Development of a Panel of Biomarkers for the Diagnosis of Systemic Lupus Erythematosus

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Introduction

Patients suffering from the autoimmune disease systemic lupus erythematosus (SLE) can present with a variety of symptoms, many of which occur in other diseases making accurate diagnosis challenging. None of the currently available tests provide a definitive diagnosis on their own and the results of multiple differing tests must be integrated to support a clinical judgement. The aim of this study was to identify biomarkers which could form the basis of a novel molecular diagnostic test for the diagnosis of SLE.

The development of autoantibodies associated with SLE is well known and their appearance can precede disease by many years, making them attractive as potential biomarkers for early diagnosis. We have developed a unique biomarker discovery platform based on a “functional protein” array which utilises correctly folded proteins. This platform has been used to detect autoantibodies in SLE samples and to identify their cognate antigens. We have applied powerful data analysis strategies to identify panels of biomarkers which may have clinical utility in the diagnosis of SLE.

Methodology

Selection of biomarker panels

We investigated several strategies for data normalisation, marker selection and classification. Rather than simply generating a list of individual markers that are generally more reactive in case vs control, the approach is focused on building panels of biomarkers where the data from each biomarker is correlated with each other, to produce a robust panel giving high sensitivity and specificity for disease.

Following the assay, arrays were scanned and raw data extracted using standard methods to identify autoantibodies bound to the array and to determine the intensity of autoantibody binding. The resulting net fluorescent intensities of all protein features on each array were then normalized to reduce the influence of technical bias by a multi-scaling procedure.

Biomarker selection was performed using genetic programming. The data from the three training set was then used with a genetic algorithm to identify classifiers which would successfully distinguish case from control samples. The performance of classifiers was determined by calculating the combined sensitivity and specificity (S+S scores) when applied to the test set. The number of biomarkers in each panel was limited to n where n = 1-15. There was a progressive increase in performance of the panels as additional markers were included in the panels until the performance reached a plateau when n=15 (Fig 2).

Data were repeatedly split into test and training sets and analysis cycles iterated until a stable set of classifiers was identified. Biomarker panels were cross-validated until the sensitivity and specificity converged. Two independent permutation assays were used to confirm that the chosen biomarker panels relate to the disease status of the sera and not to an inherent bias in the data.

Results

Study design

• Case cohort (n=160): patients diagnosed with SLE
• Control cohorts (n=156): matched healthy controls with no history of autoimmune disease

Results (cont’d)

Performance of biomarker panels

A series of panels of biomarkers were identified which can distinguish SLE from control samples with greater than 90% sensitivity and 80% specificity.

Where n=4 or greater, the best performing panels have an S+S score higher than the value of 1.5 (i.e. above the typical value for the anti-nuclear antibody test [ANA]). Some of the identified biomarkers have previously been associated with lupus in particular or more generally with diseases with an autoimmune component.

Conclusions

• We have developed a unique “functional protein” microarray to detect autoantibodies in serum samples and applied it to samples from SLE patients.

• A panel of biomarkers were identified which can distinguish SLE from control samples with greater than 90% sensitivity and 80% specificity.

• This may offer an improvement over the existing criteria-based diagnosis. Further development and validation of the biomarker panels is ongoing.

Visit www.ogt.co.uk for more information.

References