SERUM PROTEOMICS OF COVID-19 SAMPLES ANALYSED BY LIQUID CHROMATOGRAPHY AND SELECT SERIES™ CYCLIC™ ION MOBILITY MASS SPECTROMETER

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INTRODUCTION

During the pandemic which began in December 2019, COVID-19 spread across the world, infecting over 600 million people and has so far caused almost 7 million deaths [1]. Although many people are asymptomatic or experience only mild symptoms, SARS-CoV-2 can cause severe illness. The risk of developing severe illness is influenced by many factors, particularly age, comorbidities, vaccination status and access to healthcare. The emergence of this novel respiratory pathogen overwhelmed many healthcare systems globally, leading to high levels of mortality in the early stages but mortality rates have now thankfully greatly decreased with the rapid development of therapeutics and vaccines.
At the outset of the pandemic, the Manchester Allergy, Respiratory and Thoracic Surgery (ManARTS) Biobank recruited hospitalized patients with COVID-19 and collected biological samples. This was done with a view to using molecular profiling and deep phenotyping to characterize the mechanisms underlying the disruption of metabolic processes that resulted in severe disease.
Clinical proteomics is a rapidly growing area of research and involves the analysis of bodily fluids, for example plasma or serum. The amount of available sample is much greater than in a classical proteomics experiment and allows the use of analytical scale chromatography, which tends to be more robust and reproducible when compared to nanoscale LC employed in classical proteomics experiments [2,3]. In this presentation, we have analyzed pooled tryptically digested undepleted serum samples collected from a large cohort of patients suffering from COVID-19, using SELECT SERIES Cyclic Ion Mobility mass spectrometer operating in HDMS^E, a data independent acquisition mode [4].

METHODS

Cohort Demographics

The ManARTS COVID-19 cohort, **Figure 1,** comprised over 400 adults of whom 68% are male with a median age of 60. The cohort was ethnically diverse, with a higher representation of Black, Asian and minority ethnic groups compared to the general population. Other factors such as smoking status and BMI were also recorded to allow further statistical analysis based upon these metadata. From this cohort, six patient samples were pooled to represent mild or severe disease status, A QC comprising of both disease states was also created.

Sample Preparation

Undepleted sera samples corresponding to different disease states or severity were mixed with RapiGest™ SF Surfactant (Waters Corporation) and then subjected to reduction, alkylation and overnight trypsin digestion. Samples were diluted 7 times prior to injection onto the LC system.

Liquid Chromatography

Chromatography:	ACQUITY™ Premier UPLC™	Column: ACQUITY Premier CSH™ C18 1.7 µm, 2.1 mm x 100 mm reversed-phase	
Mobile phases:	A Water containing 0.1% (v/v) formic acid	B Acetonitrile containing 0.1% (v/v) formic acid	
Gradient:	5-35% mobile phase B over 20 min	Flow rate: 150 µL/min	Column temp: 55ºC

Mass Spectrometry

Mass Spec, **Figure 2**:	SELECT SERIES Cyclic IMS	Resolution:V, nominal mass res 50k FWHM	
Polarity:	Positive	Capillary Voltage:	2.2 kV
Source temp:	100ºC	Mass Range:	m/z 50 to 1990
Acquisition:	HDMS^E	Integration time:	0.15s for low and elevated energy
Elevated Energy CE:	20 - 46 eV	Lock Mass:	[Glu1]-Fibrinopeptide B, 2$^+$, (m/z 785.8421)

Data Processing

Processing and searching:	Progenesis™ QI for Proteomics, ProteinLynx Global Server	Database:	UniProtKB reviewed Human
Fixed modification:	Carbamidomethylation (C)	Variable modification:	Oxidation (M); Deamidation (N/Q)
Mass tolerances:	10 ppm precursor, 20 ppm products	FDR:	1% protein level

Metaboanalyst 5.0 and TIBCO Spotfire® were used for further data analysis and viewing

RESULTS

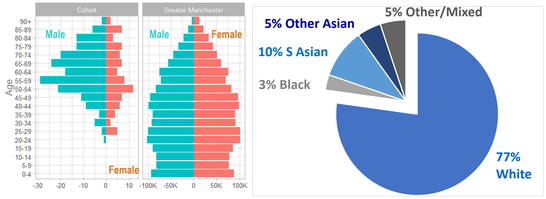

Figure 1 *ManARTS COVID-19 cohort. Population distribution (left) shows a skew in the population towards male, older adults when compared with the general Greater Manchester population. The cohort is also shown to be ethnically diverse (pie chart, right).*

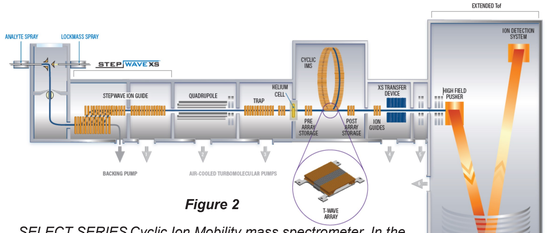

Figure 2

SELECT SERIES Cyclic Ion Mobility mass spectrometer. In the HDMS^E mode of operation, low and elevated collision energies are applied alternately to the Transfer region, after the Ion mobility separation. This allows precursor and product ions to be aligned based upon both retention time and drift time information.

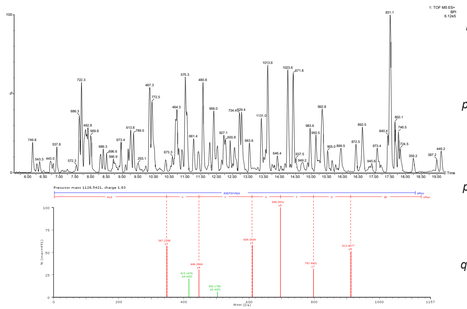

Figure 3

Example Low Energy chromatogram and elevated energy spectrum for the peptide ESDTSYVSLK from C Reactive Protein (CRP).

Analysis of the processed data shows that 96% of the identified peptides (including those not quantified), are within + - 3 ppm of their theoretical values

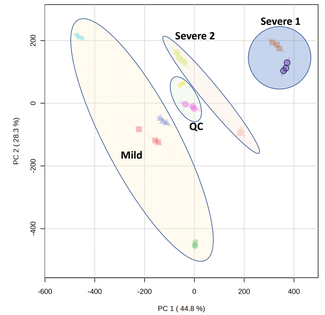

Figure 4

Multivariate analysis consisting of unsupervised principal component analysis (PCA). Data were filtered based upon Anova p value < 0.05. Based on the samples of the pilot study, clear differentiation is observed between the severe and mild patient groups. Severe patient group 1 shows the most significant separation from the other groups

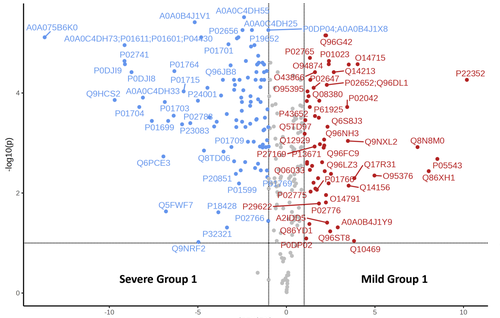

Figure 5

Volcano plot highlighting the differential regulation of proteins between Severe (Left) and Mild (right) group patients. In total, 268 protein groups were quantified.

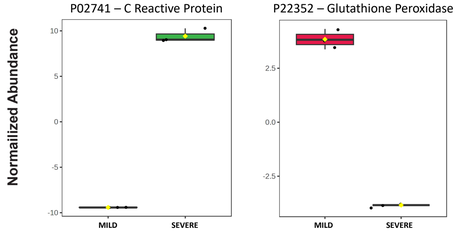

Figure 6 *Example of two regulated proteins in the severe disease patient group compared with the mild disease group. C-reactive protein (P02741) and Glutathione peroxidase (P22352).*

CRP is a signature of inflammation [5] and has been found in a parallel analysis of the depleted serum to be increased in severe patients. GPX has been linked to COVID and oxidative stress [6]

CONCLUSIONS

- **Proteomic analysis of plasma samples (undepleted & unfractionated) from COVID-19 patients has been performed using the Cyclic IMS platform and generated a large number of quantifiable proteins**
- **The pilot study highlighted the separation of patient groups based on severity of COVID-19 when using multivariate statistics.**
- **A range of proteins were identified as being statistically differential, which included CRP (P02741), for example, heavily upregulated in the severe group and Glutathione peroxidase (P22352) being heavily depleted in severe patients**
- **Further analysis will be performed on the full cohort of individual samples and offers a challenge with more diverse metadata involved in the statistical analysis**

REFERENCES

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