Identification of metabolite families in exhaled breath using secondary electrospray ionization MS and UHPLC-MS/MS

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Exhaled breath is a complex mixture of endogenous and exogenous compounds that reflect key information about the human metabolism. Therefore, knowledge of the chemical identity of metabolites in breath is crucial to establish a link between biochemical pathways and exhaled metabolites.
Secondary electrospray ionization coupled to high-resolution mass spectrometry (SESI-HRMS) is a rapid method, which enables the analysis of exhaled breath with excellent sensitivity. Clusters of correlating signals provide information on families of compounds that share a common metabolic origin. In this work, we used SESI-HRMS for the on-line analysis of exhaled breath of 171 healthy participants. Based on the data obtained, we performed correlation analysis to find respective families of exhaled metabolites.
However, unambiguous compound identification is difficult due to interferences with isobaric compounds and the occurrence of isomers. Thus, ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) of exhaled breath condensate (EBC) was utilized as complementary method to determine retention times and fragments for the confirmation of putative structures.

Real-time measurements were carried out using a home-built SESI source coupled to a high-resolution time-of-flight mass spectrometer recording from 50-450 Da in positive and negative ion mode. 171 healthy participants were asked to exhale into the ion source through a disposable mouthpiece. Correlation analysis was then carried out to find groups of correlating signals. Different online databases (HMDB, KEGG) were utilized for the determination of possible metabolite candidates. EBCs of 13 healthy participants (6 male, 7 female) were pooled and pre-concentrated by lyophylization. UHPLC-MS/MS experiments were then used to obtain retention times and fragments for the previously identified correlating families. Possible structures were then confirmed by comparison with a standard where available.

The correlation analysis revealed several strongly connected metabolite families, suggesting either transformation cascades through common biochemical pathways or homologous series. Compound identification is currently ongoing. Additionally, we were able to identify several structures of recently reported possible biomarkers of pulmonary fibrosis, offering an insight into the pathophysiology of the disease.