EVIDENCE OF NETOSIS IN CIRCULATING IL-33/ST2-MEDIATED INFLAMMATION IN ENDOTHELIAL CELLS

**Abstract**

The present study compares the relative performance of 4 IFN gene signatures in a cohort of 687 participants with self-reported SLE.

**Methods:** A centralised site, IRB-approved, SLE cohort was recruited using social media. Qualified participants with self-reported SLE were consented electronically and asked to provide medical record review consent, complete an online questionnaire regarding their disease as well as provide 3 fingertick blood samples over approximately a 6 week period. Blood samples from 687 participants were tested using a multi-modular gene expression assay containing 11 IFN response genes (primarily from the IFN-α response pathway). Normalised gene expression values were calculated, and the resulting data analysed to determine concordance between IFN gene signatures.

**Results:** 10 of the 11 IFN response genes were highly correlated with one another (r ≥ 0.80). The 4-gene signature of IFI27, IFI44, IFI44L, and RSAD2 identified 36.5% of the participants as IFN high. Three other literature reported IFN signatures provided similar classification results with participants being assigned to the same IFN sub-group over 90% of the time, and nearly identical patient distributions.

**Conclusions:** The study demonstrated that commonly used IFN gene signatures provide similar IFN subtyping to the 90th percentile. The use of social media to engage patients directly along with self-collection of blood samples provides new opportunity for testing clinical study participants and potentially 90th percentile patients without requiring an office visit.

**REFERENCES:**


**Disclosure of Interest:** None declared

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**Scientific Abstracts**

**Table 1**

<table>
<thead>
<tr>
<th>Signature</th>
<th>Genes</th>
<th>IFN High (%)</th>
<th>IFN Low (%)</th>
<th>% Agreement (95% CI) to Furie et al.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFI27, IFI44, IFI44L, RSAD2</td>
<td>36.5</td>
<td>63.5</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>IFI2AK2, IFIT1, MX1</td>
<td>36.3</td>
<td>63.7</td>
<td>94.8% (93.4–95.9)</td>
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</tr>
<tr>
<td>EIK2AK2, IFIT1, IFI44</td>
<td>36.0</td>
<td>64.0</td>
<td>95.1% (93.7–96.2)</td>
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<tr>
<td>IFI6, HERC5, IFIT1, MX1</td>
<td>36.0</td>
<td>64.0</td>
<td>94.8% (93.4–95.9)</td>
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</tbody>
</table>

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**OBJECTIVES:**

The aim of this study is to demonstrate the implication of NETosis in the pathophysiology of this disease.

**Methods:** Blood specimens from BD patients and matched healthy volunteers were collected at the American University of Beirut Medical Centre. Circulating neutrophils were isolated and cultured in Roswell Park Memorial Institute (RPMI) 1640 medium. Cultured cells were treated with colchicine, dexamethasone or corresponding vehicle, and stimulated with serum from BD patients, controls or left untreated. The kinetic and amount of NET formation was assessed by cell counting using Hoechst 33342 and Sytox green staining and fluorescent microscopy. Moreover, previously described NET-bound proteins (elastase, myeloperoxidase, citrullinated histone-3 and PR-3) were identified by immunolabelling and mRNA expression levels of PAD4, a key enzyme that promotes citrullination of arginine residues on chromatin, was evaluated in neutrophils from BD compared to controls. Finally, evidence of NETosis in vivo was assessed on paraffin embedded specimens from damaged organs of BD patients using immunolabelling and confocal microscopy.

**Results:** The percentage of unstimulated neutrophils undergoing NETosis in vitro was significantly higher in BD patients compared to controls. Treatment of unstimulated BD neutrophils with colchicine and dexamethasone resulted in significant decrease in NETs formation compared to controls. Interestingly, the percentage of NETs increased upon exposure of neutrophils to serum from BD patients. Moreover, PAD4 mRNA expression was 3 times higher in BD patients compared to controls. Immunolabelling assay demonstrated that NET-bound proteins were present in NETs scaffold of unstimulated neutrophils from BD patients in vitro. Additionally, NETs were detected in skin tissues of BD patients focally distributed in proximity to small vessels in vasculitis patients and to inflamed adipose tissue in panarctins patients. These were associated with elastase and citrullinated histone-3 proteins.

**Conclusions:** We show here for the first time that circulating neutrophils from BD patients are prone to NETosis in vitro. NET formation was inhibited by the addition of colchicine and dexamethasone reflecting their therapeutic benefits in BD. The increase of NETosis upon serum stimulation suggests the presence of soluble factors and cytokines triggering NETs in BD. Combined, these results suggest a major role of NETs in the pathophysiology of BD.

**REFERENCE:**


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**IL-33/ST2-MEDIATED INFLAMMATION IN ENDOTHELIAL CELL IS DIRECTLY AGGRAVATED BY IL-6 DURING LUPUS NEPHRITIS**

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**Background:** "Alarmins" are prototypic endogenous pro-inflammatory factors as they are released from necotic cells and provoke local damage or systemic inflammation. Evidences are accumulating to support the inclusion of "Alarmins" as targets of autoreactivity as well as inducers in the pathogenesis of Systemic Lupus Erythematosus (SLE). Interleukin (IL)-33 is a novel member of the family of "Alarmins" due to its characteristics and functions in mediating host immune responses. On this background, we sought to determine the role of IL-33/ST2 axis in lupus pathogenesis. The role of IL-33/ST2 axis has not previously been described in lupus nephritis.

**OBJECTIVES:** This project will study the followings: (1) To determine whether IL-33 was present in renal glomerular endothelial cells; (2) To assess the functional and intracellular signal transduction mechanisms regulating the link between IL-33/ST2-mediated innate immune inflammation in human umbilical vein endothelial cells (HUVECs).

**Results:** This study, for the first time, showed that IL-33 was pathologically expressed in the kidney tissue of patients with lupus nephritis and not in that of subjects with relative normal renal tissues at atrophy. However, no significant difference was observed between patients with lupus nephritis and kidney cancer. Immunofluorescence (IF) for IL-33 in kidney was performed in lupus patients. IL-33 was clearly seen in glomeruli and also in peritubular areas. To determine whether the IL-33 staining in glomerular area was in endothelium, multiple staining for IL-33, CD34 (a marker for endothelial cells) and 4'6-diamidino-2-phenylindole

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**EVIDENCE OF NETOSIS IN CIRCULATING NEUTROPHILS AND SKIN LESIONS WITH VASCULITIS AND PANNICULITIS IN BEHÇET’S DISEASE**

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**Background:** Behçet’s disease (BD) pathophysiology is poorly understood. It is characterised by recurrent episodes of acute inflammation consisting of neutrophil infiltration in affected organs and around blood vessels. Recently, NETosis, trapping and killing pathogens by activated neutrophils, has been described. These Neutrophil Extracellular Traps (NETs) were shown to promote inflammation in a number of autoimmune diseases such as Systemic Lupus Erythematosus, ANCA-Associated Vasculitis and Rheumatoid Arthritis. We hypothesise that in BD, neutrophils trigger inflammation and vasculitis via NETosis.
(DAPI) stained for cell nuclei was performed. Co-localization of IL-33 and DAPI in CD34 positive cells demonstrates IL-33 staining in the nucleus of glomerular endothelial cell of lupus kidney. Expression of intracellular but not surface ST2 was increased in plasmacytoid dendritic cells/pDC (CD16-CD14-CD85k+CD123 +) of lupus patients when compared with healthy controls. Incubation of HUVECs with IL-33 and/or IL-12 increased the production of IL-6, but IL-4, IL-5, IL-6, IL-13 and TNF-α was not produced.

Conclusions: As a result of external stimuli or infection, renal glomerular endothelial cells undergo cellular death and release the "alarm" IL-33, to alert the lupus immune system. Released IL-33 interacts with their target cells, pDC via their specific receptor ST2 to subsequently induce innate and adaptive responses, activate inflammatory pathways in the pathogenesis of lupus nephritis.

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Disclosure of Interest: None declared


SAT0045
DNA METHYLATION OF SOCS3 AS A POSSIBLE MECHANISM FOR PERSISTENT URATE INDUCED INFLAMMATION

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Background: Hyperuricemia is a metabolic condition associated with cardiovascular diseases.1 However, mechanisms for a causal relation have not been fully elucidated yet. Previously, we showed that monocytes primed with urate show a shift in the balance of cytokine production: increased proinflammatory cytokines and decreased levels of IL-1 receptor antagonist.2

Objectives: In this study we investigate if these changes to urate exposure are persistent and changes in DNA methylation serve as a molecular substrate for these effects of hyperuricemia.

Methods: DNA methylation was assessed in whole blood of 80 individuals of Maori ancestry with varying serumurate levels. Human PBMCs and monocytes from Dutch healthy volunteers were isolated and pretreated for 24 hour with urate. Cells were either directly stimulated with LPS or LPS/MSU or subjected to increasing resting days before restimulation. Cytokine levels were determined in supernatants by ELISA. SOCS3 mRNA levels were determined by qPCR after 24 hour urate priming. Phosphorylation of STAT3 was assessed after stimulation by flow cytometry with intracellular staining for pSTAT3.

Results: Human PBMCs primed with urate demonstrated increased IL-1Ra and IL-6 responses and decreased IL-1Ra production compared to controls. Although IL-1Ra production was diminished after increasing resting days, persistent effects were observed for the reduction of IL-1Ra and induction of IL-6. To investigate whether these persistent changes were mediated by epigenetic changes, differences in DNA methylation between normouricemic and hyperuricemic individuals were assessed. SOCS3 gene was higher methylated at 3 neighbouring intragenic positions in hyperuricemic individuals. In vitro, SOCS3 mRNA levels were significantly increased in monocytes after 24 hour urate treatment. Moreover, urate dose-dependently suppressed the phosphorylation of STAT3 after stimulation.

Conclusions: In this study we demonstrated that urate has persistent proinflammatory effects on human monocytes. Higher SOCS3 DNA methylation is observed in hyperuricemic individuals. In vitro, urate priming leads to increased levels of SOCS3 mRNA and consequently suppression of STAT3 phosphorylation. Interestingly, STAT3 inhibition has been reported to mediate IL-1Ra downregulation. Therefore, we hypothesize urate induced inflammation is at least partly mediated by changes in methylation of the SOCS3 gene. However, further validation of this pathway is needed to elucidate possible targets for therapy.

SAT0044
LIN28A IS OVEREXPRESSED IN OSTEOARTHRITIS AND IS ESSENTIAL FOR THE STABILITY AND HIGH LEVEL EXPRESSION OF IL-6 AND COX-2 IN HUMAN CHONDROCYTES

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Background: Osteoarthritis (OA), the most common type of joint disease, is characterised by progressive and irreversible degradation of articular cartilage. Dysregulated gene expression has also been linked to disease pathogenesis. Lin28A is an evolutionarily conserved RNA binding protein and known to play a critical role in development, metabolism and tumorigenesis. However, the role of Lin28A in osteoarthritis is not yet explored.

Objectives: To study the role of Lin28A in the regulation of genes associated with OA pathogenesis in human chondrocytes.

Methods: Primary human chondrocytes were isolated from the undamaged portion of the knee OA cartilage by enzymatic digestion and were cultured in DMEM/10% FCS. Total RNA from cartilage explants or chondrocytes was prepared using Trizol and was made DNA-free by on-column digestion method. mRNA expression levels of Lin28A, Lin28B, MMP-13, IL-6, COX2 and iNOS mRNAs were quantified by TaqMan assays. Protein expression was analysed by immuno-blotting using validated antibodies. Nucleofection was used for the siRNA mediated depletion or plasmid mediated overexpression of Lin28A gene was used to study the regulation of Lin28A mRNA and protein expression in OA chondrocytes in a time dependent as well as dose dependent manner. siRNA mediated depletion of Lin28A expression in OA chondrocytes inhibited the IL-1β-induced expression of MMP-13, IL-6, COX2 and iNOS mRNAs. Importantly, the overexpression of Lin28A resulted in decreased half-life of IL-6 and COX-2 mRNAs, while the overexpression of Lin28A had the opposite effect in IL-1β stimulated OA chondrocytes. This indicated that Lin28A contributes towards the stability of IL-6 and COX-2 mRNAs.

Conclusions: Our data for the first time demonstrate that Lin28A plays a key role in OA pathogenesis by stabilising the expression of catabolic gene transcripts in human chondrocytes under pathological conditions. These data reveal a previously unidentified role of Lin28A in chondrocytes and identify it as a potential therapeutic target for the treatment of OA.

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