Characterization of the salivary protein repertoire of Covid-19 patients during and after disease-a pilot study

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Introduction

The COVID-19 pandemic impressively demonstrated how progress in technology, world-wide data-sharing and knowledge exchange within the scientific community impacts global health. The pandemic underscored the need for multiple diagnostic strategies providing information on disease onset, progression, immunity and infection sequelae. Saliva, being a kind of virulence reservoir for the SARS-CoV-2 virus, is a remarkable fluid in terms of diagnostic possibilities. Its molecular composition and specifically its proteome contains information about the nutritional, immunological and disease/health status. The possibility of non-invasive (self-) collection facilitates straightforward multiple sampling over time, providing not just a disease "snap-shot" but offering the opportunity to monitor disease progression and therapeutic interventions.

Methods

We analysed the proteome of self-collected saliva from SARS-CoV-2 infected subjects during five days, and after disease (post-Covid), along with healthy volunteers and infection-free subjects living in the same household. Peptides were analysed on a timsTOF Pro mass spectrometer in both PASEF-DDA and diaPASEF mode. MaxQuant and dia-NN algorithms were applied for data analysis, and Perseus was used for data visualization and statistics.

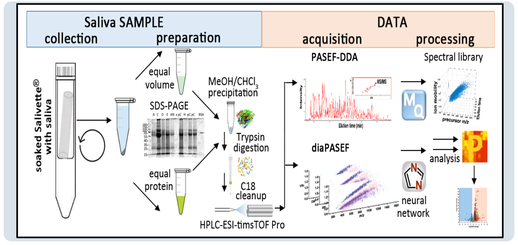

Fig. 1 Overview of workflow and time scale for proteome analysis: Saliva self-collection in Salivette, followed by proteomics-sample preparation.

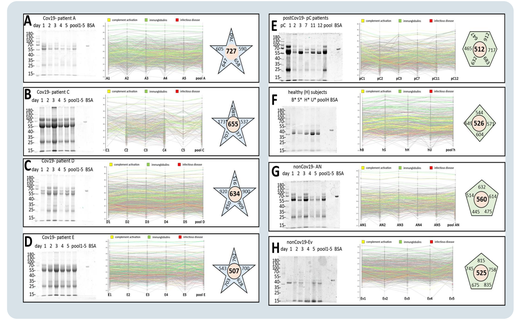

Fig. 2 Protein profiles (SDS-PAGE), LFQ-proteins abundance profiles, and Venn-diagrams of identified proteins obtained from DDA-runs. A-D) Longitudinal (day 1-5) and pooled saliva samples from Covid-19 patients, E) saliva samples from post-Covid-19 patients, F) healthy volunteers and G,H) infection-free subjects living in the same household. Enriched Reactome-terms (complement activation, immunoglobulins and infectious disease are color-coded.

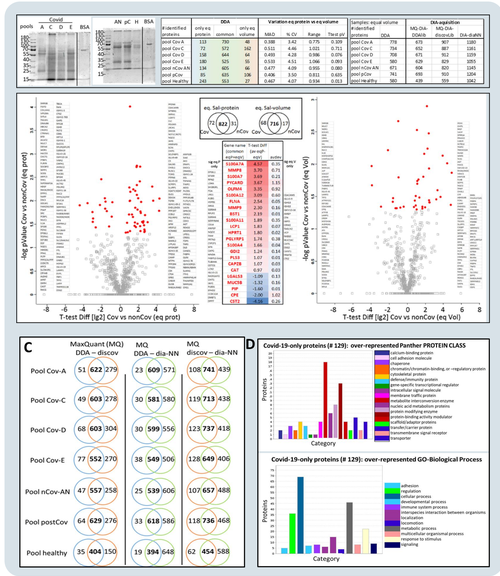

Fig 3: Differential analysis of Covid-19 vs. non-Covid-19 saliva proteomes.
A) Comparison of protein-content and volume-adjusted identifications and quantifications.
B) Significantly changed proteins in Covid (patients A,C,D,E) vs. non-Covid (post-Covid), healthy, and infection-free subjects. Left: Proteins with no p-Value (only in nonCovid) or Right: (only in Covid-19).
C) Comparison of saliva protein identifications by spectral library (MaxQuant DDA) and following DIA-analysis based on experimental and discovery-library (MaxDIA) and neural network-based DIA-analysis (dia-NN)
D) Biological interpretation-analysis of proteins only identified in Covid-19 saliva.

Results

- Since inflammatory processes present in diseased subjects cause an increased plasma protein (albumin) leakage, both volume- and protein-adjusted saliva were prepared for MS-analysis to evaluate the saliva protein repertoire.
- SDS-PAGE protein profiles of saliva samples demonstrated considerably variability in protein content, also reflected in protein identifications and abundance following proteomic analysis.
- In longitudinal protein profiles we found proteins annotated to complement activation, immunoglobulins and infectious disease variably enriched and thus decided to perform differential protein abundance analysis on pooled samples.
- We identified consistently more proteins in DIA-acquisitions. Moreover, computational spectral libraries outperformed sample-based DDA peptide libraries. The neural network based dia-NN algorithm not only outperformed MaxDia in regard to protein identifications but also in algorithm stability and speed.

Summary

This study underscores the importance of considering practical aspects – sample preparation, data acquisition and analysis- in clinical proteomics based on DIA acquisition strategies. Our preliminary results on saliva "liquid-biopsies" might be used for pharmacological targeting of protease activities that impact disease progression and saliva-aerosol disease dissemination.

Conclusions

- Preliminary results on saliva "liquid-biopsies" might be used for pharmacological targeting of protease activities that impact disease progression and saliva-aerosol disease dissemination.

Technology

For research use only. Not for use in Clinical diagnostic procedures.

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