FUNED Fundação Ezequiel Dias

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Deep Proteomics Coverage of Human Serum Samples by Trapped Ion Mobility Mass Spectrometry

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Introduction

Yellow fever is a mosquito-borne viral illness and an endemic disease found in areas such as South America and Africa. The diagnosis requires information from patient and laboratory findings such as PCR and immunoassays. The disease has an incubation period of 3-6 days and ranges from asymptomatic to severe forms. Symptoms may include headache, myalgia, nausea/vomiting and high fever. About 15% of cases have severe symptoms and the mortality can approach 50% because of renal, hepatic, neurological impairment, and hemorrhage. A limitation to detecting yellow fever outbreaks has been the complexity of the diagnostics which currently relies on serologic testing of IgM antibodies and on detection of yellow fever virus RNA in the blood from acute cases of yellow fever using laboratory protocols. Thus, the development of a rapid tests, specially using mass spectrometry, could extend yellow fever diagnostics and prognostics. Herein, we have performed proteomics analysis on serum samples from different patients who either recovered or died from yellow fever using trapped ion mobility spectrometry coupled to quadrupole-time of flight mass spectrometry to evaluate and understand the progression of this disease.

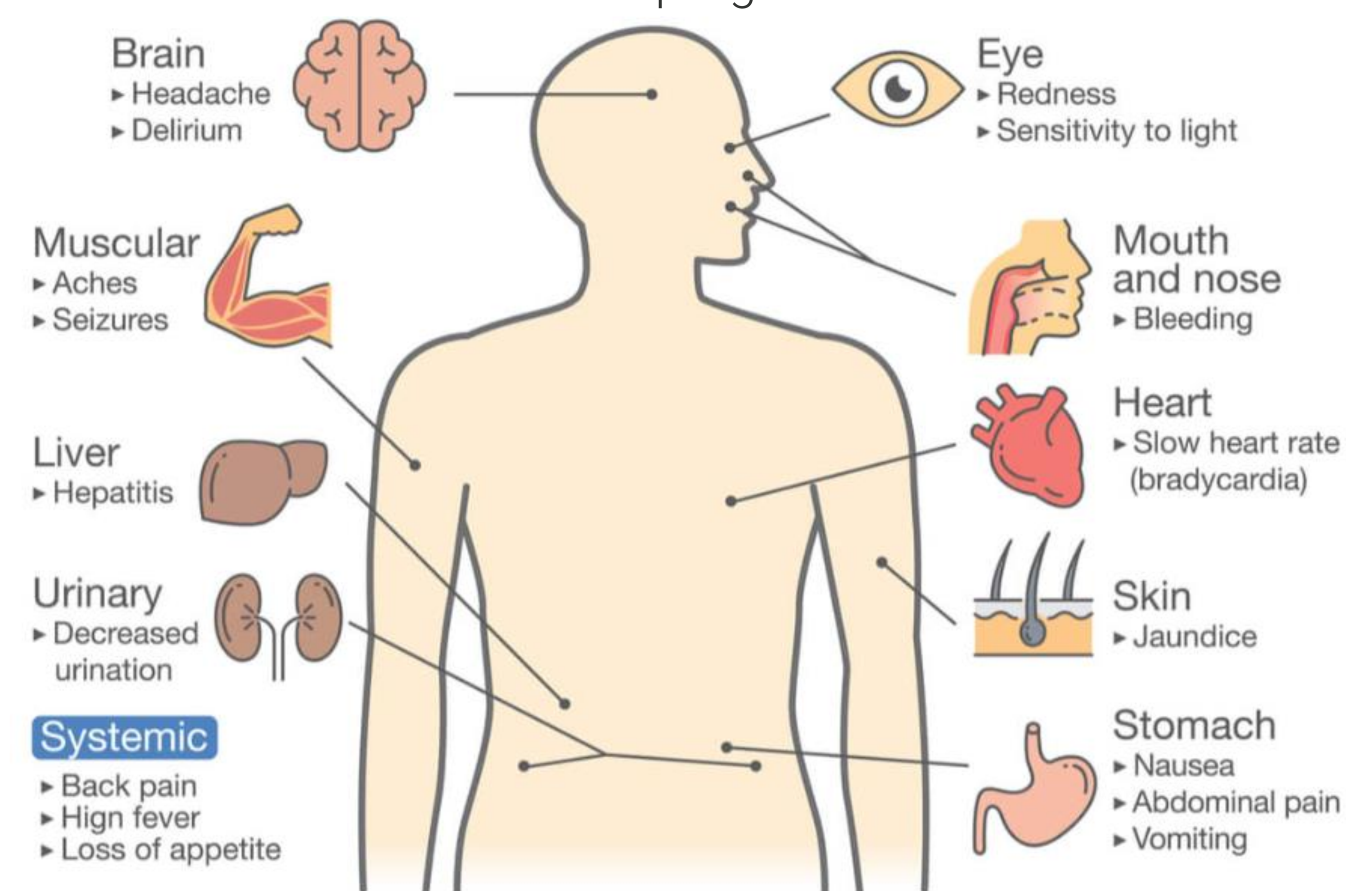

Figure 1: Symptoms of Yellow Fever. Figure from https://www.traveldoc.ca/yellow-fever-vaccine-Hamilton.

Methods

Pooled serum samples from a control group, a group recovered from infection and group from patients who died were analyzed by 2 workflows: no depletion and by depletion with acetonitrile. Serum proteome samples were digested with trypsin and 200 ng was loaded on column. LC-TIMS MS/MS data were obtained using a timsTOF Pro 2 instrument and chromatography was performed on a nanoElute (Bruker Daltonics) operating with an Aurora nano column (25 cm x 75 µm ID, C18 - IonOpticks, Australia) (Figure 2). All raw data were processed using PaSER and MSFragger (default parameters) for DDA mode and DIA-NN 1.8 – library free approach for dia-PASEF mode (at 1% FDR).

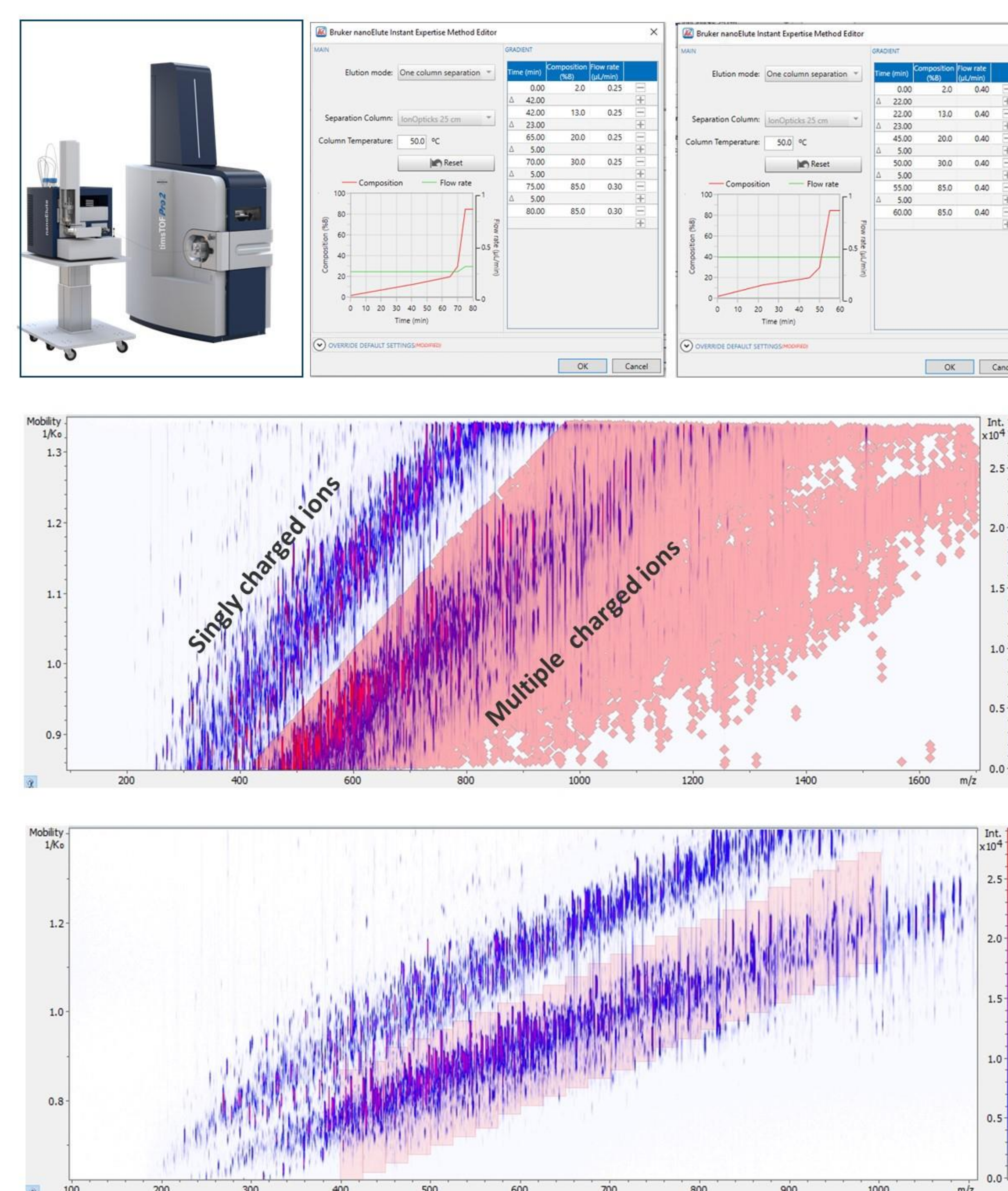

Figure 2: A) nanoElute and timsTOF Pro 2 used on the study. B) 70 minutes LC gradient with DDA acquisition. C) 50 minutes LC gradient with dia-PASEF acquisition. Heat map (m/z versus mobility 1/K0) showing peptides distribution for an entire run where red diamonds indicate precursors selected for MS/MS on a DDA MS acquisition mode (10 PASEF ramps – 1.1 second as total cycle time. E) Heat map showing dia-PASEF windows (29 windows in 0.84 seconds (50 ms as ramp and accumulation time) . 100-1700 m/z range.

Results

Global proteome analysis identified around 1,300 protein groups in DDA mode and 2,000 protein groups in dia-PASEF from depleted serum. Total number of unique peptides across all analyses was 15,000. Numbers of identified proteins according each group are shown below. Correlations between triplicates showed values above 0.97 (Pearson correlation) and protein intensity (abundance) with a dynamic range of five orders. The number of identified proteins changed significantly when different groups are compared. As expected, the number of peptides are higher for non-depleted serum, but number of proteins are lower in this sample preparation due to the presence of highly abundant proteins.

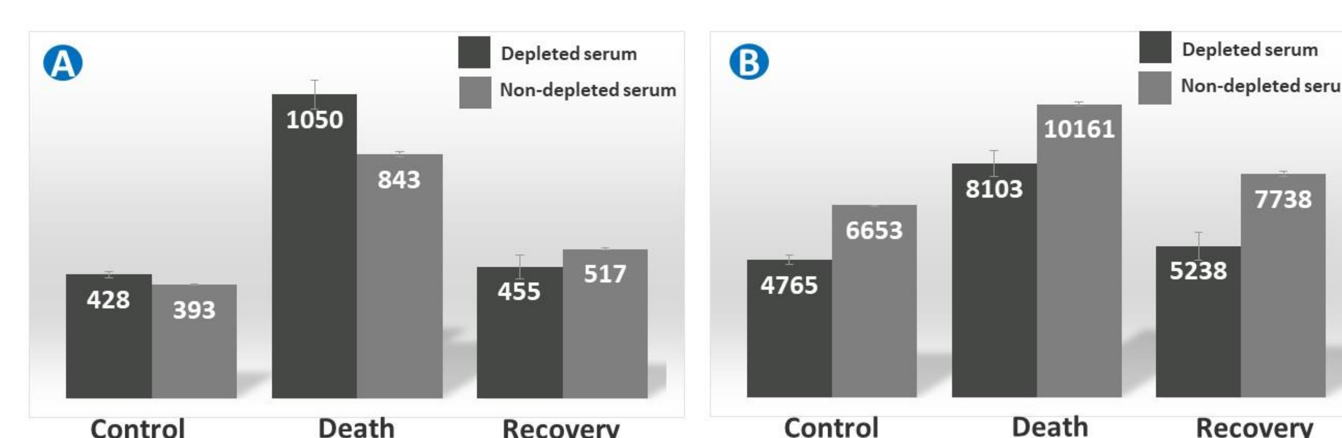

Figure 3: Number of identified Protein Groups (A) and peptides (B) according to group of patients and sample preparation (depleted and non-depleted) analyzed by DDA mode.

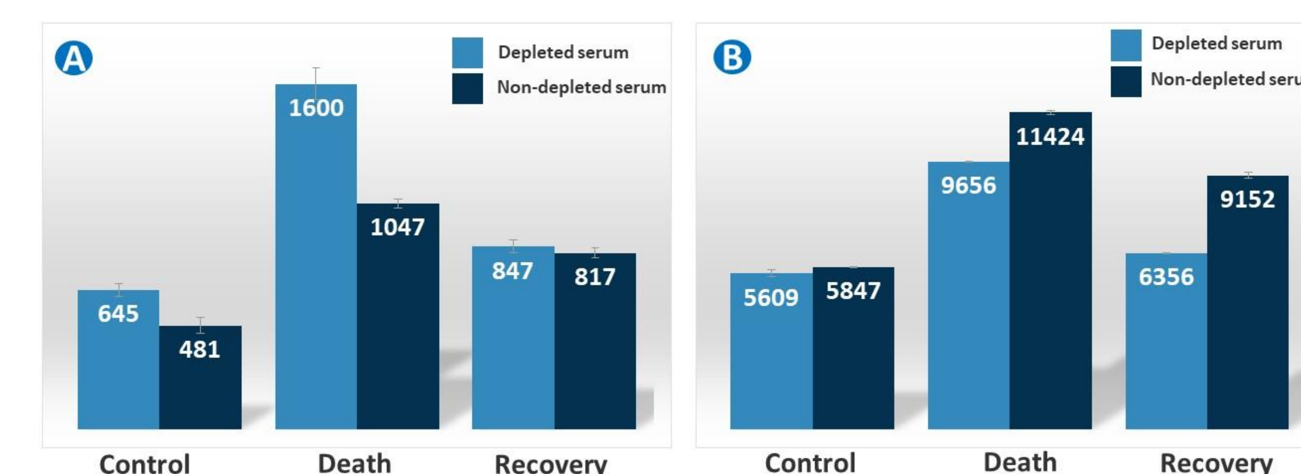

Figure 4: Number of identified Protein Groups (A) and precursors (B) according to group of patients and sample preparation (depleted and non-depleted) analyzed by dia-PASEF mode.

In addition to a high number of identifications, using label free quantification several proteins that were up or down regulated across the 3 patient groups could be quantified (figure 5). As an example of up regulated proteins in the death group when compared to the control, hemoglobin (beta subunit) is about 54x, while immunoglobulin lambda was found in the range 381x and the transferase formimidoyltransferase-cyclodeaminase was found more than 1000-fold change in this group. Bleeding episodes and liver damage are common symptoms in severe conditions and our findings reflect the clinical evidence for these patients.

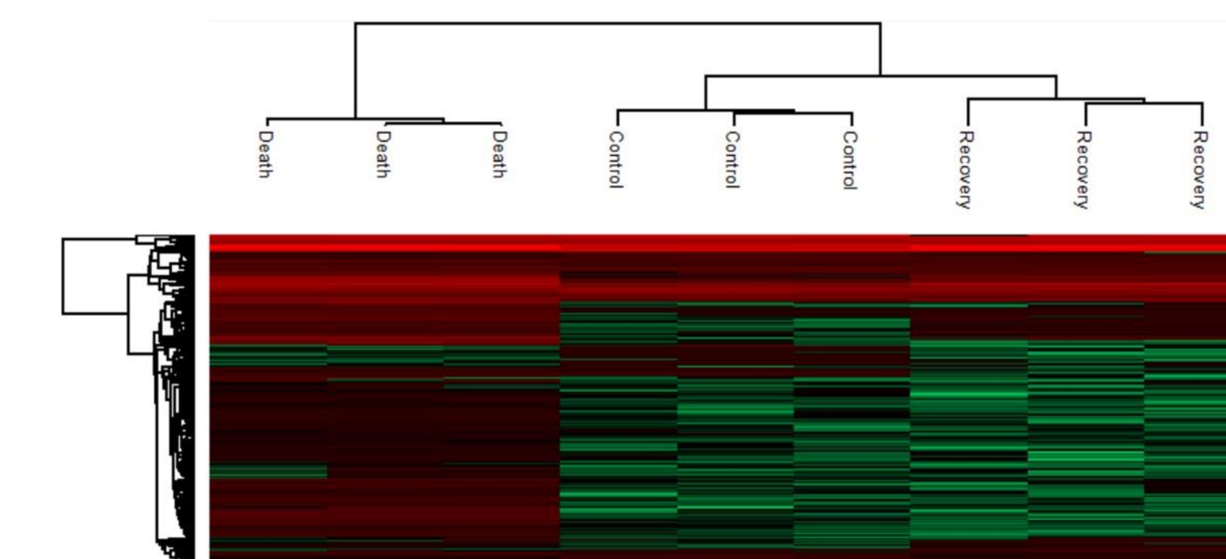

Figure 5: Hierarchical Clustering generated from depleted serum DDA mode is demonstrated as an example of label free quantification. Several proteins groups are up and down regulated according to different patient groups.

Notably this work shows a high number of identifications from serum, specially for patients related to yellow fever. Protocols using depletion were the best approach in this study and even using a short gradient, dia-PASEF (library free) identified 95% of proteins identified by DDA (overlap area – green) and still adding about 45% and 49% (blue area) further in the non-depleted and depleted treatments respectively (Figure 6).

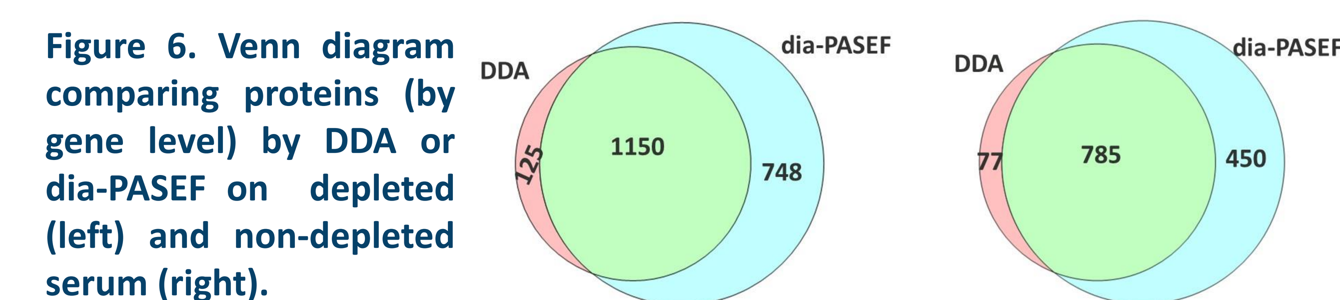

Figure 6. Venn diagram comparing proteins (by gene level) by DDA or dia-PASEF on depleted (left) and non-depleted serum (right).

Conclusion

This proteomic study has identified several proteins which can be used as biomarkers improving diagnostic and prognostics of yellow fever.

Additionally, analysis of serum reveals by mass spectrometry using timsTOF Pro enables a deep evaluation of yellow fever pathophysiology.

timsTOF

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