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Searching for biomarkers characteristic for severe stage of COVID-19 disease

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Introduction

Biomarkers, also known as biological markers, are defined as objectively measured and evaluated molecules (e.g. amino acids, peptides, proteins, enzymes, lipids, sugars, antibodies, hormones etc.) that act as indicators of typical biological processes, pathogenic processes or pharmacological responses. Their estimation can provide a valuable source of information about a patient's health condition, thus allowing for better diagnosis, prognosis and treatment options. Biomarkers analysis in physiological fluids (e.g. blood, urine, saliva, sweat, tears, faeces or exhaled air) can also offer a quick, painless, non-invasive and cost-effective method for disease detection and monitoring. However, this approach so far has many limitations due to the complexity and wide dynamic range of compounds present in bodily fluids. A promising alternative could be the direct identification of characteristic compounds from tissue sections, which would provide a comprehensive molecular view in a histology-related context [1].

Proteomics as a large-scale study of interactions, function, composition and structures of proteins could offer extensive insights into the molecular and cellular mechanisms of diseases. Nevertheless, the diversity of protein sequences and post-translational modifications makes it a challenging discipline. Fortunately, impressive technological advancements combined with bioinformatics have significantly progressed proteomics, aiding in compound characterization. The most commonly applied methods contain electrophoresis and high-throughput technologies such as liquid chromatography with mass spectrometry (tandem MS, MALDI-TOF) [2].

Under these circumstances, MALDI-MSI has gained particular attention due to its many advantages. This label-free technique generates two-dimensional ion density maps, enabling a spatial distribution of various biomolecules, including pharmaceuticals, lipids, peptides and proteins, through a tissue section in a single run. It allows for the simultaneous screening of hundreds of biological compounds, even low molecular weight compounds fig(e.g. drugs, metabolites) with a high sensitivity for multiple samples placed in a single target plate What's more, an easy sample preparation and a soft-ionization process simplifies data collection and interpretation [3].

The COVID-19 pandemic highlighted the need for accurate diagnostic tools. Utilizing modern techniques in biomarker search and recognition would help indicate the disease, assess the infection severity, and identify at-risk patients. The collected information could help in making treatment decisions and determining admission or intensive care criteria [4].

Results and Discussion

The aim of the studies conducted in the Institute of Organic Chemistry was the search and identification of potential protein and peptide biomarkers indicating the SARS-CoV-2 virus infection. The research focused on analysing the lung tissues that could indicate characteristic compounds for the COVID-19 disease, the advancement stage or other complications related to the disease course (e.g. lung fibrosis) and involved preparing lung tissue samples taken from a person who died of severe SARS-CoV-2 virus infection for MALDI-MSI imaging studies. Firstly, the samples were covered with two matrices, α-cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (DHB) to detect compounds with low and high molecular masses, respectively. Matrix-coated samples were next subjected to MALDI-MSI analysis [5]. For some values revealed in a range of molecular ions, MALDI-MSI imaging was performed to show compound distribution within the tissue sample (Figure 1). Results combined with bioinformatic methods allowed for the identification of protein fragments involved in the process of replication and degradation of the SARS-CoV-2 genome, as well as other peptides associated with inflammatory processes, or even the fragment of hACE2 enzyme, which serves as a receptor for coronavirus particles (Table 1).

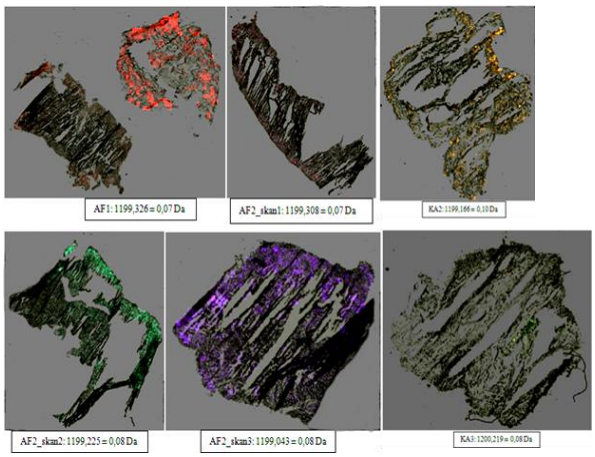

Fig. 1. *Picture showing the distribution of a ~1199 Da peptide identified in lung tissue using MALDI-MSI*

Table 1. *Representation of peptides identified during MALDI-MSI analysis as potential biomarkers for COVID-19 disease.*

	Protein	Fragment	m/z	Commentary
Inflammation	Vitamin K-dependent protein C (PROC)	HSSLER	729,29	May be involved in the pathogenesis of COVID-19 (lung damage and thrombogenicity). Involved in the coagulation process and the metabolism of elastic fibres
	Pulmonary surfactant-associated protein A1 (SFTPA1)	NCLYSR	755,37	Binding surfactant phospholipids lowering the surface tension in alveoli. Associated with lung fibrosis, a side effect of SARS-CoV-2 infection
	Fibrinogen alpha chain (FGA)	DSGEGDFL AEGGGVR	~1464	Increased levels observed for COVID-19 cases correlating with excessive inflammation and disease severity [6]
	Serum amyloid A-1/ A-2 protein (SAA1/ SAA2)	FFSFLGEAF DGAR	~1464	Increased levels of amyloid A observed in human serum of patients with severe SARS-CoV-2 virus infection [7]
	Complement component 6 (C6)	ALNHLPLEY NSALYSR		Constituent of the membrane attack complex playing a role in the innate and adaptive immune response
	Apolipoprotein A-II (APOA2)	VKSPELQAE AK	~1199	Decreased level of APOA1, APOA2 and APOC2 in COVID-19 plasma patients [8]
	Apolipoprotein A-I (APOA1)	DSGRDYVS QFEGSALG K	~1816	
	Apolipoprotein C-II (APOC2)	ESLSSYWES AK	~1286	
hACE2	Angiotensin-converting enzyme type 2 (ACE2)	LFNMLR; HEGPLHK	793,44 817,50	Receptor interacting with RBD of the S1 protein subunit of SARS-CoV-2
Replication and Replication and degradation of the viral genome	Replicase polyprotein 1ab (fragment)	NLYDK	651,64	Involved in the transcription and replication of viral RNAs
	Cystic fibrosis transmembrane conductance regulator (CFTR)	WFQMR	769,35	CFTR expression/function is involved in the regulation of SARS-CoV-2 replication
	E3 ubiquitin-protein ligase HUWE1 (HUWE1)	SMLNFLK; HLGYTLCK	853,78 935,74	Protein inducing ubiquitination and degradation of the MERS-CoV protein ORF3 (important for SARS-CoV-2 infection)

Conclusions

Progress in biomarker recognition and proteomic technologies shows great promise for a better understanding of disease pathologies and improving diagnostic procedures, particularly in the context of COVID-19. Nonetheless, it is still a developing field of study and involves significant costs and long-term examination. The analysis of samples is highly dependent on the sample collection and preparation, and the longevity of probes is also a consideration. Result interpretation is not an easy task and requires in-depth knowledge of multiple inflammatory processes. Currently, analyses are so far compared with standard approaches, but ongoing advancements may simplify the process.

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