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Aspect of COVID-19 Pandemic: Case for Mass Spectrometry

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Abstract

Fast spread of COVID-19 worldwide causing unprecedented number of coronavirus infections, Scientist community are in race to control the disease by the development of both novel effective diagnostics strategies and effective therapies. Ultimately an efficient vaccine. Research efforts has focused on recognition of the pathogen entry mechanisms to the host's cells, simultaneously characterize the host's cellular defence mechanisms in response to the pathogen attack. Early genome sequencing enlighten us on the origin of the virus responsible for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. PCR tests first implemented to recognize the presence of viral RNA. PCR tests for SARS-CoV-2 has no gold standard reference, which limits the accuracy of results and increases the percentages of false positives, besides PCR test are unable to predict disease status (progression). Alternative techniques where tested and evaluated, such as immunological based tests. Mass spectrometric methods. Which was suggested as a complementary method to the structural information gathered by molecular level test? Here, we highlight on the contributions of mass spectrometry to several areas of SARS-CoV-2 infectious disease research understanding the host's cell proteome changes including diagnostics techniques, vaccines and therapies.

Keywords: SARS-CoV-2; Proteomics; Immunoassays; Mass Spectrometry; Spike Protein; PCR

Introduction

By the end of 2019, mass cases of rapidly spreading pneumonia emerged from Wuhan, China; which was attributed to a new Coronavirus (CoV). The new virus initially short named (2019-nCoV). On January 2020, The World Health Organization (WHO) declared the COVID-19 as an international epidemic.

Coronaviruses (CoVs) are the largest identified, enveloped, positive-sense single stranded RNA viruses; that may well infect a range of mammals (including humans), birds and fish [1]. Alpha-, Beta-, Delta-, and Gamma-coronavirus are four genera classes of coronaviruses according to the international Committee on Taxonomy of viruses (ICTV). Scientist has identified six human coronaviruses [2], which belongs to alpha- and beta- coronaviruses (SARS-CoV, MERS-CoV, HCoV-NL63, HCoV-229E, HCoV-HKU1, and HCoV-OC43).

In 2003 the SARS-CoV (a lineage B Betacoronavirus) [3-5] has emerged in China with fatality percentages around 10%, this was overcame in 2012 by the detection in humans of the MERS-CoV (a lineage C Betacoronavirus) [6-8] with fatality rates almost 40%.

Scientists carried out comprehensive genetic sequencing of the novel coronavirus, and comparative analysis of already sequenced coronaviruses (CoVs) available in GenBank.

Sequenced coronavirus extracted from the first Chinese infected patient labelled HKU-SZ-005b aligned against 23 other related complete genome sequences of βCoVs strains collected from mammals (Bats, Himalayan palm civet and human) [9]. Results showed 89% similarities with bat SARS-like-CoVZXC21, and in lower percentages with human SARS coronavirus of about 82% [9].

Those numbers of high death rates by SARS-CoV and MERS-CoV-the last two human coronaviruses- show the threat potential of these highly pathogenic HCoVs.

In 2013 Chan [9]; Ge [10] in view of their studies on human coronaviruses and the interspecies –transmission ability of SARS and MERS predicted the emergence of a novel coronavirus [10,11].

The novel coronavirus SARS-CoV-2 caused up-to-the moment more than 31 million confirmed infections resulting in 960 thousand deaths worldwide since December 2019 [12]. Urgent development a high-throughput and sensitive method for effective infection diagnostic tools and new therapeutic strategies (vaccines and/or treatments) are essential to prevent further mortalities.

Unprecedented efforts worldwide in response for the COVID-19 pandemic from governments, academics, pharmaceutical companies all collaborating steering around two broad approaches. First, the development of accurate tests to identify the infected individuals, secondly, the production of antibodies that recognize or defuse the virus. Demonstrated excellent analytical performance, in terms of specificity and sensitivity, is the key to the success of these approaches.

The most common tests used for the detection of SARS-CoV-2 are the molecular tests, such as quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) [13-15], quantitative real-time RT-PCR assays (qPCR) [2,16] and the enzyme-linked immunosorbent assay (ELISA).

The antibodies approach relies on accurate descriptors of the viral antigens. The two viral proteins (antigens) most widely used in antibody testing kits are either the nucleocapsid (N) Protein, which is the protein shell that envelops the nucleic acid (RNA) genome. Alternatively, the spike (S) protein, which attaches to the host cells. Evidently, the host response is vital for the successful deployment of any intended to use approach, in addition to the number of clinical samples across different populations.

Most of the current PCR tests target a single human coronavirus gene. Which results in false negative results due to the possible mutations those occur in human coronavirus genome? The PCR [17] is considered the gold standard method for the detection of SARS-CoV-2, although, it is relatively time consuming, with sound sensitivity (71-98)% and low accuracy depending on the site and quality of sampling [18], stage of disease [19] and virus replication/clearance levels. Higher sensitivity is achievable if multiple target genes are used [20,21]. It is worth note; the lack of "gold-standard" for COVID-19 PCR testing is a handicap for the test accuracy and precision. The performance of three RNA-dependent RT-PCR assays tested; targeting (RdRp)/helicase (Hel), spike (S), and nucleocapsid (N) genes of SARS-CoV-2 [21]. The lowest detection limit was achieved by the COVID-19-RdRp/Hel assay the viral load of ranged from 220 to 47k RNA copies/ml, assay cross-reactivity was investigated with other HCoVs and other respiratory pathogens.

The structure of SARS-CoV-2 virus is a single-stranded (positive sense) enveloped RNA virus. Consists mainly of four structural proteins including S, E, M (Spike, Envelope, Membrane) glycoproteins together create the viral envelope, and N (Nucleocapsid) protein that holds the RNA genome. The virus genome has been sequenced as previously mentioned [22]. Currently considerable genetic information emerged since the start of the epidemic and also structural information are still emerging. Structural information of molecular descriptors is associated with understanding the disease progression the fast spread within diverse populations.

The rapid development of diagnostic assays and vaccine/treatment is vital.

Fast sequencing techniques developed recently granted a rapid and comprehensive genome sequencing of SARS-CoV-2 virus, which allowed the implementation of rapid PCR tests conforming the presence of viral RNA in infected individuals. In addition, in parallel many assays-based on the detection of antibodies used to the recognition of SARS-CoV-2 antigen [23,24]. Besides a bio-analytical industrial validation of those tests is required before its distribution. Having said that the successful deployment of these tests depends on accurate identification of peptides expressed uniquely to pathogens. Analytical techniques such as ELISA and other antibody based techniques are considerably in leading position compared to other analytical techniques due to its speed and specificity. ELISA proved usefulness in the detection of viral proteins in severe acute respiratory syndrome (SARS) [25-27].

Recently, remarkable evolution in mass spectrometry (MS) extended the list of identified proteins in single experiment to thousands of ID's and also their respective Posttranslational modified forms; due to the extensive advancements achieved in resolution > 450K in bench top

machines or sensitivity due to low noise signal, accuracy < 1ppm in some machines, and speed of analysis. Hand to hand with quantitative improvements allowing the accurate differentiation between diseases versus control protein profiles in large number of samples, not to forget the role of bioinformatics software's that made this possible analysing huge data sets. Mass spectrometric technology has revolutionized by the introduction of Orbitrap analyser and nano scale liquid chromatography in addition to electrospray ionization and lastly most recently the TIMS-TOF machines with gigantic improvements in speed, sensitivity, and resolution of MS instruments. Which makes LCMS methods an attractive approach in clinical settings alternative to ELISA and protein array methods for the rapid identification of peptides in clinical settings where consistency is paramount? [28-33].

Mass Spectrometry and SARS-CoV-2

Mass spectrometry-based analysis can answer questions in COVID-19 field from at least four different areas. The first and most urgent question: The identification of unique peptides that can be deployed for the development of vaccines and ELISA Diagnostic kits with specific antigens. The second important question, is the viral spike glycoprotein, and this question has two parts as for the host-cell attachment as mentioned earlier the binding-receptor (ACE2) [34], and also as a target for neutralizing antibodies [35,36] that mimics circulating viruses blocking the receptor stimulated through vaccination [37-39]. The third question the development of biomarkers for host response, understanding the disease mechanisms, the immune system response during disease stages (disease severity), and response to medications. Such data from suggested/validated biomarkers will allow us to screen/recognize high-risk populations, track disease progression and identify/prevent/treat future pandemics like SARS-CoV-2. Blood biomarkers for coronaviruses disease-related proteins were suggested, like those associated with the inflammatory response [29,30], blood coagulation [29] and cell damage [31,32]. The fourth and final question is the stability of SARS-CoV-2 on surfaces and environment. SARS-CoV-2 was detected on surfaces of both symptomatic and asymptomatic infected passengers 17 days after cabins were vacated on cruise ships (The Diamond Princess and Grand Princess) [40] and also in sewage [41]. Detection in various contaminated surfaces at different time points is vital and achievable perfectly by mass spectrometry (Time resolved identification). Answering all these questions means mass spectrometry can provide significant molecular level information of SARS-CoV-2.

Mass spectrometry contributed in COVID-19 areas answering questions resembling disease mechanism and disease severity by comprehensive protein profiling [42,43] of hosts response to the viral infection. Proteins expression in response to viral infections offers possible biomarkers for disease progression and severity.

The second area concerns the SARS-CoV-2 virus attachment to the host cell. From previous studies on earlier coronaviruses pandemics, (SARS-2002& MERS-2013) researchers believe spike (S) glycoproteins are one of the main keys to viral binding to human cells, researchers targeted spike (S) glycoproteins for vaccine designs and antibody based therapies. Yuan [44] has investigated accessibility to spike (S) proteins using cryogenic electron microscopy and some antibodies that have shown a promising binding to SARS-CoV-1 [44]. Earlier several studies revealed promising efficiency of antibodies targeting spike proteins of SARS-CoV-1 in preclinical trials. In addition to receptor-binding domain (RBD) targeting [45-49]; many structural studies proposed another therapeutic monoclonal antibody binding targets in coronavirus structure; N-terminal domain (NTD) [47,50,51] which is structural unit in coronavirus S1 subunit moreover in S2 subunits, it includes FP (fusion peptide) [51], HR1 (heptad repeat 1) [44,52,53]. Spike glycoproteins in all coronaviruses are heavily glycosylated. With the recent tremendous evolvement of high-resolution mass spectrometry, it became very easy to identify and precisely site mapping of these glycoproteins. Gouveia [54] presented a short-list of 14 peptides [54] from the main SARS-CoV-2 proteins, for targeted mass spectrometry method development of diagnostic strategies. Proteins from inactivated virus samples -initially SARS-CoV-2 virus produced in Vero E6 cells- digested and data were acquired using data-dependent acquisition (DDA) mass spectrometry-based bottom-up proteomics. These peptides selected based on set of rules that eliminates any chances of cross-reactivity and/or false positives [55].

In parallel, following a SARS-CoV-2 infection; changes occurring in the host cell proteome are a valuable information that could be collected by time-dependent proteomics, which may lead perhaps as investigated to a potential drug targets. C Ihling [56] mass spectrometric detection method of nucleocapsid N protein, as the most abundant protein in the SARS-COV-2 virus. The new method uses the same reported peptides of the N protein in SARS-CoV but improving the preparation procedure in order to achieve much better detection limits using much less virus loads. Furthermore, the method was validated in different physiological fluids.

Leshan Xiu [28] designed and evaluated amultiplexed coronaviruses test, a test combining mass spectrometry with PCR appreviated (mCoV-MS), Mass ARRAY [28] method was designed to detect 6 known already known human coronaviruses, with a potential to discover any novel human coronaviruses. The method relays on the PCR amplification

strength combined with the extensive capabilities of MALDI-TOF mass spectrometry to identify microorganisms accurately and the ability to measure large number of samples rapidly. This method is limited when human coronaviruses load is very low. Also unrecognized protein spectral signals can provide a clue for novel coronaviruses.

Finally, the joint comprehensive mass spectrometry-based proteomics research efforts with molecular level collected information on human SARS-CoV-2 will reveal pathophysiological and structural information to treat COVID-19. The computational extracted information is very well regarded data in vaccine and drug development processes; helping to understand the mechanisms behind antigen response.

From our experience with SARS and MERS coronaviruses outbreaks, a health threatening potential with high fatality rates and the actual SARS-2 global outbreak, an ideal analytical method for the diagnostic of coronaviruses should identify the HCoVs accurately and capable of predicting any potential emerging novel coronaviruses [28].

Proteomics-based mass spectrometry with its proven ability to detect numerous proteins per run rapidly, precisely, reproducibly, and site mapping proteins post-translational modifications precisely can provide a considerable complementary information to the genomic information gained by molecular techniques. In addition, mass spectrometry has proven success in diseases-related protein biomarkers.

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