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Abstract

Understanding Autoimmune Pathogenesis in Pediatric Pulmonary Conditions by a 30-marker Spectral Flow Cytometry assay on Bronchoalveolar Lavage

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Introduction

Autoimmune lung diseases in children, such as asthma and interstitial lung disease, can significantly affect long-term health outcomes. However, the mechanisms underlying the development of these diseases, particularly the role of autoimmunity, are not yet fully understood. In the INSPIRE CHILD Project (Investigating Novel Strategies in Pediatric Immune Responses and Microbiome Environment: Comprehensive Lung Disease Investigation in Children) one sub aim is to characterizing immune cell infiltration in the lungs using bronchoalveolar lavage (BAL) samples from children in the early stages of lung disease. This study seeks to identify unique immune profile changes associated with various pulmonary conditions, providing novel insights into the pathogenesis of these diseases.

Methods

BAL was used to collect saline samples from 27 pediatric patients, aged 0–17 years, diagnosed with a range of autoimmune pulmonary disorders. After completing the daily BAL procedures at Aarhus University Hospital, the samples were transported to the Department of Biomedicine at Aarhus University for further processing. Mononuclear cells (MNCs) were isolated from BAL samples using ficoll-paque gradient centrifugation.

The MNCs were stained using a 30-color spectral flow cytometry panel, incorporating markers for lineage, differentiation, immune checkpoints, activation, and exhaustion. Sample analysis was performed using the SONY ID7000™ Spectral Cell Analyzer, with spectral unmixing executed using the ID7000 Acquisition and Analysis Software (version 2.0.2). Following spectral unmixing, the data were exported as FCS files, batch corrected and analyzed using the Cytobank platform.

Results

A blinded analysis was conducted to generate immune profiles for each BAL sample, using a manual gating technique. The immune profiles were classified into 5–6 groups based on one or more dominant immune cell populations. To enable dimensionality reduction and clustering analysis, batch correction was applied to all 27 BAL samples. This approach facilitated unbiased characterization of global immune cell characterization, allowing for a more detailed analysis of immune cell subsets and the assessment of activation and exhaustion states across all samples simultaneously.

The immune profiles obtained through manual gating were consistent with the results from the dimensionality reduction and clustering analysis. Preliminary comparisons of these global immune profiles with patient diagnoses revealed promising correlations. However, a comprehensive, in-depth analysis is still ongoing and will be presented at the DSPAP Annual Meeting.

Conclusion

This study is the first time where a high-dimensional spectral flow cytometry assay has been applied on BAL samples for diagnostic proposes. Preliminary results suggest that this technique holds promise in exploring the autoimmune components contributing to the pathogenesis of various pediatric pulmonary conditions. With further in-depth immunological analysis of the collected data, spectral flow cytometry could potentially contribute to the development of personalized therapeutic strategies, improving prognosis and quality of life for children with severe pulmonary diseases.