC, BCMA expression was similar on all B cell subsets, except on aNB where it was higher in BT (p<0.001). The frequency of aNB among total B cells was reduced in BT compared to ST (p<0.001) and was comparable to HC. Siglec-1 expression on monocytes did not differ significantly between BT and ST; both groups showed a rise compared to HC (each p<0.001). There was no significant difference after belimumab treatment. Furthermore, serum BAFF levels in ST and BT were higher than in HC (each p<0.001), but did not differ significantly between BT and ST. Serum levels of sBCMA (p<0.05) and sTACI (p<0.001) were lower in BT compared to ST and also after belimumab treatment (each p<0.05). BT’s sTACI levels were lower than in HC (p=0.01).

Conclusions This study provides deeper insights into the impact of belimumab on several pathogenetic pathways of SLE activity. Regarding the inhibition of B cell hyperactivity, one key pathogenetic element of SLE, belimumab treatment showed distinct advantages. Furthermore, these results suggested that belimumab treatment did not impair the type-I IFN pathway.

THE INTERFERON BIOMARKER SIGLEC1 REFLECTS DISEASE ACTIVITY IN PAEDIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS

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Introduction SIGLEC1 (sialic acid-binding Ig-like lectin 1, CD169) is a monocytic adhesion molecule induced by interferon – α. In adult systemic lupus erythematosus (SLE), SIGLEC1 correlates cross-sectionally and longitudinally with disease activity. The aim of this work was to examine whether SIGLEC1 also reflects the disease activity in paediatric SLE.

Methods Over a period of 29 months the disease activity was clinically evaluated using SLEDAI (SLE-Disease Activity Index-2000). In 28 consecutive paediatric SLE patients (mean age 16 years, range 3–38 years, 86% female, 14% male), the number of SIGLEC1 molecules per CD14+ blood monocyte was quantified using flow cytometry. At the same time, the level of anti-ds DNA-antibody titer (ELISA) and the concentration of complement factors C3 and C4 (nephelometry) were determined. The association between SIGLEC1, C3, C4 and dsDNA-antibody with SLEDAI was estimated using a mixed linear model to model the repeated measurement of parameters within a patient. The cut-off for the change in SIGLEC1 between two consecutive visits to predict minimal clinical improvement or worsening in SLEDAI was chosen on the maximum Youden Index.

INTRODUCTION SIGLEC1 CORRELATES WITH DISEASE ACTIVITY IN ADULT SYSTEMIC LUPUS ERYTHEMATOSUS

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Introduction The expression of Sigle-1, a type-1 interferon biomarker, on monocytes was shown to correlate with disease activity in adult systemic lupus erythematosus (SLE). This study provides new insights into the possible role of sigle-1 in the pathogenesis of paediatric SLE.

Methods 28 consecutive paediatric SLE patients (mean age 16 years, range 3–38 years, 86% female, 14% male) were recruited. Siglec-1 expression on monocytes representing IFN signature, BCMA expression on different B cell subsets and the frequency of activated naive B cells (aNB) in PBMCs were analysed by FACS. Serum levels of BAFF plus soluble receptors sBCMA and sTACI were determined by ELISA.

Results Compared to ST, BCMA expression was reduced in BT on naive B (p<0.001) and memory B cells (p<0.05) but not on aNB, plasmablasts and plasma cells. In comparison to HC, BCMA expression was similar on all B cell subsets, except on aNB where it was higher in BT (p<0.001). The frequency of aNB among total B cells was reduced in BT compared to ST (p<0.001) and was comparable to HC. Siglec-1 expression on monocytes did not differ significantly between BT and ST; both groups showed a rise compared to HC (each p<0.001). There was no significant difference after belimumab treatment. Furthermore, serum BAFF levels in ST and BT were higher than in HC (each p<0.001), but did not differ significantly between BT and ST. Serum levels of sBCMA (p<0.05) and sTACI (p<0.001) were lower in BT compared to ST and also after belimumab treatment (each p<0.05). BT’s sTACI levels were lower than in HC (p=0.01).

Conclusions This study provides deeper insights into the impact of belimumab on several pathogenetic pathways of SLE activity. Regarding the inhibition of B cell hyperactivity, one key pathogenetic element of SLE, belimumab treatment showed distinct advantages. Furthermore, these results suggested that belimumab treatment did not impair the type-I IFN pathway.