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FRI0258 COMPARISON OF ELISA AND MULTIPLEX TECHNIQUES FOR QUANTIFYING A URINE BIOMARKERS PANEL FOR LUPUS NEPHRITIS IN CHILDREN

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Background: A urine 'biomarker panel' comprising alpha-1-acid-glycoprotein (AGP), ceruloplasmin (CP), transferrin (TF) and lipocalin-like-prostaglandin-D synthase (LPGDS) has been shown to cross-sectionally perform to an 'excellent' level for Lupus Nephritis (LN) identification in children.¹ Quantification of all four biomarkers by enzyme linked immunoabsorbant assay (ELISA) techniques is time consuming and costly. Therefore, novel methods of biomarker panel quantification are required to facilitate future urine biomarker led monitoring studies.

Objectives: The main objective was to compare the ability of ELISA and multiplex biomarker quantification techniques to differentiate active versus inactive LN when the biomarkers are considered individually and in combination.

Methods: The urinary biomarkers were quantified by both ELISA and a newly developed, custom multiplex platform in participants of the UK Juvenile Systemic Lupus Erythematosus (JSLE) Cohort Study. Multiplex assay development involved identification of appropriate antibody pairs, assessment of JSLE urine sample matrix effects and range finding in JSLE patient samples. Patients were categorised as having active LN (renal domain BILAG score of A, B and previous histological confirmation of LN) or inactive LN (renal BILAG score D or E). Firth's penalised binary regression with AUC ROC analysis was used to compare the ability of multiplex and ELISA assays to detect active LN disease state univariately and in combination.

Results: Biomarker analysis was undertaken on 54 JSLE patients (13 active, 41 in-active). Assessment of each biomarker univariately demonstrated similar AUC values regardless of the biomarker quantification technique; LPGDS (ELISA AUC 0.826, multiplex AUC 0.829), TF (ELISA AUC 0.829, multiplex AUC 0.996), CP (ELISA AUC 0.901, multiplex AUC 0.983), AGP (ELISA AUC 0.934, multiplex AUC 0.979) (see table 1). Combining the multiplex biomarker data in the same order as the original ELISA based study¹ led to a similar progressive increase in AUC as biomarkers were added to the model (optimal model including AGP +CP + LPGDS+TF ELISA AUC=0.951, multiplex=0.995). For all biomarker combinations, the multiplex-derived AUC was higher than the ELISA AUC.

Abstract FRI0258 – Table 1. AUC values generated looking at each biomarker univariately

Individual biomarkers	ELISA AUC	Multiplex AUC
LPGDS	0.826	0.829
TF	0.829	0.996
CP	0.901	0.983
AGP	0.934	0.979

Abstract FRI0258 – Table 2. Comparison of biomarker combination AUC ROC values

Biomarker combinations	ELISA AUC	Multiplex AUC
AGP	0.880	0.979
AGP+CP	0.937	0.986
AGP+CP+LPGDS	0.942	0.985
AGP+CP+ LPGDS+TF	0.951	0.995

Conclusions: This new LN urine biomarker panel multiplex assay has been shown to display a comparable ability for active LN disease state identification as compared to existing ELISA techniques. The major advantage to this approach is that it reduces cost, processing time and the volume of sample required, as compared to ELISA techniques, representing a key enabler for future clinical studies.

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FRI0259 IN VITRO INDUCED REGULATORY T-CELLS CAN AMELIORATE SEVERITY OF PRISTANE INDUCED LUPUS (PIL)

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Background: Pristane induced lupus (PIL) is an established murine model of induced systemic lupus erythematosus (SLE). Mice develop specific autoantibodies and show symptoms of SLE including arthritis, glomerulonephritis and haemorrhagic pulmonary capillaritis.

Objectives: To investigate the therapeutic effects of *in vitro*-induced regulatory T cells (iTreg) in the murine model of PIL.

Methods: BALB/c mice were injected i.p. with 0.5 ml of pristane (PIL) or PBS (control). Naive CD4⁺ thymocytes were sorted and cultured and cell suspensions with >80% CD4⁺FoxP3⁺ cells (iTreg) were injected i.v. (i) once when PIL was induced (5 × 10⁶ iTreg (iTreg-single)) or (ii) every 4 weeks (1 × 10⁶ iTreg, iTreg-rep). Mice were monitored for paw swelling and grip strength. After 8 months, histological analysis quantified cartilage degradation, number of osteoclasts, extent of inflammation and bone erosion. Glomerulonephritis and pneumonitis were quantified using the kidney biopsy score and a histomorphometric image analysis system; inflammatory tissue was analysed by tissue cytometry. Serum levels of auto-antibodies were measured by ELISA. Frequencies of B cells, activated and regulatory CD4⁺ T cells and Th1, Th2, Th17 cells were measured by flow cytometry. FlowCytomix Pro was used to measure serum cytokines. RT-qPCR was used to measure expression levels of interferon (IFN)-signature and T-cell subset related as well as inflammation-associated genes.

Results: Monthly injections of 1 × 10⁶ iTreg reduced the clinical and histological severity of PIL-arthritis. This was seen by a higher mean grip strength, less mean paw swelling, retardation of symptom onset and a lower summative arthritis severity score (figure A). There was significant reduction of arthritis severity in all histological parameters and in the percentage of affected mice with erosive arthritis (33% of iTreg-rep mice vs 62% of PIL-mice). A single boost of 5 × 10⁶ iTreg could not prevent joint manifestation, however a slight retardation in 'loss of grip strength' and significantly less erosive area was seen. In regards to cellular composition of the inflammatory tissue in paws, a significantly increased relative amount of CD4⁺Foxp3⁺ cells was seen in the iTreg-rep group compared to PIL group.

Repeatedly injected mice (iTreg-rep) had significantly less renal disease (glomerular activity score) (figure B) and pulmonary involvement (perivascular inflammatory area) (figure C) compared to PIL. iTreg-rep mice had significantly lower serum levels of disease-associated auto-antibodies (figure D). Upon restimulation, splenic CD4⁺ cells in iTreg-rep showed a less pronounced Th1, Th2 and Th17 response as well as lower percentages in B- and activated T-cells, whereas the percentage of Treg was higher than in all other groups (figure E). FlowCytomix analysis showed reduced cytokine production after repeated iTreg administration. Correspondingly, iTreg-rep showed decreased expressions of IFN-signature-, T-cell subset- and inflammatory cytokine related genes (figure F).

Conclusions: Repeated injections of iTreg ameliorate the clinical, histological, serological as well as the genetic severity of PIL-manifestations. A single boost of iTreg at time of disease induction does not prevent manifestations, but retards the

onset of symptoms. Thus iTreg have significant positive effects on PIL, which may have consequences for future approaches in treating SLE.

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FRI0260

POLYMORPHISMS OF STAT4 AND MIR146A PREDICT THE ACHIEVEMENT OF 5 YEARS REMISSION IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with a complex pathogenesis in which genes and environmental factors interact leading to a protean clinical picture. Treat-to-target recommendations have identified 'remission' as a target in SLE, since achievement of remission improves the outcome and is associated with decreased damage progression. Nonetheless, predicting factors for the achievement of remission are lacking. It is likely that genes associated with SLE pathogenesis may influence the disease course.

Objectives: Thus, our aim was to analyse previously identified loci associated with SLE in a cohort of SLE patients to evaluate their influence on remission achievement.

Methods: We recruited 117 Italian SLE patients. A panel of 34 SNPs in 19 genes involved in immune response, autophagy and inflammation, was selected. SNPs genotyping was performed by allelic discrimination assay by TaqMan assays (Applied Biosystems, Foster City, CA, USA) and ABI PRISM 7000. The main clinical/laboratory features (including injury index and disease activity) were collected on an electronic platform. Remission was defined according to Zen et al.¹ and evaluated over 5 years. A genotype/phenotype correlation analysis was performed.

Results: The variant alleles of rs7574965 (STAT4) ($p<0.001$) and rs2910164 (MIR146a) ($p=0.031$) were significantly associated with lack of achievement of 5 years remission in SLE. Specifically, patients carrying the C allele of MIR146a were less likely to achieve 5 years remission ($p=0.01$, OR 0.235, 95% CI 0.074–0.752) as well as to achieve remission after 1, 2 and 3 years of evaluation ($p=0.002$, $p=0.001$, $p=0.002$, respectively). Among the clinical and laboratory features, 5 years remission was less likely to be achieved by patients who had arthritis in their clinical history ($p=0.007$), and who tested positive for anti-dsDNA ($p=0.005$). In a multinomial logistic regression analysis, arthritis ($p=0.022$, Exp(B)=0.255, 95% CI 0.079–0.820), anti-dsDNA ($p=0.003$, Exp(B)=0.166, 95% CI 0.051–0.537) and MIR146a rs2910164 gene variant ($p=0.046$, Exp(B)=0.250, 95% CI 0.064–0.974) were confirmed to be independent risk factors for unachieved 5 years remission (table 1).

Abstract FRI0260 – Table 1

REMISSION	Exp (B)	Exp (B) 95% CI	
		Lower	Upper
Arthritis	0255	0079	0,82
anti-dsDNA	0166	0051	0537
STAT4	0354	0,11	1139
mir146A	0,25	0064	0974

Conclusions: We describe for the first time the contribution of STAT4 and MIR146a SNPs as predicting factors for the achievement of 5 years remission in SLE. No genetic study has been performed so far in SLE, while a genetic profile of patients may be useful to predict the disease outcome.

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[1] Zen, et al. Ann Rheum Dis. 2017 Mar;76(3):562–565.

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FRI0261

ASSESSMENT OF AUTOPHAGY FUNCTION IN SYSTEMIC LUPUS ERYTHEMATOSUS IN RESPECT OF HYPERLIPIDEMIA AND IMMUNOSUPPRESSIVE DRUGS

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Background: Autophagy is an orchestrated homeostatic process to eliminate unwanted proteins and damaged organelles. Lipid turnover, as well, is controlled by autophagy through a process described as lipophagy. Defective lipophagy has been already linked to important metabolic disorders such as fatty liver, obesity and atherosclerosis.

Objectives: Assessment of autophagy focusing on lipids regulation in untreated newly diagnosed systemic lupus Erythematosus (SLE) patients and after three months of treatment with immunosuppressive drugs.

Methods: Subjects in this study have been divided into three groups. Group 1 included 60 newly diagnosed SLE patients before receiving any treatment, group2 included the same subjects of group 1 after three months of treatment with immunosuppressive drugs and group 3 included 30 healthy donors of matched age and sex as a control group. For each subject, disease activity was assessed by (SLE-DAI) score, lipid profile was measured in addition to evaluation of lipids uptake, enhanced phagocytosis and intracellular killing ability of monocytes and neutrophils using Sudan Black B stain and Nitroblue tetrazolium stain mixed with latex particles coated with antibodies. Microscopic pictures were captured and quantified by ImageJ.

Results: 95% of patients were females(57/60) with mean of age(39.7±8.6). Mean of SLEDAI score in group 1 was (18.6±3.4) decreased in group 2 (3 months after treatment) to (10.4±4.2). There was a positive correlation between total cholesterol, LDL and triglycerides and disease activity(SLEDAI score) ($r=0.677$, $r=0.603$ and $r=0.718$; respectively). On the contrary, There was a negative correlation between HDL and disease activity($r=-0.396$). Furthermore, there was a negative correlation between lipid content of cells and intracellular killing and disease activity($r=-0.258$ and $r=-0.324$; respectively). After 3 months, 100% of patients were taking Corticosteroids and Hydroxychloroquine(60/60).18.3% of patients received Azathioprine(11/60), 40.0% received Cyclophosphamide(24/60) and 15% received Mycophenolate(9/60) besides Corticosteroids and Hydroxychloroquine. Comparing group 2 to group 1, there was significant increase in cholesterol, LDL and triglycerides ($p=0.027$, $p=0.021$ and $p=0.017$; respectively) while HDL showed insignificant difference($p=0.0740$). Lipid content in cells and intracellular killing significantly decreased($p=0.0322$ and $p=0.0271$; respectively).

Conclusions: Autophagy is deficient in patients with SLE aggravated by immunosuppressive drugs so they are more susceptible to infections and dyslipidemia. Consequently, lipid lowering drugs are definitely required to decrease comorbidity.

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FRI0262

MONITORING DISEASE ACTIVITY IN SYSTEMIC LUPUS ERYTHEMATOSUS WITH DIGITAL ELISA QUANTIFICATION OF SERUM INTERFERON- α

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Background: To date, anti-dsDNA–Ab titration, better achieved with the Farr test, has been used to monitor global disease activity in systemic lupus erythematosus (SLE). Indeed, anti-DNA–Ab-positivity is associated with overall SLE activity. However, the sensitivity and specificity of that association are relatively low. The close association between Interferon alpha (IFN α) expression and SLE activity suggests that monitoring this cytokine might help physicians better evaluate