Cracking the Code: Reproducibility Challenges in Metabolomics for Asthma Models

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Context: Asthma represents the most common chronic respiratory disease, affecting 235 million people worldwide. Asthma is a non-curable complicated disease. Currently, asthma monitoring relies on invasive sputum analysis or empirical approaches. The development of personalized medicine strategies would positively impact patients' quality of live. In this study, we developed a multi-omics workflow to identify metabolic pathways in in vitro asthma models to identify breath biomarkers origin.

Method: We used A549 lung epithelial cell line as a proof-of-concept in vitro model compatible with a multi-omics workflow. The cells were stressed with H_2O_2, lipopolysaccharide (LPS), and sputum supernatant to trigger inflammatory responses. Next, we investigated the metabolic profile using headspace monitoring for volatile molecules, metabolite and lipid double derivatizations. The three types of extract were all analysed using comprehensive multidimensional gas chromatography coupled to mass spectrometry (GCxGC-MS). Resulting profiles were processed independently and using data fusion approaches.

Results: In the literature, we observed high variability in the reported stress incubation conditions for H_2O_2 and LPS challenges. Moreover, day-to-day reproducibility was challenging, especially for H_2O_2. To gain a better understanding, we developed an in situ H_2O_2 quantification method for different culture conditions. In the end, only the LPS and the sputum conditions met the replication requirements.

For the three optimized analytical conditions, the resulting metabolite profiles were processed for pathway discoveries and cross-compared with our asthma breath marker data base. From there, we identified specific aldehyde-related pathways.

Conclusion: In this study, we performed a thorough investigation of the reproducibility of oxidative stress conditions for an in vitro asthma model in a multi-omics setting. We developed three analytical workflows, each focusing on a specific group of molecules: volatile organic compounds, semi-volatile metabolites and semi-volatile lipids. The generated data were cross compared with our breath marker data base.