SLE-KEY™ RULE-OUT SERLOGIC TEST FOR SLE USING THE IMMUNARRAY iCHIP®

Introduction

SLE is associated with a large spectrum of autoantibodies, but currently there is no single adequate serologic diagnosis. Each autoantibody individually fails to discriminate with sufficient specificity and/or sensitivity SLE patients from healthy controls or from subjects afflicted with other autoimmune diseases. Currently a diagnosis of SLE is based on multiple criteria and can take years of concerned monitoring.

Aim

We developed the previously described iCHIP® (figure 1) as an effective SLE rule-out diagnostic test by profiling with an antigen microarray multiple, distinct autoantibody reactivities in the sera of SLE patients compared to healthy controls and by using informatics analysis to rule out a diagnosis of SLE. An initial set of 200 antigens associated with SLE was selected from the literature bolstered with sets of proprietary markers developed by ImmunArray (figure 2). Here we report verification and validation of our SLE-Key™ Rule-Out Serologic Test.

Method

We collected serum samples from 246 SLE patients from four independent sources [Albert Einstein College of Medicine, Medical University of South Carolina, Johns Hopkins University and Emory University] and compared them with sera of 252 healthy control samples independently sourced (clinical data is shown on tables 1, 2). We tested these samples using the ImmunArray iCHIP® - a proprietary microarray that displays multiple antigens representing a range of SLE-associated biochemical pathways (figure 2). Training was performed on a subset of 150 SLE patients and 150 healthy controls using 4 independent classification methods: SVM, Logistic regression, QDA and LDA. Verification and validation were performed on additional sets of 50 SLE patients and 50 healthy control samples each.

Results

Based on comparing the ROC curves of the four models (figure 3) LDA was selected as it displayed the best balance between sensitivity and specificity. LDA classification method differentiated SLE patients from healthy subjects with a sensitivity of 94% and specificity of 75% (table 3) using a selected subset of six auto-antigens. The informative antigens represented some known to be associated with SLE, as well as some proprietary antigens not previously known to have an association with the disease. SLE-Key™ test out put is presented in figures 4.5 and 6.

Validation Table 2: Patient samples clinical data

Table 3: The LDA Validation performance

<table>
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<th>Validation</th>
<th>LDA</th>
<th>Area under Curve (AUC)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>Positive Predictive Value (PPV)</th>
<th>Negative Predictive Value (NPV)</th>
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<td></td>
<td></td>
<td>0.94</td>
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References (1) Fattal, I, et al; Immunology 2010, 130, 337-343

Figure 1: iCHIP® - ImmunArray proprietary antigen micro-array platform

Figure 2: ImmunArray’s iCHIP® antigen biochemical pathways representation

Figure 3: ROC curves for the four classification models on validation data set, indicating test performance at selected threshold (left panel). LDA model validation probability distribution (right panel).

Figure 4: SLE-Key™ test output, LDA validation probability distribution. LDA threshold is shown as dotted horizontal purple line. The patient SLE-Key™ score is represented as a red “X”.

Figure 5: LDA classifier antigens individual intensity histograms

Figure 6: SLE associated antigens individual intensity histograms

Conclusion

The SLE-Key™ Rule Out serologic test can be used as a decision support tool for physicians in ruling out a diagnosis of SLE with a sensitivity of 94%, specificity of 75% and NPV of 93%. Work comparing this testing performance directly to standard serologic testing is ongoing.