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A non-targeted metabolomics analysis of SARS-CoV-2 and influenza infection in children

Emily G Armitage[1]; Parthena Savvidou[2]; Olga Begou[3], Alan Barnes[1], Elias Iosifidis[2], Helen Gika[3], Neil J Loftus[1], Emmanuel Roilides[2], Charalampos Antachopoulos[2]

[1]Shimadzu Corporation, Manchester, UK; [2]Infectious Disease Unit, 3rd Department of Pediatrics, Aristotle University School of Medicine, Hippokration General Hospital, Thessaloniki, Greece; [3]Biomic AUTh, Center for Interdisciplinary Research and Innovation, Thessaloniki, Greece

Overview

- Application of an innovative software tool for non-targeted analysis to analyze metabolomics data acquired from the analysis of blood samples from patients with SARS-CoV-2 or influenza compared to controls.
- Metabolomics revealed statistically significant phenotypic differences in cases and controls including 75 features significant by ANOVA that could be putatively identified as metabolites or lipids by MS/MS library matching.
- Phosphatidylcholines were most significantly reduced in the blood profiles of influenza and SARS-CoV-2 relative to controls and SARS-CoV-2 levels were also significantly lower than influenza.

1. Introduction

Acute respiratory illnesses (ARIs) are a leading cause of morbidity and mortality in paediatrics; however, diagnosis and prognosis can be challenged by children presenting with similar clinical symptoms, regardless of the underlying infection. Being able to distinguish between infections caused by viruses such as SARS-CoV-2 and influenza could lead to a more accurate diagnosis and clinical outcome. In this study, non-targeted metabolomics analysis of blood samples from children admitted to hospital with an ARI later diagnosed as either SARS-CoV-2 or influenza infection were compared to controls with the aim of phenotyping and enhancing the distinctions between the disease profiles.

2. Materials and Methods

Age and gender matched whole blood samples collected from donors with SARS-CoV-2 (n=21), donors with influenza A (n=28) and controls (n=10) were extracted in ice cold methanol. Pooled QC samples were prepared by mixing equal aliquots from each extract.

All samples were analyzed with a non-targeted metabolomics method using high resolution reversed-phase LC-MS/MS (LCMS-9050 Q-TOF, Shimadzu Corporation, Japan) and a novel software application (Insight Profiler, Shimadzu Corporation) for data processing and analysis to differentiate metabolic differences between the donor groups. Data processing in Insight Profiler software considered feature detection, alignment, statistical analysis and compound identification in a single method. Compound identification used an in-house metabolomics library, as well as third-party repositories including MassBank, LipidBlast and HMDB.

2.1 Data acquisition

Reversed phase LC Separation.

- C18 BEH (2.1x100mm 1.7µm); 50ºC, flow rate 0.4 mL/min.
- Binary gradient; water + 0.1% formic acid and acetonitrile + 0.1% formic acid.
- Sample cycle time 35 minutes.

LC-MS/MS Mass Spectrometry Detection.

- Positive ion mode TOF MS survey scan (m/z 60-1000; 100 msecs).
- 27 DIA-MS/MS mass scans (m/z 40-1000; 33msec; precursor isolation 35 Da)
- Collision energy spread 5-55V
- External mass calibration
- Total cycle time <1 sec.

2.1 Automated data processing in Insight Profiler

Insight Profiler is a novel software solution to streamline non-targeted analysis workflows for QTOF data, providing an end-to-end solution for non-targeted analysis and metabolomics.

Feature Detection and Alignment
Detects and aligns all ion signals that behave as a peak.

Statistics and filters
Applied to find ion signals of significance and to remove ions of high variance.

Compound identification
Using large scale screening lists and multiple libraries to identify ion signals of interest.

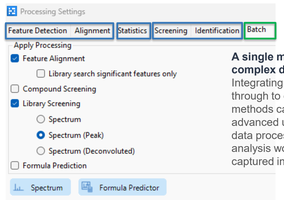

Figure 1. The Insight Profiler processing application method editor, designed to create a single workflow for feature detection of unknowns to compound identification.

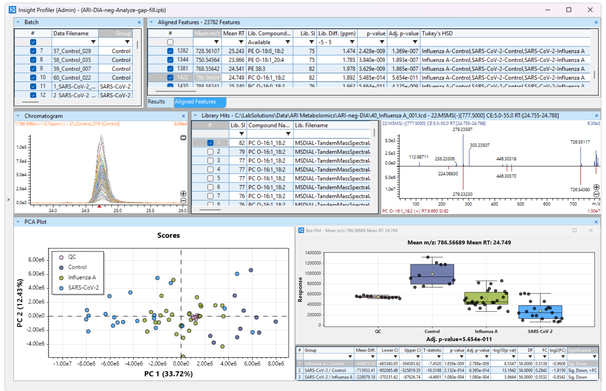

Figure 2. Insight Profiler was applied to the analysis of whole blood extracts from children with Influenza A, SARS-CoV-2 or controls following non-targeted metabolomics analysis (LC-DIA-MS/MS). Interactive and intuitive data review enables analysis of statistically significant features. Chromatogram, spectrum and library identification are linked with statistical analysis, including PCA/box plots, helping data interpretation to find potential biomarkers. The example highlights the putative identification of PC O-16:1_18:2 in negative ion mode data which was significant in both negative and positive ion mode datasets.

3.2 Metabolomics reveals potential biomarkers of ARIs

Non-targeted metabolomics analysis of whole blood extracts revealed statistically significant phenotypic differences in cases and controls. Figure 2 highlights the six most significant features by ANOVA, putatively identified as PC O-16:1_18:2, PC 18:2_18:2, PC 18:2_20:4, PE O-16:1_18:2, PC O-16:0_18:2*, PC 17:0_18:2*. Tukey's HSD post hoc analysis revealed that all of these were significantly diminished in both infections versus the control group, while PC O-16:1_18:2 and PC O-16:0_18:2 were also significantly lower in SARS-CoV-2 relative to Influenza A.

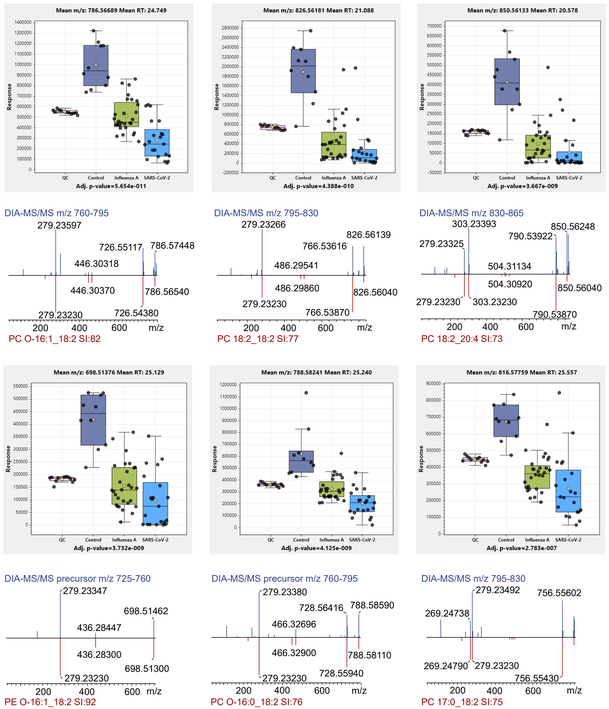

Figure 3. Insight Profiler revealed 75 annotated metabolite and lipid features that were significantly different between groups (ANOVA, FDR adjusted p<0.05). PCs were significant in both positive and negative ion mode datasets. Boxplots and MS/MS library identifications for the top 6 most significant annotated features are displayed here.

3.3 Metabolomics workflow reveals potential biomarkers

Current diagnostic strategies for ARIs in children can suffer from a lack of sensitivity and specificity required to make an accurate and timely assessment enabling an optimal care plan. Metabolomics may be a useful approach to improve our understanding of different ARI phenotypes and potentially reveal specific biomarkers that could enhance care plans.

In this study, blood samples from patients aged between 1 month and 16 years who presented at hospital with ARI symptoms and tested positive for SARS-CoV-2 or influenza were compared to age matched controls who tested negative for both illnesses. Children with immunosuppression or comorbidities were excluded from the study.

Consistent with the clinical presentation of each illness, metabolic differences were similar for children infected with either SARS-CoV-2 or influenza A. Insight Profiler revealed 75 annotated metabolite and lipid features that were significantly different between groups (ANOVA, FDR adjusted p<0.05). As depicted in the Upset plot in Figure 3, Tukey's HSD post hoc analysis revealed that 73 of these were significantly different between SARS-CoV-2 and controls, 35 of which were also significant between Influenza A and controls and 9 of which were significantly different between the infections as well.

Upset plot shows the number of annotated significant metabolites/lipids in each pairwise comparison (set size) and the number of annotated significant metabolites/lipids common to pairwise comparisons (intersections).

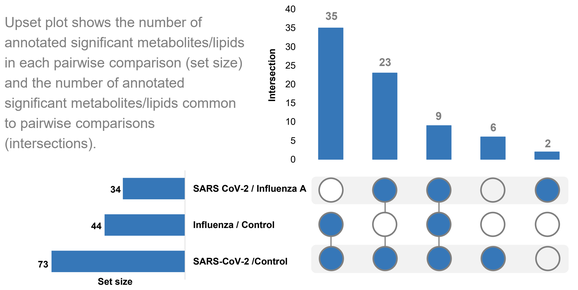

Figure 4. Upset plot highlighting interactions in annotated metabolites and lipids found to be statistically significantly different according to Tukey's post hoc analysis between each pair of groups: SARS-CoV-2 vs control, Influenza A vs control and SARS-CoV-2 vs Influenza A.

4. Conclusions

- Non-targeted metabolomics analysis of whole blood extracts revealed statistically significant phenotypic differences in cases and controls.
- Consistent with the clinical presentation of each illness, metabolic differences were similar for children infected with either SARS-CoV-2 or influenza.
- Insight Profiler software enabled a streamlined automated approach to revealing metabolic signatures of each infection highlighting statistically significant features (PCA and ANOVA) putatively identified with integrated MS/MS library searching.