HIGH TYPE 1 INTERFERON ACTIVITY IS ASSOCIATED WITH ACTIVE CLASS 3/4 LUPUS NEPHRITIS IN EUROPEAN-AMERICAN LUPUS PATIENTS INDEPENDENT OF ANTI-DSDNA ANTIBODIES

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Background/purpose Lupus nephritis (LN) is one of the most severe types of organ involvement in systemic lupus erythematosus (SLE), despite the recent advances in immunosuppressive therapies. High type 1 interferon (IFN) is a heritable risk for SLE, and some previous studies have suggested a link between high IFN and lupus nephritis. However, little is known about the relationships between high levels of IFN and the subtypes of LN, and whether IFN is more associated with anti-dsDNA antibodies or with clinical nephritis.

Methods We studied 244 European-American (EA) SLE patients and measured type 1 IFN in sera by performing WISH IFN bioassay as described previously. Subtypes of LN were confirmed by renal biopsy review. Complements, anti-dsDNA and other auto-antibodies were measured in the clinical laboratory, and standard clinical cut-offs were used to define a positive result. Non-parametric analyses were used to compare IFN data with the clinical data.

Results IFN level and SLEDAI score was positively correlated (r=0.26, p=0.0001, Spearman) in our cross-sectional evaluation. EA subjects with a high levels of IFN (IFN score >2) were more likely to have renal manifestations compared to the subjects with a low levels of IFN (IFN score <2) (p<0.001, OR=3.0, Fisher’s exact test). In addition, the incidence rate of class 3/4 LN was significantly higher among patients with a high levels of IFN compared to patients with low levels of IFN (p<0.01, OR=5.5, Fisher’s exact test). Notably, IFN level was significantly higher in active class 3/4 LN compared to inactive class 3/4 LN (p<0.05 Mann-Whitney U) and this was not observed in non-class 3/4 LN populations. Positivity of ds-DNA antibody did not show significant difference between inactive class 3/4 LN and active class 3/4 LN.

Conclusion Our data support an association between type 1 IFN and class 3/4 nephritis that is independent of overall SLEDAI and anti-dsDNA antibodies, suggesting that IFN is involved in renal pathogenesis. These data also suggest that IFN could predict renal disease activity or the future risk of developing LN, especially class 3/4 LN in EA SLE patients.

DEVELOPMENT OF A MULTIMARKER MODEL FOR THE DETECTION OF SYSTEMIC LUPUS ERYTHEMATOSUS BASED ON NEW AND TRADITIONAL AUTOANTIBODIES

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Background Autoantibodies (AAbs) are central to the pathogenesis of systemic lupus erythematosus (SLE). While the presence and intensity of AAbs are important, they are not sufficient for diagnosis or assessment of disease activity. A novel approach utilizes a group of AAbs not previously studied in SLE. Multimarker models of autoantibodies (AAbs) may provide an enhanced understanding of disease activity.

Methods We recruited 244 European-American (EA) SLE patients and measured type 1 IFN in sera by performing WISH IFN bioassay as described previously. Subtypes of LN were confirmed by renal biopsy review. Complements, anti-dsDNA and other auto-antibodies were measured in the clinical laboratory, and standard clinical cut-offs were used to define a positive result. Non-parametric analyses were used to compare IFN data with the clinical data.

Results IFN level and SLEDAI score was positively correlated (r=0.26, p=0.0001, Spearman) in our cross-sectional evaluation. EA subjects with a high levels of IFN (IFN score >2) were more likely to have renal manifestations compared to the subjects with a low levels of IFN (IFN score <2) (p<0.001, OR=3.0, Fisher’s exact test). In addition, the incidence rate of class 3/4 LN was significantly higher among patients with a high levels of IFN compared to patients with low levels of IFN (p<0.01, OR=5.5, Fisher’s exact test). Notably, IFN level was significantly higher in active class 3/4 LN compared to inactive class 3/4 LN (p<0.05 Mann-Whitney U) and this was not observed in non-class 3/4 LN populations. Positivity of ds-DNA antibody did not show significant difference between inactive class 3/4 LN and active class 3/4 LN.

Conclusion Our data support an association between type 1 IFN and class 3/4 nephritis that is independent of overall SLEDAI and anti-dsDNA antibodies, suggesting that IFN is involved in renal pathogenesis. These data also suggest that IFN could predict renal disease activity or the future risk of developing LN, especially class 3/4 LN in EA SLE patients.

This includes a score of vitality level (a lower score is suggestive of more fatigue). RDW was recorded, in addition to standard markers of lupus disease activity including Erythrocyte Sedimentation Rate (ESR), Complement C3, anti-double-stranded DNA binding (anti-dsDNA), C-reactive protein (CRP) and SLEDAI/BILAG. Spearman’s rank was used to analyse variables with a p-value of <0.05 considered significant.

Results In cohort 1, 72 patients aged 14–42 (median 21) were recruited. FACIT score did not correlate with anti-dsDNA (p=0.4), C3 (p=0.06), ESR (p=0.06), CRP (p=0.1) or SLEDAI (p=0.6). There was a strongly significant correlation between FACIT and RDW (p≤0.001; r=−0.44); figure 1. In cohort 2, 106 patients were recruited aged 18–75 (median 44.3). RDW correlated with ESR (p=0.03; r=−0.20), BILAG (p=0.002; r=−0.30) and vitality scores (p=0.02; r=0.23); figure 2. In cohort 3, 47 patients aged 19–75 (median 46) were recruited. FACIT correlated with RDW (p=0.03; r=−0.32); figure 3.

Conclusions An elevated RDW correlates with higher levels of fatigue. For the first time a serologically marker has shown strong association with fatigue in patients with lupus. This is demonstrated in three groups of varying age, ethnicity and geography and using two different fatigue scores.
Purpose Given the heterogeneity of clinical presentations, the diagnosis of Systemic Lupus Erythematosus (SLE) can be challenging, in particular in those patients presenting with early or incomplete disease, or with overlapping or atypical features. Autoantibodies (AABs) are important in aiding the clinical diagnosis of SLE, with some few AABs, anti-double-stranded DNA (dsDNA), anti-Smith (Sm), and anti-ribosomal P (riboP) being highly associated with SLE. As none of the traditional AABs has sufficient sensitivity to achieve diagnosis of SLE, current testing is based on measuring multiple AAB assays either in parallel or serial. We have recently identified novel AABs in SLE, which hold promise for improving diagnostic testing of SLE (1). We have developed quantitative ELISA prototypes for five new AABs, which were tested in combination with traditional AABs. The objectives of this study were to evaluate the diagnostic value of novel AABs and to screen for an optimised combination of novel and traditional AABs using logistic regression to increase the diagnostic accuracy of SLE testing.

Methods Serum samples were obtained from 156 SLE patients with European ancestry at the rheumatology department of the Heinrich-Heine University (Düsseldorf, Germany), and Hannover Medical School (Hannover, Germany). SLE samples were compared against 126 samples from autoimmune diseases (AID; myositis: n=20; Sjögren’s syndrome (SjS): n=31; rheumatoid arthritis (RA) n=36; systemic sclerosis (SSc): n=39), and 77 healthy control samples. Prototype bead-based ELISAs were developed for recently identified novel antigens. Traditional diagnostic AABs were measured using IVD ELISAs and included: SSA/Ro60, SSA/Ro52, La/SSB, Sm, RNP, dsDNA, ScI70, CENPB, Jo-1, CCP, phospholipid and dsDNA. Optimised marker combinations of new and traditional markers were tested using logistic regression and receiver operating curve analysis (ROC).

Results When comparing 156 SLE patients with 203 control samples, the area under the curve (AUC) of the five novel SLE ELISAs ranged from 0.63 to 0.75. A cut-off was set at a specificity of 95% and yielded a sensitivity ranging from 13.5% to 21.2% for the five novel assays. The sensitivity and specificity of new ELISAs was comparable to traditional ELISAs, which was in this cohort for anti-dsDNA 35% and 97%, anti-Sm 15% and 97%, and anti-RiboP 26% and 97%. A logistic regression model was used to combine the results of multiple tests. Compared to a logistic regression with traditional assays, a logistic regression with novel markers achieved higher sensitivity by pertaining high specificity. The logistic regression model based on a multimarker IVD assay with ten extracted nuclear antigens (ENA) yielded an AUC of 0.87 and a sensitivity of 58% at a specificity of 95%. By contrast, the optimal combination of traditional and novel ELISAs reached an AUC of 0.92 and a sensitivity of 75% at a specificity of 95%.

Conclusions This study demonstrates the feasibility of combining test results of novel and traditional AABs using logistic regression to increase the diagnostic accuracy for SLE. Further studies are required to assess the impact of different ethnicities on marker selection and algorithm performance.

PS1:3 ANALYSIS OF C9ORF72 EXPANSIONS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND RHEUMATOID ARTHRITIS: PRELIMINARY DATA

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Background The most frequent genetic cause of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Dementia (FTLD) is a large hexanucleotide expansion (mostly hundred/thousand repeats) within a non-coding region of the C9orf72 gene. The cut-off to distinguish normal and pathogenic expansions has not yet been defined, but most healthy individuals have 2–20 repeats. The pathogenic mechanism of the dominant mutation is most probably toxic gain of functions. Nonetheless, C9orf72 reduced expression has been observed in post-mortem brains of mutated patients. Interestingly, while gene haploinsufficiency alone seems insufficient to cause neurodegeneration, decreased transcriptional activity with increasing numbers (>7) has been demonstrated in vitro and knockout mice developed features of systemic lupus erythematosus (SLE). We investigated C9orf72 gene in a cohort of patients with rheumatoid arthritis (RA) and SLE; as a control group we studied 49 ALS patients without pathogenic expansion.

Methods 29 SLE and 50 RA pts were screened, by the use of a PCR-based protocol, validated in our laboratory. A cut-off of ≥9 repeat units was considered in our analysis.

Results No patients with large expansions were found. The average and median values of repeat units were 5.29 and 6 in SLE, 4.73 and 2 in RA and 4.8 and 5 in the control population. We individuated ≥9 repeat units in 5/30 (16.7%) SLE patients and 7/50 (14%) RA patients; a prevalence higher than ALS group (8.16%). We searched for clinical or serological differences among SLE pts with the normal and ≥9 repeat. Although those differences were not statistically significant, we reported a higher prevalence of kidney involvement in patients with a number of repeats ≥9 (5/6; 83.3% vs 7/23; 30.4%), p=0.056.

Conclusion Our preliminary results indicate that ≥9 repeats within the C9orf72 gene are detectable in a non negligible number of patients with systemic autoimmune disease, confirming the possible role of C9orf72 in autoimmune system. The possible association with specific subset of disease must be confirmed in a larger cohort of patients.

REFERENCES