

Abstracts

2. 50% of patients with SLE and serositis manifested with renal lupus and high SLICC scores, which is higher compared to other studies on SLE.

Conclusion Cardiopulmonary involvement is increasingly detected in patients with SLE.

REFERENCE

1. Turaka V, Koshy K, George T, et al. Clinical outcomes in patients with cardiac lupus: a retrospective study. *Indian Journal Of Rheumatology* 2020;15(2).

PO.3.64 OPTIMISING TYPE I INTERFERON GENE EXPRESSION ASSAYS: ADDRESSING THE RESEARCH AGENDA OF A EULAR TASK FORCE

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Purpose Type I interferon (IFN-I) assays are an important emerging biomarker in SLE. A EULAR Task Force for IFN-I assays proposed a research agenda, which included key questions concerning the use of IFN-I stimulated gene (ISGs) and protein assays. These include: (i) influence of sample type; (ii) validated reference genes that are not influenced by IFN-I; (iii) for both gene expression and protein assays: confirmation that ISGs or proteins are specifically responsive to IFN-I, not IFN-II.

Methods To compare sample types, we extracted RNA from paired whole blood (TEMPUS) and PBMC samples obtained from 10 healthy controls, 7 at risk non-progressor, 6 at risk progressor, 10 inactive SLE and 12 active SLE. These were assessed for published IFN Scores A (module 1.2 and 3.4) and Score B (modules 3.4 and 5.12) using TaqMan. Bland-Altman agreement plots compared results.

To assess reference genes, we analysed the same set of samples for expression of a panel of 16 reference genes from the literature using TaqMan. Results were analysed using RefFinder software.

To compare the specificity of IFN response of candidate ISGs and proteins, we performed in vitro stimulation of healthy whole blood and PBMCs using IFN- α , IFN- β , IFN- κ , IFN- λ , IFN- γ , IL-1, IL-6, IL-10 and TNF. Samples were analysed at 0, 6 and 24h using TaqMan for a panel of 26 ISGs (summarised as IFN-Score-A and IFN-Score-B) as well as the transcripts for 17 IFN-stimulated proteins (mostly chemokines) described in the EULAR Systematic Literature Review.

Results IFN-Score-A correlated well between whole blood and PBMC samples ($r^2=0.8614$). IFN-Score-B showed weaker correlation ($r^2=0.2024$) and Bland-Altman plot showed greater deviation from line of agreement than for Score A.

There were marked difference in stability of published reference genes. Across several algorithms, the most consistently stable genes were: YWHAZ, PGK1 and GUSB. The least stable were RPLPO, HMBS and ACTB (β -actin). Calculation of IFN Scores using the least stable reference genes demonstrated greater variability between samples and poor separation of SLE and HC samples compared to calculation using the most stable reference genes.

IFN- α strongly induced IFN-Score-A, IFN-Score-B and expression of CCL3, CCL4, CCL5, CCL7, CCL8 and CXCL12 as compared to IFN- γ . CXCL9 and CCL26 were more responsive to IFN- γ stimulation than IFN- α . CCL2,

CCL19, CCL20, CCL21, CCL23 and CXCL11 demonstrated similar levels of response to IFNs. Expression of CXCL1, CXCL8 and CCL13 were suppressed by IFN- α . The chemokine transcripts CCL2, CCL7, CCL13, CCL20, CCL23, CXCL1 and CXCL8 were more responsive to IL-1 than IFN- α . CCL3, CCL19, and CCL21 responded to IL-1 similarly to IFNs.

Conclusions (i)The relative expression of different sets of ISGs varies between PBMCs and whole blood sample types. (ii)Some reference genes used in published IFN-I assays are not stable. (iii)Some gene expression and serum protein assays reported to measure IFN-I include components that are either not ISGs, or are more responsive to IFN-II or other cytokines than IFN-I. Our future work will develop a whole blood IFN-I assay optimised to avoid these artefacts and confounders.

PO.3.65 THE LEPTIN AND ADIPONECTIN LEVELS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND THEIR RELATIONSHIP WITH CARDIOVASCULAR RISK FACTORS

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Purpose To determine the leptin and adiponectin levels in women with systemic lupus erythematosus (SLE) and clarify the relationship of adipocytokines with cardiovascular risk factors.

Materials and Methods The study included 48 women with SLE (median age 40 [31;49] years, disease duration 3 [1;9] years), mostly with low and moderate activity (SLEDAI 2K index = 5[2;8]). The majority (83%) received glucocorticoids (GC) at the time of the examination, hydroxychloroquine was taken by 75%, immunosuppressants – by 21%, biological preparations – by 10% patients. The median daily GC dose in terms of prednisone was 10 [7.5; 10] mg/day. The control group included 35 women matched in age and body mass index (BMI) with SLE patients. The levels of adipocytokines (leptin and adiponectin) were assessed by ELISA. The following cardiovascular risk factors were studied: age, smoking status, BMI, waist circumference (WC), blood pressure (BP), insulin resistance (according to Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index), hypertriglyceridemia (according to the apolipoprotein B (ApoB) levels).

Results The leptin levels in SLE were 28.4 [9.4;71.6] ng/ml, and in the control group - 13.0[7.9;16.5] ng/ml ($p<0.001$), the adiponectin concentrations were 9.1[5.3;10.1] μ g/ml and 7.7[5.5;10.3] μ g/ml, respectively ($p=0.9$). Leptin levels correlated with BMI ($r=0.73$, $p<0.0001$), WC ($r=0.69$, $p<0.0001$), HOMA-IR ($r=0.57$, $p<0.001$), SLEDAI 2K ($r=-0.47$, $p<0.001$), SLICC damage index ($r=0.3$, $p=0.04$), duration of GC use ($r=0.3$, $p=0.04$). There were weak negative correlations of adiponectin levels with systolic BP ($r=-0.29$, $p=0.04$) and ApoB concentrations ($r=-0.31$, $p=0.03$). Relationships between adiponectin and BMI, SLE activity, and therapy were not found.

Conclusions In women with SLE, serum leptin levels were higher and adiponectin concentrations were similar to those in the control group. Leptin levels increased with decreased SLE activity, long-term GC use, and were associated with obesity

and insulin resistance. Adiponectin, on the contrary, was independent of SLE activity, but had a favorable, although weak, effect on blood pressure and lipid profile.

PO.3.66 TLR9 PROTEIN LEVEL IS ASSOCIATED TO PROINFLAMMATORY CYTOKINE LEVEL OF IL10 AND INF1A IN SLE PATIENTS

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Purpose The aim is to investigate the association among TLR7 and TLR9 serum levels with previous viral infections, disease activity and proinflammatory cytokine levels in SLE patients.

Methods Cross-sectional observational study in SLE patients (SLICC/ACR 2012 criteria) and healthy controls (HC). Previous infection data with RNA (HCV) and DNA virus (CMV, Epstein-Barr, Herpes simplex, Parvovirus B19 or HBV), disease activity and clinical data were collected. Biological samples of SLE patients and HC from the medical visit were supplied to the TLR7, TLR9, IL10 and INF1A determination by enzyme-linked immunoassay.

Results 94 SLE patients (91.5% female) with a mean age of 51 (13) years old and 35 HC (80% female) and 42 (12) years old were recruited. Mean age at diagnosis was 33 (14) years old and mean disease evolution was 19 (10) years. Mean SLEDAI index was 5.35 (4.58).

The 48.94% of patients reported almost one DNA virus infections, the 2.13% reported HCV infection, the 4.25% with HCV and DNA virus, and the 31.92% not reported any infection. HC had no history of acute (3 months) or lasting chronic infections with viruses or bacteria.

TLR7 and TLR9 did not correlate between them. TLR9 levels were significantly higher in SLE patients than HC ($P<0.001$). Even though TLR7 levels did not show any difference between both groups, an association with the age of individuals was observed ($P<0.001$).

No association among TLR7 or TLR9 levels with CRP, ESR, anti-dsDNA, ENAs or antiphospholipid antibodies was observed, and nor with disease activity, age at diagnosis and disease evolution time. In contrast, however, we reported low TLR7 levels in SLE patients and antiphospholipid syndrome in comparison to those without antiphospholipid syndrome ($P=0.001$).

High TLR9 levels were significantly associated to increased levels of IL10 and INF1A in SLE patients ($P<0.001$). TLR7 levels were not associated with INF1A levels but it is noticeable that there is a tendency to increase TLR7 levels in cases with increase of IL10 levels.

Conclusions TLR9 is increased in SLE patients in comparison to HC. TLR7 increases with age. No evidence of association between previous infections and TLR levels was found. Nor do we observe any difference in TLR level according to auto-antibodies presence or disease activity, probably due to the long-term SLE evolution and a good control of the disease.

There was, however, an association between high TLR9 levels and increase of IL10 and INF1A.

PO.3.67 MEASURING IFNA2 LEVELS BY A SINGLE-MOLECULE ARRAY IN CLINICAL PRACTICE OF CHILDHOOD-ONSET SLE PATIENTS DOES MATTER; RESULTS FROM A SINGLE CENTER LONGITUDINAL STUDY

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Introduction Type-I interferon (IFN-I) pathway activation plays a pivotal role in the pathogenesis of SLE and has been proposed as biomarker for disease activity. IFN-I pathway activation can be measured by determining the expression of IFN-I stimulated genes or a so-called IFN signature. Ultrasensitive single-molecule array (Simoa) technology enables measurement of IFN protein concentrations at subfemtomolar concentrations. Parallel use of these measuring methods in longitudinal cohorts of childhood-onset SLE (cSLE) patients in relation to disease activity could help in translating the most relevant technique for use in clinical practice.

Objective To determine the association of serum IFNa2 levels and whole blood IFN-I stimulated gene expression with disease activity and study their potential to mark specific disease activity states in a longitudinal cohort of cSLE patients.

Methods Serum IFNa2 levels were measured in 338 samples from 48 cSLE patients and 67 healthy controls using an IFN-a2 Simoa assay (Quanterix) on an HD-X analyser. A 5 gene IFN-I signature was measured by RT-PCR in paired whole blood samples. Disease activity was assessed by the clinical SELENA-SLEDAI (cSLEDAI) and BILAG-2004. Low disease activity was defined by the Low Lupus Disease Activity State (LLDAS) and flares were characterized by the SELENA-SLEDAI flare index. Analysis was performed using linear mixed effect models.

Results A clear positive correlation was present between serum IFNa2 levels and the IFN-I gene signature ($r=0.78$, $p<0.0001$). Serum IFNa2 levels and the IFN-I gene signature showed the same significant negative trend in the first three years after diagnosis. In this timeframe, mean baseline serum IFNa2 levels decreased with 55.1% (delta 172 fg/mL, $p<0.001$) to a mean value of 164 fg/mL, which was below the calculated threshold of 219.4 fg/mL. In the linear mixed model, serum IFNa2 levels were significantly associated with both the cSLEDAI and the BILAG-2004 ($p<0.001$ and $p<0.01$), while the IFN-I gene signature did not show this association ($p=0.35$ and $p=0.23$). Moreover, 69.7% of the time points in LLDAS had a serum IFNa2 level under the calculated threshold, while only 31.9% of the time points in LLDAS reached an IFN-I gene signature below the calculated threshold. Both techniques were equally capable of marking disease flares (79.2% above threshold vs 87.5% above threshold).