5.49; $p < 0.001$ respectively. Δ MFI and SI on DHR between patients and healthy controls were comparable. There was no difference in oxidative burst activity between active and inactive SLE patients. Two patients showed a double peak in DHR flow cytometry consistent with mosaic pattern associated with a probable carrier state for X-linked chronic granulomatous disease. Both oxidative burst activity and phagocytic activity did not show significant correlation with the dose of prednisolone.

Conclusion: This study suggests that there is impaired neutrophil function in childhood SLE and also there may be a correlation between neutrophil dysfunction and disease activity. The *E. coli* based phagocytic functions of neutrophils were significantly reduced in pediatric SLE patients compared to healthy controls. Phagocytic activity of neutrophil was significantly lower in patients with disease activity and coexistent infection in patients with pediatric SLE. Oxidative burst activity was reduced in patients with pediatric SLE compared to healthy controls. However, there was no significant correlation of oxidative burst activity to the age, disease activity and coexistent infections.

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Abstract THU0510 – Figure 1. Phagocytic functions in patients with active SLE versus inactive disease

Abstract THU0510 – Figure 2. Correlation of SLEDAI and phagocytic activity

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THU0511

COMPARISON OF PAXGENE AND TEMPUS WHOLE BLOOD RNA COLLECTION AND ISOLATION SYSTEMS FOR THE QUANTIFICATION OF TYPE I INTERFERON-STIMULATED GENE EXPRESSION

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Background: Type I interferons (IFN) have important roles in many pediatric and adult rheumatic diseases and are a new therapeutic target for which several "anti-interferon (anti-IFN)" treatments are currently in use or in development. Since the direct detection of these proteins in biological samples has proved challenging, indirect methods are often used to infer the presence of type I IFN. Most commonly this involves quantification of the relative expression of interferon-stimulated genes (ISGs) that are used to calculate an interferon score (IS) (1). This score has been used for example to assess type I IFN activity in pediatric patients with type I interferonopathies, systemic lupus erythematosus, dermatomyositis and systemic juvenile idiopathic arthritis (2). Both qPCR and Nanostring technology have similar sensitivity and reproducibility for IS determination (3). The use of different whole blood RNA collection systems on the IS have not been evaluated however despite evidence of method-dependent changes in gene expression (4).

Objectives: The aim of the study was to compare expression of six common ISGs (*IFI27*, *IFI44L*, *IFIT1*, *ISG15*, *RSAD2*, *SIGLEC1*) and the corresponding IS in RNA derived from two commonly used whole blood RNA collection systems (PAXgene and Tempus).

Methods: Whole blood was collected from ten healthy individuals (median age 25.5 years) in sodium heparin tubes and incubated without or with recombinant human interferon alpha 2b (rhIFN α , 2 IU/ml, 4 hrs, 37°C, 5% CO₂). Next, samples were divided between PAXgene (PreAnalytiX, Becton Dickinson) and Tempus (Applied Biosystems) tubes and RNA was isolated according to the manufacturer's protocols. cDNA was synthesized (500ng input RNA; qScript cDNA synthesis kit) and ISG expression measured on a QuantStudio 6 Real-Time PCR instrument using a TaqMan Fast Advanced Assay. For each ISG, expression was normalized against the geometric mean of two housekeeping genes (18s rRNA and HPRT1) and calculated using the formula $2^{-\Delta\Delta Ct}$. Relative gene expression is reported as the normalized expression of each ISG divided by the median of normalized expression of the same ISG in unstimulated samples. The median relative expression of all six ISGs was used to calculate the IFN score for each sample.

Results: There was no statistically significant difference in the normalized expression of any of the six ISGs in either the rhIFN α -stimulated or unstimulated samples derived from PAXgene or Tempus tubes. The greatest difference in mean normalized expression in both unstimulated and stimulated samples was observed for *ISG15* (difference in mean normalized expression was 0.0034 and 0.11, respectively). Overall there was a strong correlation of the IFN score between PAXgene and Tempus tubes for both the unstimulated ($R^2 = 0.9117$, $p < 0.0001$) and rhIFN α -stimulated samples ($R^2 = 0.8529$, $p = 0.0001$).

Conclusion: Despite reported differences in gene expression patterns associated with samples collected in PAXgene versus Tempus tubes, our results demonstrate that 6-gene interferon scores do not differ significantly between RNA samples obtained with these two systems. These results suggest that health care and research centres can use either tubes for IFN score determination using these 6 ISGs and results can be directly compared irrelevant of the RNA collection system employed.

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