Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Review

Mass spectrometry applied to diagnosis, prognosis, and therapeutic targets identification for the novel coronavirus SARS-CoV-2: A review

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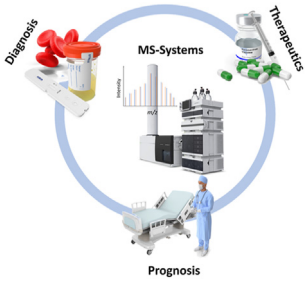
HIGHLIGHTS

- Trending strategies on MS analysis of biological samples infected with SARS-CoV-2.
- Omic approaches applied to COVID-19 diagnosis, prognosis, and therapeutic targets.
- Compilation of SARS-CoV-2 infection insights through MS-acquired data.
- MS feasibility to generate valuable molecular information for medical purposes.

GRAPHICAL ABSTRACT



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ABSTRACT

Mass spectrometry (MS) has found numerous applications in medicine and has been widely used in the detection and characterization of biomolecules associated with viral infections such as COVID-19. COVID-19 is a multisystem disease and, therefore, the need arises to carry out a careful and conclusive assessment of the pathophysiological parameters involved in the infection, to develop an effective therapeutic approach, assess the prognosis of the disease, and especially the early diagnosis of the infected population. Thus, the urgent need for highly accurate methods of diagnosis and prognosis of this infection presents new challenges for the development of laboratory medicine, whose methods require sensitivity, speed, and accuracy of the techniques for analyzing the biological markers involved in the infection. In this context, MS stands out as a robust analytical tool, with high sensitivity and selectivity, accuracy, low turnaround time, and versatility for the analysis of biological samples. However, it has not yet been adopted as a frontline clinical laboratory technique. Therefore, this review explores the potential and trends of current MS methods and their contribution to the development of new strategies to COVID-19 diagnosis and prognosis and how this tool can assist in the discovery of new therapeutic targets, in addition, to comment what could be the future of MS in medicine.

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Contents

List of abbreviations

AA	Arachidonic acid
ACE2	Angiotensin-converting enzyme 2
API	Active pharmaceutical ingredient
APO	Apolipoproteins
ATP	Adenosine Triphosphate
CID	Collision induced dissociation
COVID-19	Coronavirus Disease 2019
CQ	Chloroquine
EI	Electron Ionization
ELISA	Enzyme-Linked Immunosorbent Assay
ESI	Electrospray ionization
FA	Fatty acid
FAC-MS	Frontal Affinity Chromatography-Mass Spectrometry
FDA	Food and Drug Administration
FTICR	Fourier-Transform Ion Cyclotron Resonance
GM3s	Monosialodihexosyl ganglioside
HCoV	Human coronaviruses
HCQ	Hydroxychloroquine
HDL	High Density Lipoprotein
HESI	Heated Electrospray Ionization
HILIC	Hydrophilic Interaction Liquid Chromatography
HPLC	High Performance Liquid Chromatography
HR	High-resolution
HRFAB-MS	High Resolution Fast Atom Bombardment Mass Spectrometry
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IT	Ion Trap
LA	Linoleic acid
LC	Liquid Chromatography
LC-MS	Liquid Chromatography coupled with mass spectrometry
LC-MS/MS	Liquid Chromatography coupled with tandem mass spectrometry
LIT	Linear Ion Trap
LoD	Limit of Detection
LPV	Lopinavir
LysoPC	Lysophosphatidylcholine
LysoPE	Lysophosphatidylethanolamine
MALDI	Matrix-Assisted Laser Desorption/Ionization
MERS-CoV	Middle East respiratory syndrome coronavirus
M^{pro}	Main protease
MS	Mass spectrometry
MS/MS	Tandem Mass Spectrometry
MTD	Monitoring of Therapeutic Drugs
NanoLC-MS/MS	Nanoscale Liquid Chromatography coupled with tandem mass spectrometry
PBMCs	Peripheral blood mononuclear cells
PCR/ESI-MS	Polymerase chain reaction coupled with electrospray ionization mass spectrometry
PL^{pro}	Papain-like protease
POCTs	Point-of-cares tests
PRM	Parallel Reaction Monitoring
PS-MS	Paper Spray Mass Spectrometry
QFPD	Qingfei Paidu Decoction
QOrbitrap	Quadrupole Orbitrap
QqQ	Triple quadrupole
QTOF	Quadrupole Time of Flight
RBD	Receptor binding domain
RdRp	RNA-dependent RNA polymerase
RDS	Respiratory Detox Shot
RDV	Remdesivir
RNA	Ribonucleic acid
RP	Reversed Phase
RTIs	Respiratory tract infections

RT-qPCR	Real Time quantitative Reverse Transcription Polymerase Chain Reaction	TAGs	Triacylglycerols
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus	TDF	Tenofovir disoproxil fumarate
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2	TFC	Turbulent Flow Chromatography
SMs	Sphingomyelins	TOF	Time of Flight
S-protein	Spike protein	UHF	Ultra-High-Field
SRM	Selected reaction monitoring	UHPLC	Ultra High-Performance Liquid Chromatography
		WHO	World Health Organization

1. Introduction

Mass Spectrometry (MS) has been widely used in the detection and characterization of biomolecules, especially due to its versatility, mainly related to soft ionization techniques as Matrix-Assisted Laser Desorption/Ionization (MALDI) and Electrospray Ionization (ESI). Thus, MS has found numerous applications in medicine through the analysis of biological signatures associated with viral infections such as Coronavirus Disease 2019 (COVID-19).

COVID-19 is an infectious disease first spotted in Wuhan City, in the Hubei Province of China, and was declared a pandemic by the World Health Organization (WHO) in January 2020. The disease, whose etiologic agent is the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has affected nearly 260 million people including more than 5.2 million fatal victims (data from December 2021), and it is considered the most severe pandemic since the Spanish flu [1–8].

For being highly contagious and considerably lethal, efforts are being globally made to find ways to control the pandemic, including the search for effective drugs and vaccines. In addition, the lack of information about the viral replication process and the emerging of other viral strains has led scientists to a ceaseless race against time in the search for new, accurate, fast, and effective methods to improve diagnostic tests, to monitor the prognosis of patients and to develop new therapeutics alternatives [9]. In this context, a collaboration among researchers specialized in MS around the world, named COVID-19 MS coalition, represents an effort to share crucial information, such as molecular and structural data, sample collection, method protocols, and data generating and treatment through open datasets so they could help each other to achieve their goals in the fight against COVID-19 [10].

Researches focused on the aforementioned directions is usually motivated by MS-based omic strategies, aiming to identify, characterize and quantify all relevant biological molecules involved in the dynamics of an organism (Fig. 1). This approach has proven to be extremely powerful for health-related purposes due to its unrivaled potential to monitor sets of molecules to describe cellular metabolism and perturbations. Therefore, these studies have substantially advanced our understanding of the human organism [11–13].

Therefore, this review aims to gather information and discuss the MS feasibility to "scan" the human body at a molecular level so answers could be given to society on what happens to the patient throughout all SARS-CoV-2 infection phases, and how that fresh information could help manage the pandemic. Recently, Griffin & Downard [14] published a review also focused on describing the applications of MS to COVID-19 ongoing researches. Our approach is also contributing to the discussion of what was searched and discovered throughout the first year of the pandemic, how specialists worked, and the trending analytical instrumentation so far. However, we give critical and interpretative considerations onto diagnosis, prognosis, and therapeutic target discovery, in addition to giving a different perspective on how MS could fit in the future regarding medical applications and why it is not yet implemented as a clinical routine tool.

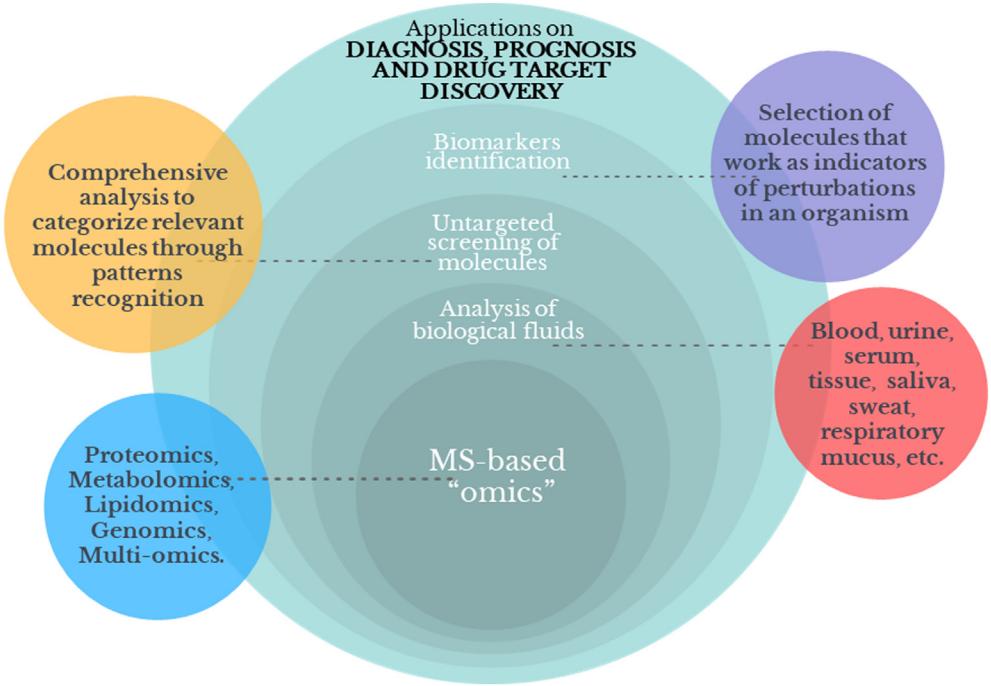

Fig. 1. Mind map on MS-based "omics" workflow, applications and possibilities.

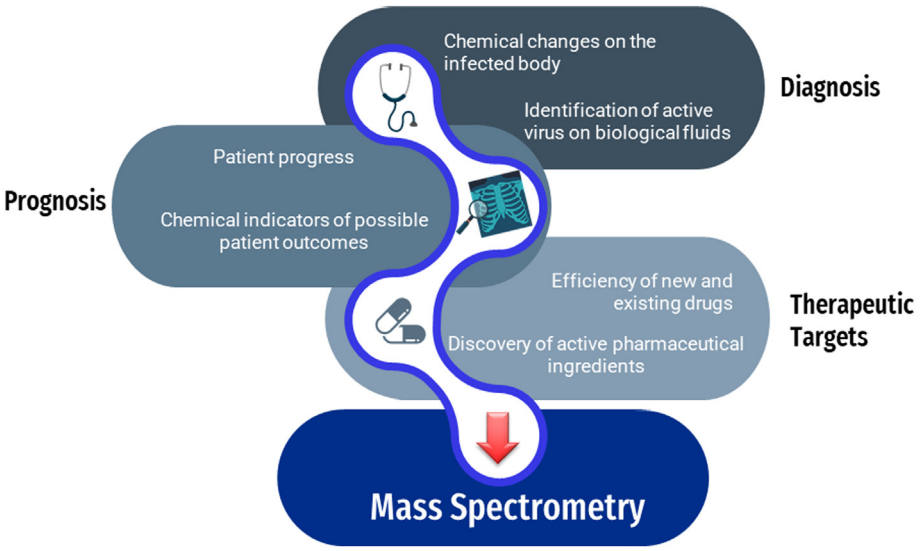

Fig. 2. Graphical flowchart of MS potentialities to elucidate key clinical challenges on diagnosis, prognosis and therapeutic targets.

2. Methodology

Metadata on applications of MS on prognosis, diagnosis, and therapeutic target identification of the novel coronavirus SARS-CoV-2 were gathered using different search engine databases including Web of Science, PubMed, Science direct, WHO website, Google Scholar, Scopus, and SciFinder.

3. MS applied to COVID-19 diagnosis, prognosis and therapeutic targets: an overview

After a comprehensive compilation of publications, we came across some patterns on the three main clinical applications of MS focused on in this review. Given the urgency of getting information about the novel coronavirus, the potential of MS is being diversely explored to try answering some key questions about how SARS-CoV-2 is affecting the infected human body (Fig. 2).

For diagnosis, the main emerging paths for trying to develop revolutionary methods to diagnose a disease is evaluating the molecular changes caused in the human body due to an external pathogen entry or detecting active viruses, considering COVID-19. We believe MS is not in a place to substitute Real-Time quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR), Lateral Flow, or any other COVID-19 test that is being used over the current pandemic. Not only because its high instrumentation acquisition cost or because it is still not an easy-to-operate

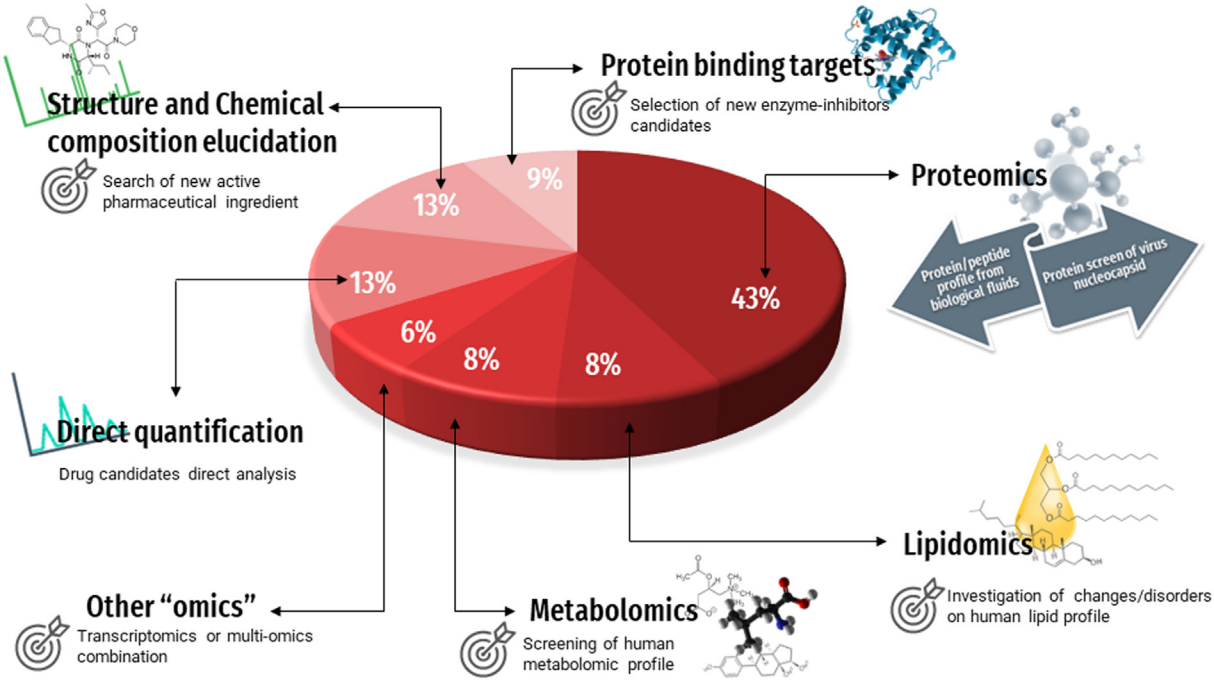

Fig. 3. A statistical overview of the main courses of action and scientific targets of COVID-19 related studies using MS (dark-red shades representing mostly diagnosis and prognosis approaches; light-red shades representing exclusively therapeutic target assignments). Statistics was calculated based on the total of publications. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

analytical tool available in clinical facilities, but it is mostly due to the fact that its investigation potential can provide more than a simple positive or negative response.

Despite some publications involve the development of diagnosis methods by MS, including one already approved by the Food and Drug Administration (FDA), we observed that the potential of MS, as far as diagnostic protocols are concerned, is more indirectly considered, which means, it has been indeed applied to distinguish infected from non-infected individuals assertively based on differential molecules. These molecules, otherwise, could be used as a reference in the development and optimization of new methods for fast, simple, and direct diagnostic, through low-cost device assemblings, such as microchips, sensors, and microfluidics.

By screening biological fluids from patients infected with SARS-CoV-2 and comparing this information against non-infected material, physicians could not only establish the number of positive and negative diagnostics but also have a good prospect on what is happening in the human body regarding metabolic changes and how the virus presence affect human homeostasis.

That led us to the infection prognosis phase. On this matter, the molecular information obtained from the MS analysis is considered to evaluate possible treatment procedures to improve the outcome of patients. Since the prognosis is usually based on clinical exams or complete blood work, as COVID-19 affects multisystem in the organism and can cause severe damage to vital organs, any additional molecular information acquired from a more comprehensive tool, such as MS, can help understand the delayed symptom manifestation.

The entire MS acquired information about the difference in the organism before and after infection by a pathogen and its progress helps scientists to identify therapeutic targets, which consequently is highly valuable in the process of formulating a new antiviral agent. The main studies on the subject are focused so far within two different directions. First, scientists have been dedicating themselves to discovering novel active pharmaceutical ingredients (API) on existing synthetic drugs or natural products, and on monitoring the response of the body exposed to these new drug candidates by MS analysis.

Regarding the selected workflow and instrumentation behind all studies, it is possible to observe some tendencies (Fig. 3).

By the statistical analysis through the compiled data, proteomics was undoubtedly the standout course of action. Within this approach, some authors focused their efforts on describing the protein/peptide composition of the SARS-CoV-2 nucleocapsid, mainly on the diagnosis inclined studies. Besides, a considerable amount of works has been performed to describe the proteomic profile of infected patients for biomarker discovery for both diagnostic and prognostic purposes.

The metabolome and lipidome screening of infected patients was also an interesting strategy, used mostly for prognosis assignments. By accessing this information through MS, specialists were able to find some tendencies on human structural lipid levels that stood out as possible alerts for clinicians. Amino acids, organic acids, and other minor structures leaning for the same path.

Some non-omic approaches were also verified especially in the therapeutic section. In this case, a full screening of natural products was a highlight for new API discovery, and rather than executing a complete omics approach, direct quantification or direct search for specific biomolecules were proposed. To be more thorough, MS is being used in this particular field to screen drug absorption, formation of potentially toxic metabolites, protein binding, and metabolic pathway analysis.

Considering the instrumentation specifics, ESI-MS was a highlight among the aforementioned publications. The fact is not surprising since it provides excellent coverage of a wide range of molecules especially with high molecular masses, such as proteins. This resource not only provides the possibility of obtaining information about the molecular mass of peptides and proteins with great precision but also generates amino acid sequences and analyzes molecules with lower molecular masses, such as the ones targeted on metabolomics, lipidomics, and non-omic approaches. All that comprehensive information that qualifies biochemical profiles is better received when interfaced with tandem mass spectrometry (MS/MS). In this case, among the most employed mass analyzers are the quadrupole combined with time-of-flight (QTOF) and the quadrupole or linear ion trap (LIT) combined with Orbitrap [15–17].

QTOF is a hybrid mass analyzer that combines the compound fragmentation efficiency of quadrupole collision cells and the high resolution of time-of-flight. It simultaneously offers high sensitivity, accuracy, and dynamic range so it could be possible to acquire more information from the samples and even quantify specific molecules. For targeted strategies, on the other hand, triple-quadrupole (QqQ) analysis improve the characterization of proteomic (or any other "omics" approach) profiles and, consequently, the understanding of biochemical pathways [18,19].

In the last few years, Orbitrap has become the mass analyzer of the first choice. Orbitrap was developed in the early 2000s and inherited some features from previously available mass analyzers such as the principle of image current detection from Fourier-transform ion cyclotron resonance (FTICR-MS), the use of ion trapping from its ion trap (IT) precursor, and the electrostatic field from TOF mass analyzers. Altogether, these features resulted in a powerful and unique combination that allow obtaining high resolution, high mass accuracy, and good dynamic range, which gives us a hint on why this mass analyzer is becoming so popular [20,21].

MALDI-MS did not pass unnoticed either and appeared among some diagnosis and therapeutic targets inclined studies, which have demonstrated the feasibility and potential of MALDI-TOF coupling for biomarker discovery assays once it also provides fast determination of the molecular mass of proteins with up to 100 kDa [15].

Undoubtedly, hyphenated techniques were the most explored MS systems overall, especially with high- or ultra-high-performance liquid chromatography (HPLC or UHPLC) coupled with MS or MS/MS setups. The direct infusion was also the choice for some studies; however, for complex samples as biological fluids, we believe that a separation method as liquid chromatography (LC) even coupled with high-resolution MS instruments is highly preferable. We also observed a tendency of miniaturized devices as nano-electrospray ionization sources and even nano-chromatographers as a primary coupling separation technique.

The publications gathered on the related issues will be thoroughly discussed in the following sections.

4. Diagnosis

In this section, we will discuss diagnosis-focused studies. First, the proteomics dedicated ones will be approached, followed by multi-omics, untargeted metabolomics, and transcriptomics. We will also discuss some previous publications dedicated to describing other coronaviruses and some perspectives.

4.1. Current diagnostic strategies and MS assignments

Considering that COVID-19 is a complex and multisystemic disease that evolves in phases very quickly, together with the high number of asymptomatic and pre-symptomatic infected people who can transmit the virus, having a proper diagnosis in the early stages of infection is the bottom-up of trying to prevent the disease from progressing [22].

Table 1
Summary of MS-based studies on new alternatives for COVID-19 diagnosis.

Omics strategy	MS system	Targeted/Untargeted study design	Sample	Object of investigation	Ref.
Proteomics	nanoLC-Orbitrap	Targeted	Nasopharyngeal swabs	Peptides from the SARS-CoV-2 nucleocapsid protein	[33]
	nanoLC-ESI-QOrbitrap and TFC-HESI-QqQ	Untargeted and targeted	Nasopharyngeal and oropharyngeal swabs	Peptides of SARS-CoV-2	[37]
	nanoLC-nanoESI-Orbitrap	Targeted	Highly diluted gargle solutions	Nucleoprotein	[34]
	nanoLC-ESI-QOrbitrap	Targeted	Nasopharyngeal and oropharyngeal swabs	Tryptic peptides of SARS-CoV-2 proteins	[38]
	nanoLC-ESI-TOF	Targeted	Nasopharynx epithelial swabs	Viral nucleocapsid N protein	[35]
	LC-ESI-Orbitrap	Untargeted and targeted	Infected Vero E6 cells	Tryptic peptides of SARS-CoV-2 proteins	[39]
	nanoUHPLC-UHF-Orbitrap and nanoUHPLC-LIT	Untargeted	Urine	Proteomic profile	[31]
	LC-ESI-Orbitrap	Targeted	Nasopharyngeal swabs	Nucleocapsid protein of SARS-CoV-2	[36]
	UHPLC-ESI-TripleTOF	Untargeted	Serum and plasma	Proteomic profile	[32]
	nanoLC-ESI-QTOF	Targeted	Nasopharyngeal and oropharyngeal swabs	Peptides of SARS-CoV-2	[40]
	MALDI-TOF	n.m.	Nasopharyngeal swabs	Specific discriminatory peaks to differentiate positive samples from negative samples	[45]
	MALDI-TOF	Targeted	Swab and gargle samples	Viral envelope glycoproteins	[44]
	MALDI-TOF	Targeted	Nasopharyngeal swabs	Specific discriminatory peaks to differentiate positive samples from negative samples	[46]
	LC-MS	Targeted	n.m	Peptides of SARS-CoV-2	[41]
Multi-omics	nanoLC-ESI-QOrbitrap	Untargeted	Respiratory Specimen	Global proteome/metaproteome/metabolome profile	[48]
	UHPLC-HESI-QOrbitrap	Untargeted	Serum	Proteomic profile	[42]
	nanoLC-TripleTOF and UHPLC-MS/MS	Untargeted	Serum	Protein and metabolites analysis	
Metabolomics	ESI-QOrbitrap	Untargeted	Plasma samples from peripheral venous blood	21 discriminant multiclass biomarkers	[50]
Transcriptomics	MALDI-TOF	Targeted	Nasopharyngeal and oropharyngeal swabs	SARS-CoV-2 nucleocapsid genes	[51]

*TFC: Turbulent Flow Chromatography; HESI: Heated Electrospray Ionization; UHF: Ultra-High-Field; n.m.: not mentioned.

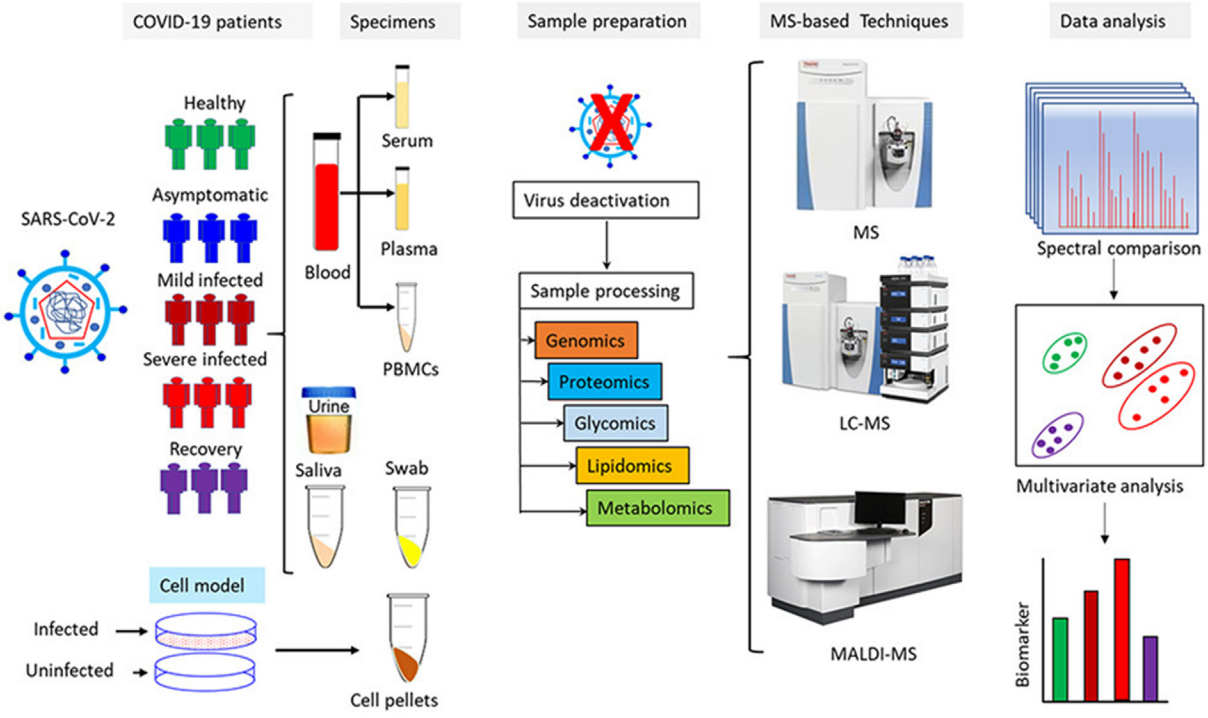

Fig. 4. MS-based multi-omics universal workflow to study new alternatives for COVID-19 diagnosis. Source: Mahmud & Garrett 2020 [30].

Currently, molecular diagnosis of COVID-19 primarily relies on RT-qPCR assay, which is based on the qualitative detection of SARS-CoV-2 ribonucleic acid (RNA) in upper and lower respiratory specimens such as nasopharyngeal or oropharyngeal swabs [7,23,24]. Serological antibody-based techniques have been introduced as complementary tools to RT-qPCR [23–26]. Immunological methods are based mostly on chemiluminescent assays of immunoglobulin IgG and IgM for SARS-CoV-2 from blood or

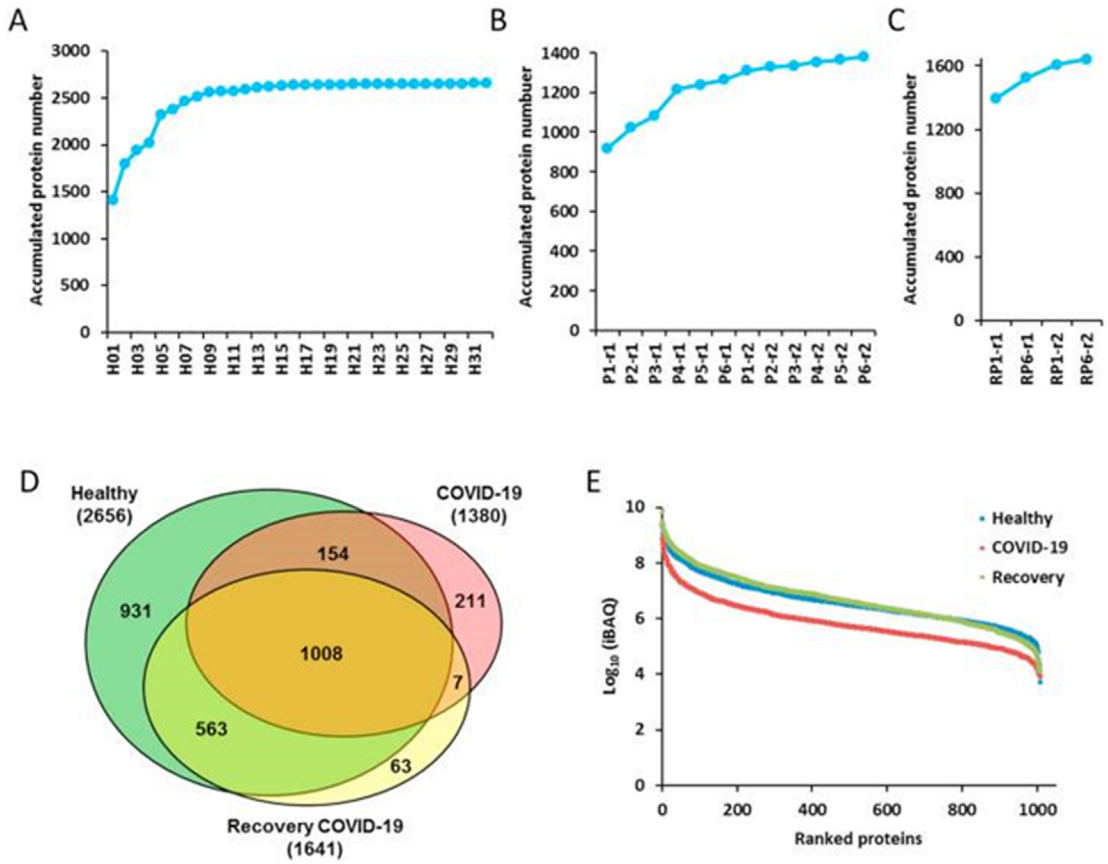

Fig. 5. Identification and relative quantification of urine samples from COVID-19 patients and healthy controls. The graphics present the accumulation curve of the quantified proteins from (A) 32 healthy volunteers, (B) 6 COVID-19 patients and (C) 2 recovered patients. The Venn diagram (D) shows the identified urine proteins from the healthy volunteers, COVID-19 patients and recovered patients. The graphic (E) presents the dynamic range of the absolute quantitative information (iBAQ) of identified proteins from healthy volunteers, COVID-19 patients and recovered patients [31].

immunochromatographic assessment in the form of rapid point-of-care tests (POCTs), not requiring additional analytical equipment [27]. Until this point, there are several FDA-approved commercial kits of serological immunodiagnostic COVID-19 tests available (updated in December 2021).

However, all diagnostic tests have limitations. For the patients, the RT-qPCR test is invasive and uncomfortable, besides it could take until 3 days to get the results or even longer depending on local testing capacity [27,28] and the serological ones have suboptimal sensitivity, often resulting in false-negative diagnostic despite their substantial specificity [27,29].

Facing the current necessity of improving available diagnosis assays and/or developing new diagnosis alternative that allies high accuracy, low cost, and less invasive sampling, MS-based omic strategies could help achieve some answers. Screening analysis of biologic fluids of COVID-19 positive-tested patients crossed against biologic fluids from non-infected people could define new molecular indicators that hopefully can be adapted to new POCTs in the future, for instance. A substantial number of studies discussed within the following sections points to this course of action. Table 1 presents the MS-based studies involving new alternative methods for COVID-19 diagnosis.

4.2. Proteomic-based studies on biomarker identification in human body fluids

All MS-based omic methods that search for new biomarkers in biological fluids comprise a general workflow (Fig. 4). First, the samples are collected and manipulated according to a virus deactivation protocol, followed by the appropriate handling for each type of analysis. Then, the pre-treated samples are injected into a mass spectrometer by direct infusion or by LC at most. Liquid chromatography coupled with tandem MS (LC-MS/MS) has been the standout instrument for these approaches since the system enables the analysis of peptide mixtures in complex biological fluids, as the analyzed samples on these cases. Multivariate statistical analysis of the acquired data is then performed for biomarker selection and target quantification when applied.

Initially, some reviewed studies looked for accessing a proteomic profile of urine, serum, and plasma samples, aiming to discriminate positive-diagnosed patients from healthy individuals. A specific COVID-19 proteome sequence was detected in urine samples through full MS experiments carried out in an ultra-high-field (UHF) Orbitrap mass analyzer followed by MS/MS scans acquired on a LIT analyzer by the Top20 method [31]. The object of investigation were urine samples donated by 32 healthy volunteers, 6 COVID-19 patients, and 2 recovered individuals, aiming to find discriminative features in the proteome among these three groups (Fig. 5) [31]. In a healthy control group, a total number of 2656 proteins were identified (Fig. 5A), followed by 1380 proteins in the COVID-19 positive group and 1641 in the recovery group (Fig. 5B and C). The authors also found 1008 proteins being commonly identified and quantified among all groups (Fig. 5D). The average abundance of identified proteins for COVID-19 samples was lower than the other two groups (Fig. 5E).

Their study was the first one to access COVID-19 features in

urine, which were consistent with a previous blood assay. Even though experimental data about urine proteome, in this case, is yet limited, it could be a promising approach further on. This study is a good example of how MS data could be helpful in the understanding of human metabolism before and after being infected by SARS-CoV-2. The biological interpretation of these data could be a handful of valuable information for the diagnosis and prognosis of patients.

Following a similar workflow, 27 biomarkers were identified in serum and plasma samples, which are differentially expressed depending on the severity grade of the infection considering WHO parameters. Several proteins were associated with COVID-19 severity that has not been previously mentioned, such as alpha-1B-glycoprotein (A1BG), beta and gamma-1 actin (ACTB; ACTG1), lipopolysaccharide-binding protein (LBP), galectin 3-binding protein (LGALS3BP), leucine-rich alpha-2-glycoprotein (LRG1), and others [32]. In this case, the presence of the above-mentioned and some other proteins on the patient could be also an alert of a severe prognosis. The prognosis insights about this study will be discussed in section 5.3. According to the authors, this work provides solid evidence that proteomic signatures have the potential to overcome conventional clinical assays since it provides more than a yes or no response [32].

4.3. Proteomics-based studies on virus peptides/proteins identification in human body fluids and Vero cells

A majority of authors developed analytical methods to find specific peptides for straight identification of the virus. Two major strategies were pursued, some studies were focused on the detection of main proteins from the virus nucleocapsid [33–36] while other research groups opted to search for a more comprehensive peptide/proteome profile [37–41].

For nucleocapsid protein detection, the focus of two studies was a targeted assay capable of detecting them in nasopharyngeal swabs samples, both using nanoLC-Orbitrap equipment. In the first study, the authors highlighted two peptides (ADETQALPQR and GFYAQGSR) as the most promising ones for the development of quick and robust diagnostic assays. It was observed that the number of identified peptides decreased as the viral load decreased in the sample, which means these peptides are directly related to the infection. Hence, samples with low viral load require more sensitive analytical methods for the quantification of these specific molecules to be applied. For this study, MS analyses were based on scan cycles initiated by a full scan of peptide ions carried out by an Orbitrap mass analyzer followed by high-energy collisional dissociation and MS/MS scans on the 20 most abundant precursor ions (Top20 method) [33].

In the second study, focused on the detection of viral antigens, the authors commented on the high specificity and comparable sensitivity as advantages of their SARS-CoV-2 detection method when compared to the RT-qPCR test [36]. If economically viable, this approach has the potential to be used as diagnostic assays in clinical facilities with an analytical frequency of 100 samples per day. However, the high cost of acquisition and maintenance of high-performance MS instrumentation is still a challenge to overcome.

A peptide originating from SARS-CoV-2 nucleoprotein, comprising the sequence RPQGLPNNTASWFTALTQHGK was identified also using a nanoLC-nanoESI-Orbitrap MS-based method [34]. However, only three gargle solutions samples from patients with COVID-19 positive diagnosis were analyzed and only two out of them presented the aforementioned peptide, making this study very superficial. A study with more patients and more samples is required, especially using non-infected patients as a control group, so this nucleoprotein can be certified as a possible biomarker for diagnosis purposes. In a similar work, the developed method was based on the detection of the viral nucleocapsid N protein in nasopharynx epithelial swabs [35]. The N protein is the most abundant in virion being the best option for the MS detection of the infection. Depending on the viral load available in the samples, 1 to 17 peptides would be detected and identified. Both studies commented that the strategy for a more comprehensive detection together with the quantification of a larger number of virus proteins, considering yet lower limits of detection, is indeed using targeted proteomics with a MS/MS possibility [34,35].

Besides the nucleocapsid protein of SARS-CoV-2, some studies were focused on finding other peptides that could spot the virus using nasopharyngeal or oropharyngeal swabs [38,40,42] and Vero cells [39] as samples. An initial peptide screening was performed using nanoLC-ESI-QOrbitrap MS system, followed by a target analysis carried out by turbulent flow chromatography (TFC) coupled with a QqQ mass spectrometer fitted with HESI source system [37]. The authors narrowed it down to three main peptides (DGIIWVATEGALNTPK, IGMEVTPSGTWLTYTGAIK, and WYFYYLGTGPEAGLPYGANK), which were considered as the most efficiently focused on the analytical system. 855 naso- and oropharyngeal samples were analyzed using the method with an analytical frequency of 500 samples per day.

Additionally, the authors evaluated the SARS-CoV-2 cross-reactivity against specimens of other human coronaviruses. About that, a similar study considering cross reactivity against other human viral diseases, such as those caused by influenza or arboviruses could be interesting and could lead to new important discoveries. The method sensitivity and specificity were compared against the gold standard RT-qPCR results for SARS-CoV-2, (83.8% and 96.1% respectively), which overall can be considered a good accuracy making these peptides promising biomarkers candidates to new alternatives for SARS-CoV-2 diagnosis [37]. This is the most thorough study when compared to similar publications so far, which is important to obtain more conclusive results.

Using a similar analytical platform, another research group implemented a targeted analysis focused on a comparative quantitative proteomic analysis of five positive and five negative samples. An average of 1100 proteins from each sample were detected [38]. Besides the nucleoprotein of SARS-CoV-2 in all positive samples, the authors identified the tryptic peptide (RGPEQTQGNFGDQELIR). The number of proteins uniquely detected was 32 and 50 in positive and negative samples, respectively. Regarding the proteins presented in both groups, the authors state that 57 proteins increased relative abundance in positive samples and 24 proteins increased relative abundance in negative samples. In this case, we believe a better-designed statistics assessment could narrow the proteins down to a point of selecting a promising molecular biomarker [38].

Two different peptides specific to the SARS-CoV-2 virus were identified through a targeted method developed in a nanoLC coupled with a high-resolution MS system [40]. These peptides were also detected in patients who have recovered from the infection and tested negative by RT-qPCR afterward. According to the authors, an important advantage of this method is the rapid diagnosis of symptomatic and asymptomatic patients, a very important aspect when the aim is to decrease virus circulation.

Some peptides were also identified and quantified from samples of Vero cells infected with viral strains by both untargeted and targeted proteomics performed using an LC-ESI-Orbitrap MS method [39]. The authors evaluated the limit of detection (LoD) by parallel reaction monitoring (PRM) for target tryptic peptides (NCAP, VME1), which was found to be approximately 10,000 SARS-CoV-2 particles. This approach offered a substantial increase in sensitivity. Their findings suggest that the sensitivity associated

with targeted proteomics is sufficiently high for detecting viral material in swabs and possibly other types of body fluids even with lower viral loads, which was considered a limitation on previously commented studies [39].

An *in silico* approach was adopted to identify unique peptides from SARS-CoV-2 as well [41]. The method was based on a stepwise elimination process to remove peptides with sequence homology or similar chemical features in clinically relevant systems, or that may be present in diseases with similar symptoms, to eliminate targets that could produce false-positive tests on healthy people. According to the authors, this method can be employed for both diagnostic assays and vaccine development. Following a similar aim, theoretical data were generated towards the definition of prototypic peptides of the virus and their corresponding transitions [43]. This study provides useful information for the development of targeted proteomics experimental assays considering the detection and/or quantification of SARS-CoV-2 peptides in complex clinical samples.

MALDI-TOF MS methods were developed to identify viral enveloped glycoproteins [44] and to specify discriminatory peaks to differentiate positive from negative samples [45,46]. Enveloped viruses have the unique biological feature of using cell membrane as the outer coating for its core containing the RNA genome, therefore, the SARS spike or viral spike protein (S-protein) is a unique target as a marker of the infection. Iles et al. [44] discovered that the S-protein was cleaved into S1, S2b, and S2a although held together by disulfide bridges. The S1 peak measurement has proven to be an excellent indicator of COVID-19 infection with 100% sensitivity, according to the authors.

The combination of MALDI-TOF MS acquired data with machine learning was used to discriminate 362 nasal swabs samples donated from three different clinical laboratories (211 positives and 151 negatives by RT-qPCR) [45]. A similar study also reported the potential of this approach on establishing discriminatory *m/z* between SARS-CoV-2 positive and negative samples [46]. In both papers, the authors state that MALDI-TOF MS can be an alternative as a fast tool for virus detection. From this result, in the future, with the advancement of miniaturization of analytical instruments, MALDI-equipped devices could hopefully be an option for direct diagnosis [47].

4.4. Multi-omics

Undoubtedly, proteomics has been the first-choice course of action to pursue the identification of promising biomarkers as an alternative for the diagnosis of SARS-CoV-2 infection. However, a multi-omics approach has been considered for a more comprehensive investigation. A global proteome/metaproteome/metabolome study was performed by using respiratory specimens of 20 positive and 20 negative diagnosed individuals, in addition to another 5 samples from H1N1 positive-diagnosed patients [48]. For proteomics and metaproteome (microbiome) profiles, the samples were injected through a new-developed method meanwhile for the metabolomic profile, the samples were analyzed in an RP-UHPLC-high-resolution mass spectrometer (not specified) following the protocol previously optimized by Boudah et al. (2014) [49]. Based on the multi-omic profile, the authors identified MX1 (MX Dynamin Like GTPase 1) and WARS (Tryptophan-tRNA ligase) as key proteins for a rapid and reliable viral diagnosis of SARS-CoV-2 infection. This is an interesting protocol when the point of the study is to find specific molecular features for diagnosis or any other purpose. Even though the remarkable discovery is still a protein, the use of a multi-platform to collect new multi-omic data or even new statistics treatment of the already acquired data could lead to new finds.

Serum from 28 patients with severe symptoms were considered to the identification of specific proteome and metabolome features for COVID-19 infection [42]. Preliminary untargeted proteomic data interpreted by the authors demonstrated the downregulation of platelet-related proteins in severe patients and upregulation of complement system proteins. Meanwhile, their untargeted metabolomics data showed a difference in the level of more than 100 molecules in COVID-19 positive patients. Furthermore, targeted validation procedures were performed in a nanoLC-tripleTOF system applying SRM mode for protein analysis, and the metabolites were individually analyzed using specific instrumentation according to their chemical characteristic considering polarity and ionization mode. This kind of study indeed offers a landscape view of molecular changes induced by SARS-CoV-2 infection on human blood that can provide powerful information for not only diagnostic but also prognosis and therapeutic further investigations. However, for more accurate conclusions, the analysis of a larger group of samples should be considered as well.

4.5. Untargeted metabolomics

Using the untargeted metabolomics strategy, a group of researchers developed a machine learning-based algorithm combined with the MS data to create a platform able to discriminat COVID-19 markers in plasma samples [50]. Samples from 728 individuals were directly infused in a HESI-QOrbitrap instrument. The authors proposed 21 discriminant biomarkers for COVID-19 infection using this approach. Out of the 21 molecules, eight compounds belong to the glycerophospholipid class, three glycerolipids, three fatty acids, two cholesterol derivatives, one purine metabolite, one prostanoid, one plasmalogen, and two unknown peptides. The remaining ten molecules have not been identified yet, which is not ideal but is a common pitfall of untargeted metabolomics. This study considered a larger group of samples, so these achievements could be a promising result for biomarker assignment and so should be more explored going further.

4.6. Transcriptomics

A transcriptomics approach was implemented also towards the identification of promising biomarkers of SARS-CoV-2 [51]. The study, which analysis was based on RNA isolated from naso- and oropharyngeal swabs (22 samples tested positive and 22 samples tested negative) by an MS-based method, targeting SARS-CoV-2 nucleocapsid genes (N1, N2, N3 ORF1ab/nsp3 and ORF1ab/nsp10). MS experiments were carried out in a MALDI-TOF system. The results were compared to the classic RT-qPCR one, which also targeted ORFlab and N genes depending on the brand of the kit [52]. Both results were corroborated in all samples. Using these the MALDI-TOF approach against the RT-qPCR could indeed lead to an automatized process with superior analytical frequency and, however, as commented in previous sections, the expressive cost of this equipment makes the investment not very worthy.

4.7. Coronavirus screening methods

By definition, coronaviruses are large, enveloped, positive-sense RNA viruses that can cause respiratory, enteric, hepatic, and neurological diseases in a wide range of animals, including humans. Until the 2019 pandemic outbreak caused by the SARS-CoV-2, there were six types of human coronaviruses (HCoV). Among them, SARS-CoV and MERS-CoV are known for being significant threats to human health [53].

The recent pandemic brought attention to this kind of infection; thus, the scientific community has been focusing on researches on

Table 2
A comparison of relevant assays and their respective limits of detection. Source: adapted from Orsburn et al. (2020) [41].

Assay	Matrix	Detection limit	Assay time
SARS-CoV Nucleocapsid ImmunoSwab	Nasopharyngeal aspirate medium	10 pg/mL	45 min
SARS-CoV-2 Nucleocapsid ImmunoSwab	Nasopharyngeal aspirate/urine	Unstated/based on assay above	10 min
SARS-CoV Nucleocapsid ELISA	Nasopharyngeal aspirate	2.5 ng/mL	4-h EST
SARS-CoV Nucleocapsid ELISA	Stool	9.0 ng/mL	4-h EST
SARS-CoV Protein Microarray	Human serum	1:64,000 Dilution of Positive Serum	~2 h
Inflammatory Mediator Protein Microarray	Human serum	20 pg/mL	~3 h
CDC-SARS-CoV-2	Nasopharyngeal aspirate	3.2 copies/μL	4 h
SARS-CoV-2 RT-Lamp/CAS12	Nasopharyngeal aspirate	10 copies/μL	45 min
Enkephalin LCMS	Plasma	3.2 pg/mL	6.5 min
Orexin LCMS	CSF[a]	4 pg/mL	7 min

[a] CSF: Cerebrospinal fluid.

that issue. Therefore, MS-based studies towards the understanding of the general profile of HCoV, and other pathogens of respiratory diseases, were already being pursued. In 2017, was developed a method, named mCoV-MS, to identify known HCoVs and provide clues to the scientific community for emerging HCoVs infections [53]. The method uses a multiplex PCR conjugated with MALDI-TOF technology to achieve high-throughput CoVs detection. To detect six HCoVs, the analysis targeted RNA-dependent RNA polymerase (RdRp) and nucleocapsid (N) genes of HCoVs. Additionally, for SARS-CoV and MERS-CoV, the ORF1b gene and upstream regions of the E gene were also targeted. For alpha-coronavirus and beta-coronavirus detection, the target selected was also the RdRp gene. According to the authors, despite the mCoV-MS method having presented some limitations regarding its sensibility, it can still be useful to provide some clues for alternative methods of HCoV detection.

In similar research, viral etiologies of respiratory tract infections (RTIs) were the focus of a multilocus polymerase chain reaction coupled with electrospray ionization mass spectrometry (PCR/ESI-MS) study [54]. The method was used to detect enteroviruses and rhinoviruses in respiratory samples. Based on the study, the authors conclude that the method improved the diagnostic yield for viral RTIs with a lower possibility of false-positives outcomes, when compared with virus isolation.

Considering other types of coronaviruses, in 2005, a group of researchers were able to characterize the IVB N protein in infected Vero cells by MS back then [55]. Avian Infection Bronchitis Virus (IBV) is also a member of the *Coronaviridae*. At the time, the experiments were carried out on an HPLC coupled with a QqQ mass spectrometer system equipped with a Z-spray ion source.

Finally, considering the severity of the current pandemic and the efforts being made to control SARS-CoV-2, all the MS-based methods mentioned in this review are extremely important and promising to select fast, sensitive, accurate, and alternative methods for COVID-19 diagnostic. LC-MS-based methods appear amongst the most sensitive and faster ones to even directly quantify SARS-CoV and SARS-CoV-2 in comparison with some other relevant analytical assays designed for such goal (Table 2) [41].

Recently, the FDA granted to a specific laboratory an emergency use authorization for SARS-CoV-2 RNA detection, based on RT-qPCR and MALDI-TOF-MS assay, which is the first full MS-based system approved for use. This method consists of the analysis of the upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens) and bronchoalveolar lavage specimens from individuals suspected of COVID-19, which represents a great advance in the use of MS for direct virus detection [56].

As shown in Table 2 [41], LC-MS is indeed a good direct diagnostic alternative; however, as previously mentioned, we believe the direct identification of this or any other kind of infection by MS is nowadays too powerful, but too expensive to be used in clinic facilities. The studies described so far demonstrate how MS is promising for diagnosis purposes, but still has a lot to be explored. For example, expanding sample types beyond nasopharyngeal and oropharyngeal swabs can help increase testing for the population, and, consequently, implement measures to withhold the coronaviruses from progressing. Therefore, studies involving the identification of virus biomarkers in urine, saliva, and plasma are the starting point for said purpose, since these sample types provide a less invasive setup. Additionally, most of the studies discussed in this section are still in preliminary stages, which is understandable given the severity of the pandemic and the hush for results, thus much more should be explored further regarding sampling, analytical instrumentation, data treatment, as we believe that will be.

5. Prognosis

In this section, prognosis-focused studies will be discussed. Starting with lipidomics, we will be also commenting on metabolomics and proteomics dedicated studies in that particular sequence.

5.1. Lipidomics

When infesting a cell, viruses take control of the energy of host cells pathways, using the adenosine triphosphate (ATP) generated in lipid metabolism for its replication. Therefore, the lipidomic profile of the host cell is changed by differentially regulating key pathways of lipid synthesis, remodeling, and utilization [57]. The studies discussed in this topic report the use of MS to visualize changes in the lipidomic profile of patients infected with the virus, employing methodologies that provide rapid and promising results. An important observation is that, in most works, it is evident the need to increase the number of analyzed samples to make statistical inferences.

Using only commercial swabs for taking samples directly from the nasopharynx and oropharynx of positive, negative, and blind patients to COVID-19 (Fig. 6), synthetic polyolefin-silica paper (Teslin®) and 1 μL of chloroform as an extracting agent, the identification of 17 lipids was possible. Furthermore, phosphatidylcholine, serine, ethanolamine, lipid sterols and diglycerides were considerably altered [58]. In this study, a paper spray mass spectrometry (PS-MS) with a LIT mass analyzer was the chosen system. Although it returned promising results, it is still necessary to considerably expand the number of samples analyzed to carry out a statistical evaluation. It is interesting to report that due to the total time of analysis (60 s) and the minimal effort to sample preparation, this method can assist the screening of severe and mild cases of COVID-19.

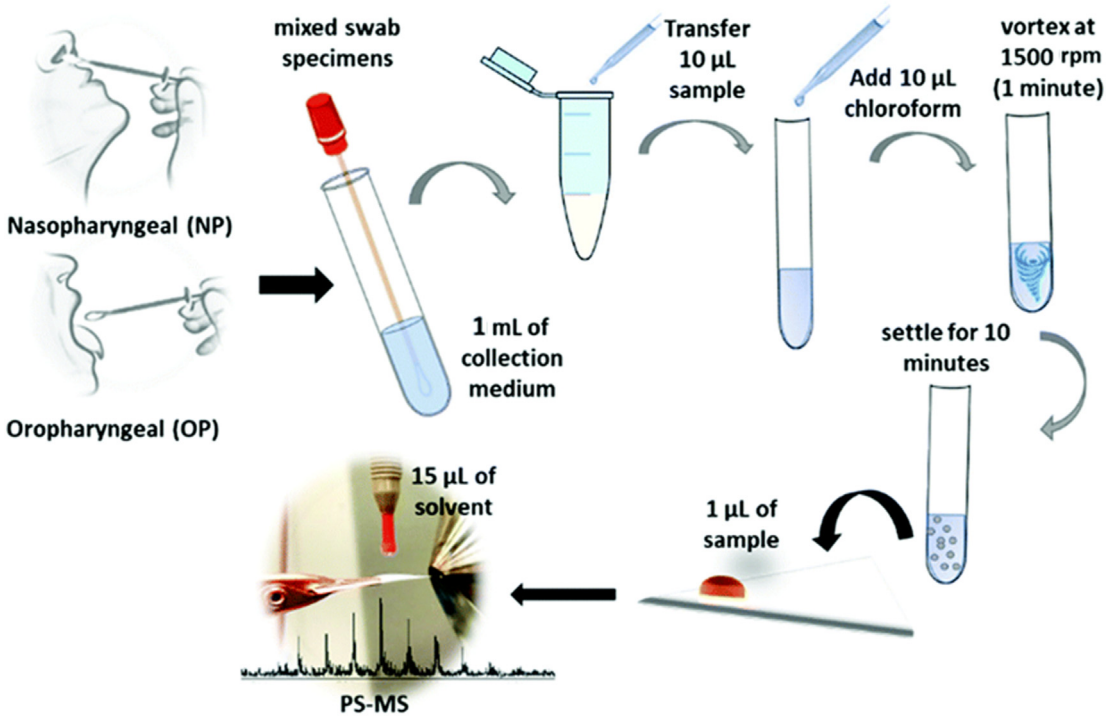

Fig. 6. Schematic representation of sample pre-treatment based on paper spray mass spectrometry (PS-MS) on a Teslin® substrate. Source: adapted from Silva et al. (2020) [58].

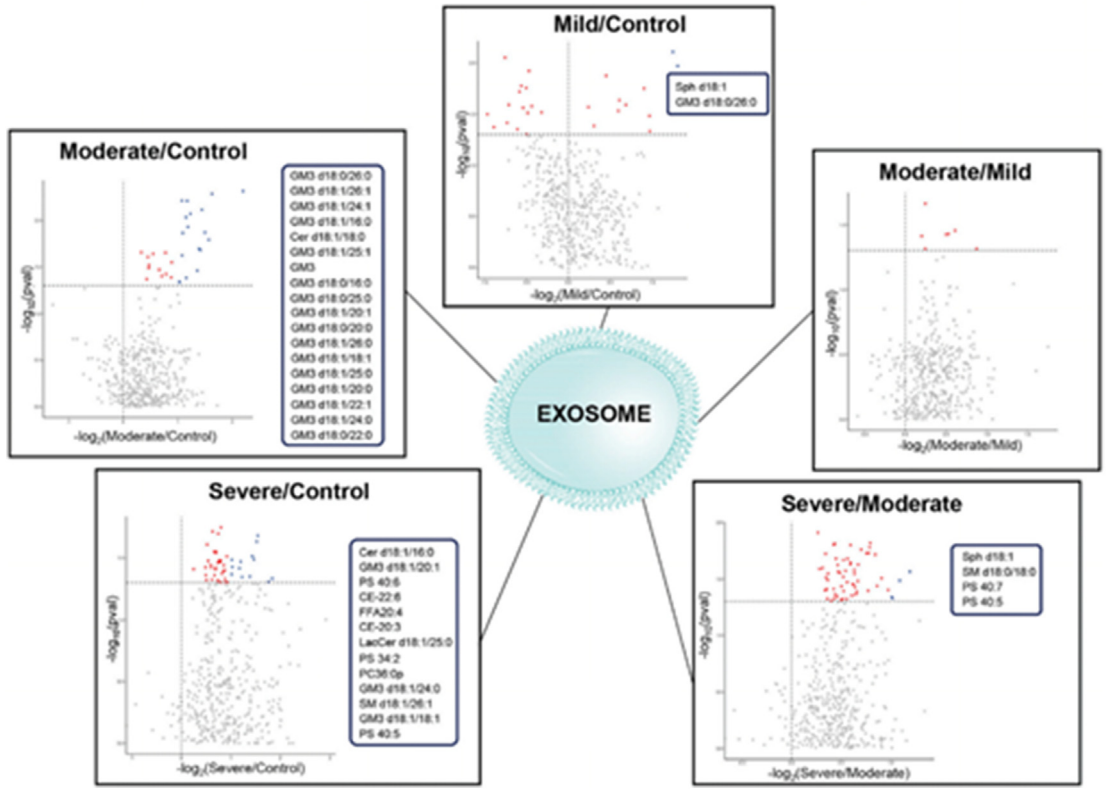

Fig. 7. Lipid profile changes in exosomes for different degree of severities of COVID-19 patients. Blue (≥2 fold) and red (<2 fold) dots indicate dramatic change in lipids. Source: adapted from Song et al. (2020) [59]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

The association of the LC with MS has proven to be effective in monitoring lipidomic dysfunctions in patients affected with COVID-19 [59]. The use of such a setup allowed the observation of several connections between altered lipid classes and disease severity

(Fig. 7). Among these changes, the reduction in the largest classes of plasma glycerophospholipids stands out, suggesting a reduction in the circulation of high-density lipoprotein (HDL) as the severity of COVID-19 increases. In mild cases, a reduction in the levels of medium and long-chain triacylglycerols (TAGs) was observed, and in cases of severe infection, a progressive increase in the number of sphingolipids, such as sphingomyelins (SMs) and monosialodihexosyl ganglioside (GM3s), was observed.

The authors highlighted that the plasma GM3s were strongly and negatively correlated with T cell and $CD4^+$ T cell count, i.e., as the disease severity increased these counts progressively decreased. The authors suggested that this might be associated with the immune response dysregulation reported for COVID-19.

Another important contribution in the verification of lipid disorders caused by COVID-19 using the association between LC and MS/MS is the increase in lipid concentration in the course of disease deterioration in fatal cases. The high levels of diglycerides, triglycerides, and free fatty acids were similarly altered in individuals who presented mild and severe cases of the disease, regardless of the gender and age of the patient tested [60]. Due to this phenomenon, it is evident that greater attention is needed to patients who meet hospital discharge criteria, as this increase in blood lipids can lead to dyslipidemia. It is interesting to highlight the observation of the demographic independence of this lipid dysfunction, which adds another positive factor for the use of MS-based systems in the search for novel biomarkers [10].

Similar observations were made considering disorders caused by two viruses of the same class as SARS-CoV-2, called HCoV-229E and MERS-CoV, known to cause mild respiratory infections and severe pneumonia, respectively [61]. An increase in the level of three main classes of lipids (fatty acid (FA), lysophosphatidylcholine (lysoPC), and lysophosphatidylethanolamine (lysoPE)) was observed. LysoPC was the predominant lipid class with 60% of all identified lipids with an enhanced concentration level. Arachidonic acid (AA), linoleic acid (LA), palmitic, and oleic acid, which belong to the FA class, showed a 7.1, 5.03, 2.61, and 4.21-fold increase, respectively. Upon HCoV-229E infection, glycerophospholipids, the main component of cell membranes, was metabolized to lysoPEs and FAs. AA and LA demonstrated a strong modulation effect on the replication of HCoV-229E and MERS-CoV, suggesting that coronavirus replication required a specific composition of cellular lipids [61].

It is important to emphasize that the aforementioned works are related to the changes caused by the new coronavirus, SARS-CoV-2, and the other most known coronaviruses. The main changes were observed in the levels of diacylglycerol, triacylglycerol, and fatty acids, and these lipids can be used as biomarkers for disease severity. It should be noted that relation was observed between the levels of GM3s and the $CD4^+$ T cell count and the evolution of the disease. These cells are part of the adaptive immune system and modulate the body immune response and memory [62–65] with the reduction of these cells, its functions are impaired, causing a severe case of inflammation, and not generating long-lasting immunity, thus explaining the possible causes of reinfection by COVID-19 [66–69].

5.2. Metabolomics

The assessment of metabolic dysfunctions caused by COVID-19 infection can serve as a tool for the prognosis of the disease, making it possible to propose interpretations regarding the general picture and clinical progress of patients. The use of analytical techniques based on LC separations and MS detection has proven to be useful in obtaining this information [42,59,60].

An evaluation of the metabolic changes associated with COVID-19 through untargeted metabolomics in blood plasma samples was firstly considered [59]. Increased levels of biliverdin, the oxidized form of bilirubin, were observed, indicating a possible relationship between oxidative stress and the disease. Polar metabolites, such as acylcarnitines, which participate in the oxidative pathways of energy production, were reduced in COVID-19 in critically ill patients, which may reflect the depletion of lung functions and lower availability of oxygen for cellular energy production. This decrease in metabolite levels in patients with COVID-19 was also observed in a similar study [60]. In this new work, 431 metabolites were identified by UHPLC-ESI-QqQ-LIT, a hybrid MS system. In the lethality group, the most metabolic variations were in the thyroid hormone synthesis and signaling pathways, purine metabolism, and autoimmune thyroid, suggesting that the alterations in these pathways are associated with the progress and deterioration of the disease.

The relationship between metabolic changes and the disease aggravation inferred in these studies can serve as a biological signature for monitoring the general clinical picture. However, there is still a long way to go, as so far there is only a limited number of studies devoted to exploring and correlating the metabolites changes with COVID-19 progression using MS, which opens a research opportunity and a gap for a better understanding of the interaction between SARS-CoV-2 and the alterations of the metabolic pathways caused in the human body.

5.3. Proteomics

The changes that SARS-CoV-2 can cause in the levels of proteins present in biological fluids can be used as an alternative for discovering biomarkers with potential employability in the prognosis of the disease [34,42]. In this context, the high use of blood samples and their derivatives in research is notorious. As examples of this statement, three distinct studies stand out as they present important results using LC-MS-based methods for proteomics of blood serum and plasma and peripheral blood mononuclear cells (PBMCs) [32,70,71].

Similar results for serum proteome and blood plasma were obtained employing a UHPLC-TripleTOF system able to perform approximately 180 analyses in one day [32]. Forty-eight patients with COVID-19 and 15 healthy volunteers were evaluated. Several proteins had their levels altered recurrently of the presence of the virus; amongst them, the groups A1BG, ACTB, ACTG1, ALB, APOA1, APOC1, CIR, C1S, C8A, CD14, CB, CFH, CFI, CRP, FGA stood out. FGB, FGG, GSN, HP, ITIH3, ITIH4, LBP, LGALS3BP, LGR1, SAA1, SAA2, and SERPINA10 showed biomarker features for disease prognosis. In addition to information on protein alterations in COVID-19 patients, it shall be noted that this work proposes the automatization of pretreatment of samples, considerably reducing the risk of contagion for the analyst.

Peripheral blood samples from 35 patients confirmed with COVID-19, 13 patients with severe symptoms, and 22 patients with mild symptoms, in addition to 6 healthy volunteers used as controls, were analyzed using LC-UHF-Orbitrap and LC-IT instruments for a proteome profile acquisition [70]. Similar to the previously commented ones, this work aimed to find relationships between the virus and protein dysregulation recurrent to contamination. The results showed that the COVID-19 virus is capable of developing mechanisms that disrupt fundamental cellular processes and, thus, evade the host immune system. More than 286 vectors of proteins directly linked to SARS-CoV-2 were verified and more than 350 human proteins were disturbed due to the presence of the virus.

Aiming at identifying potential protein markers present in blood samples from infected, recovered, and deceased patients, seven

Table 3
Summary of MS-based studies on new alternatives for COVID-19 prognosis.

Omics strategy	MS system	Targeted/Untargeted studies design	Sample	Object of investigation	Reference
Lipidomics	PS-LIT	Targeted	Nasopharyngeal, oropharyngeal swabs	Phosphatidyl choline, serine, ethanolamine, sterol lipids and DIG[a]	[58]
	LC-MS/MS	Targeted and untargeted	Blood plasma	Glycerophospholipids, sphingolipids, TAGs[a], SMs[a], GM3s[a]	[59]
	UHPLC-ESI-QqQ- LIT	Targeted	Blood plasma	DIG, TRIG[a], and FA[a]	[60]
	UHPLC-ESI-QTOF	Targeted	Huh7 and Vero E6 cells	FA[a], lysoPC[a], lysoPE[a]	[61]
Metabolomics	UHPLC-ESI-TOF	Targeted and untargeted	Blood plasma	Polar metabolites, amino acids	[59]
	UHPLC-ESI-QqQ-LIT	Targeted	Blood plasma	Thyroid hormone metabolites, MA[a], AA[a], CP[a]	[60]
	nanoLC-QTOF	Untargeted	Serum and blood plasma	Metabolomic profile (204 metabolites)	[32]
Proteomics	nanoLC-nanoESI-Orbitrap	Targeted	Gargle solutions	Nucleoprotein SARS-CoV-2	[34]
	UHPLC-HESI-QOrbitrap	Untargeted	Serum and blood plasma	Proteomic profile (93 proteins)	[32]
	UHPLC-QTOF	Targeted	Serum and blood plasma	27 protein groups	[45]
	UHPLC-ESI-QTOF	Untargeted	PBMCs[a] and blood	IREB2, GELS, POLR3D, PON1, ULBP6 and Gal-10 proteins	[71]

[a] DIG: diglycerides; TAGs: triacylglycerols; SMs: sphingomyelins; GM3s: monosialodihexosyl ganglioside; TRIG: triglycerides; FA: free fatty acids; lysoPC: lysophosphatidylcholine; lysoPE: lysophosphatidylethanolamine; MA: malic acid, AA: aspartic acid; CP: carbamoyl phosphate; PBMCs: peripheral blood mononuclear cells.

proteins with potential biomarker application in the prognosis of the disease, which is directly associated with the immune response and cytokine storm, were observed as well [71]. When comparing three groups of patients (infected, recovered, and deceased), the researchers obtained similar results to the other studies using the same type of sample, where dysregulation was observed in the levels of the proteins IREB2, GELS, POLR3D, PON1, STFPD, ULBP6, SAA1, SAA2, and S-TREM. In severe cases, the proteomic profile showed a marked elevation of the Gal-10 protein, which is exclusive to this type of patient, suggesting a relationship between this and the impairment of the immune system.

The use of less invasive methods to obtain samples for disease prognosis is a major challenge to overcome. The use of methods focused on protein detection by LC-MS-based systems can be a valuable alternative due to its high sensitivity.

In this sense, a nanoLC-nano-ESI-Orbitrap system, capable of determining SARS-CoV-2 proteins in gargle solutions (20 mL of 0.9% NaCl solution) from patients diagnosed with COVID-19, was initially proposed [34]. The samples presented an abundance of human proteins, including a nucleoprotein considered highly viral, which can be used as a prognostic marker able to assess the patient clinical status and the progression of the disease. Although there is a need to significantly increase the number of samples tested to carry out a statistical evaluation, this methodology presents a minimally invasive and highly sensitive method.

Another attempt of the use of samples collected in a less invasive manner to detect protein traces from COVID-19 contamination was described [31]. In this particular study, previously discussed in section 4.2, even though no virus proteins were identified, the human protein load present in samples from patients with mild and severe COVID-19 could be differentiated from healthy controls. The results were validated after a new proteome study of urine samples from recovered patients, showing that the developed method has potential applicability to distinguish and predict disease severity

Table 4
Summary of MS-based studies for therapeutic targets identification against COVID-19.

MS System	Targeted/Untargeted studies design	Sample	Objects of investigation	Ref.
UHPLC-ESI-QqQ	Targeted	Human plasma	Remdesevir and GS-441524	[76]
LC-ESI-MS/MS	Targeted	Human plasma, urine and peripheral blood mononuclear cells	Remdesivir, GS-704277 and GS-441524	[77]
HPLC-MS	Targeted	Blood	Hydroxychloroquine	[78]
LC-MS/MS	Targeted	Human plasma	Lopinavir and ritonavir	[79]
LC-ESI-QqQ	Targeted	Drugs	Tenofovir disoproxil fumarate	[80]
LC-HRMS	Targeted	Drugs	Chloroquine	[81]
GC-QIT	Untargeted	Geranium and lemon essential oils	Citronellol, geraniol, neryl acetate, limonene	[87]
LC-ESI-QTOF	Untargeted	Qingfei Paidu decoction	Flavonoids, glycosides, carboxylic acids, saponins, alkaloids, terpenes	[85]
UHPLC-ESI-QTOF	Untargeted	Lung-toxin Dispelling Formula No. 1 (Respiratory Detox Shot)	Luteolin, licoisoflavone B, fisetin, quercetin, glyasperin F, isolicoflavonol and semilicoisoflavone-B	[86]
zESI-QTOF	Targeted	SARS-CoV-2 M^{pro}[a] incubated with GC376	GC376	[89]
UHPLC-Orbitrap	Targeted	SARS-CoV-2 M^{pro}[a]	M^{pro} mass determination and inhibitor candidates (carmofur, ebselen and PX-12) analysis	[90]
nanoESI-QOrbitrap	Targeted	SARS-CoV-2 M^{pro}[a]	Molecular mass and characterization of the binding of SARS-CoV-2 M^{pro}[a] with the inhibitors (boceprevir, GC-376 and calpain inhibitors II and XII)	[91]
Native MS				
n.m.	Targeted	SARS-CoV-2 M^{pro}[a]	Characterization of the functional unit of M^{pro}[a] Dissociation constant determined for SARS-CoV-2 M^{pro}[a]	[92]
MALDI-TOF and LC-MS/MS	Targeted	Drugs	Disulfiram and ebselen (Zn-ejector drugs)	[93]
ESI-QTOF	Targeted	Solid culture of *Aspergillus versicolor*	Diketopiperazines	[94]
nanoESI-QTOF	Targeted	SARS-CoV-2 S-protein[a] RBD[a]	ACE2[a]/RBD[a] complexes	[95]
LC-MS/MS	Targeted	Vero E6 cells infected with SARS-CoV-2	Disturbances in protein abundance and phosphorylation during SARS-CoV-2 infection	[97]

[a] M^{pro}: viral main protease; S-protein: viral spike protein; RBD: receptor binding domain; ACE2: angiotensin-converting enzyme 2; Gal-10: galectin-10; IREB2: iron-responsive element-binding protein 2; n.m.: not mentioned.

and prognosis, in addition to helping to understand the pathogenesis of COVID-19. This work is a motivation for future studies since the number of analyzed samples was quite limited.

The articles concerning COVID-19 prognosis were summarized in Table 3.

6. Therapeutic targets

In this section, therapeutic targets related studies will be focused on. The ones dedicated to accessing existing drugs quantification and degradation pathways will be approached and, in the following subsections, studies focused on finding new alternatives for COVID-19 treatments - starting with new API and specific inhibitor search - will be described.

6.1. Current overall strategies

To date, there is no specific antiviral for the treatment of COVID-19. Thus, in addition to trying to develop a completely new drug, what it is currently being done is the redirection of existing ones. According to specialists, this is performed based on genomic sequence information coupled with protein structure modeling [72]. In this context, the identification of therapeutic targets is an important step for redirection of existing pharmaceuticals or on the development of new anti-SARS-CoV-2 agents [72,73]. Several studies are designed to demonstrate their potentials, such as bioavailability evaluation, protein binding, and metabolic pathway determination, and the latter of great importance for drug safety due to the formation of toxic metabolites, among others. Therefore, MS is presented as the first-choice technique for drug discovery assignments, since it provides this comprehensive information as well as provide data that enable us to make inferences on impurities, metabolites, and degradation products [30,74,75].

Table 4 summarizes the studies discussed in this section that was focused on the analysis of biomolecules and natural products by MS techniques that could guide scientists and clinicians on the search for novel therapeutic salvages against COVID-19.

6.2. Pharmaceutical quantification, pharmacokinetics and fraud monitoring of new synthetic drugs

Several studies employed LC-ESI-MS/MS systems to quantify promising drugs in plasma, urine, or peripheral blood mononuclear cells were pursued [76–79]. Selected reaction monitoring (SRM) strategy in the positive was used mode to analyze Remdesivir (RDV) [76,77]. The quantification of both RDV and its metabolite GS-441524 in human plasma was possible through an original validated method that took less than 2 min [76]. At the time this study was concluded, RDV was still in Phase-2 of usability evaluation so no real-life samples were analyzed. However, this study represents an important first step to ensure RDV pharmacokinetics and quality control.

Afterward, a more thorough analysis to determine RDV, GS-704277, and GS-441524 in plasma, in addition to RDV and GS-441524 in urine and total concentration of GS-441524 in peripheral blood mononuclear cells was performed [77]. The objective was to describe the pharmacokinetics of solution and lyophilized formulations of intravenous RDV. As a result, both formulations were comparable considering pharmacokinetics parameters. According to the authors, this preliminary result supports continued investigations of RDV usage in COVID-19 patients.

The same workflow were followed to analyze pharmacokinetics and dosage of hydroxychloroquine (HCQ) as well [78]. The concentration of Lopinavir (LPV) and Ritonavir (RTV) were monitored in plasma of PCR-positive patients admitted to an Intensive Care Unit (ICU) [79]. The experiments were successfully carried out through LC-MS/MS analysis of samples every 72 h to record the risk of bradycardia in COVID-19 patients treated with the LPV and RTV in a fixed-dose combination.

Closing up the synthetic drugs related studies, MS was also utilized to perform degradation screening and identification of drug adulteration. Tenofovir disoproxil fumarate (TDF), an antiretroviral belonging to the same class as RDV, was analyzed by LC-ESI-QqQ using SRM after the full scan mode [80]. The study of forced degradation was carried out to assess the stability of the drug and to identify unknown degradation pathways, which is required by regulatory agencies. This study presented a new degradation product, which could be a contribution to the development of further quality control routines. To exemplify the quality control importance, a study on suspected counterfeit chloroquine (CQ) tablets distributed in Africa during the pandemic was conducted to identify possible *m/z* of adulterants [81]. Through this method, the authors found paracetamol and metronidazole in tablets that should exclusively contain CQ.

6.3. New API-candidates search in natural products

Regarding the use of natural products as active ingredients of new drugs, we have found many reports showing the potential of MS in the qualitative and quantitative analysis of these samples. The main point of these studies is trying to identify the chemical composition of these products and elucidate structures aiming at discovering novel therapeutic targets. For instance, in 2007, LC-ESI-IT-MS was used focused on elucidating the structure of metabolites in *Boenninghausenia sessilicarpa* (Rutaceae), a Chinese herbal medicine rich in coumarin, aiming at discovering active anti-SARS-CoV ingredients [82]. In previous work, the structure of active components from more than 200 Chinese medicinal herb extracts was elucidated for the same purpose [83]. Back in 2004 [84], were developed methods for screening new therapeutic targets from 121 Chinese herbs with antiviral activities to identify drugs that can interfere specifically with SARS-CoV entry into host cells using frontal affinity chromatography coupled to mass spectrometry (FAC-MS).

In this context, similar or even more advanced instrumentation has been applied towards the identification of anti-SARS-CoV-2 molecules in natural products. A study, whose main purpose was finding the chemical composition of Qingfei Paidu decoction (QFPF) by LC-MS and also suggesting its pharmacological mechanism was design [85]. ESI was used as an ion source in both positive and negative modes. This study allowed to putatively identify 129 compounds in QFPD, which included 58 flavonoids, 20 glycosides, 13 carboxylic acids, 7 saponins, 6 alkaloids, 4 terpenes, among others (Fig. 8).

On the other hand, another study identified the active constituents by UHPLC-MS/MS using ESI only in positive mode [86]. The phytochemical analysis of Lung-toxin Dispelling Formula No. 1, known as Respiratory Detox Shot (RSD), provided the identification and confirmation of seven bioactive compounds: luteolin, licoisoflavone B, fisetin, quercetin, glyasperin F, isolicoflavonol and semilicoisoflavone-B. Gas chromatography coupled to a QIT mass spectrometer was used to identify the chemical composition of essential oils and evaluate their inhibitory effect on angiotensin-converting enzyme 2 (ACE2) receptor (host cell receptor) activity in HT-29 cells [87]. In this particular work, the authors have found 22 compounds in geranium oil and nine compounds in lemon oil with potent antiviral activity.

6.4. New viral-inhibitors-candidates search

Another important set of works has been performed focusing on

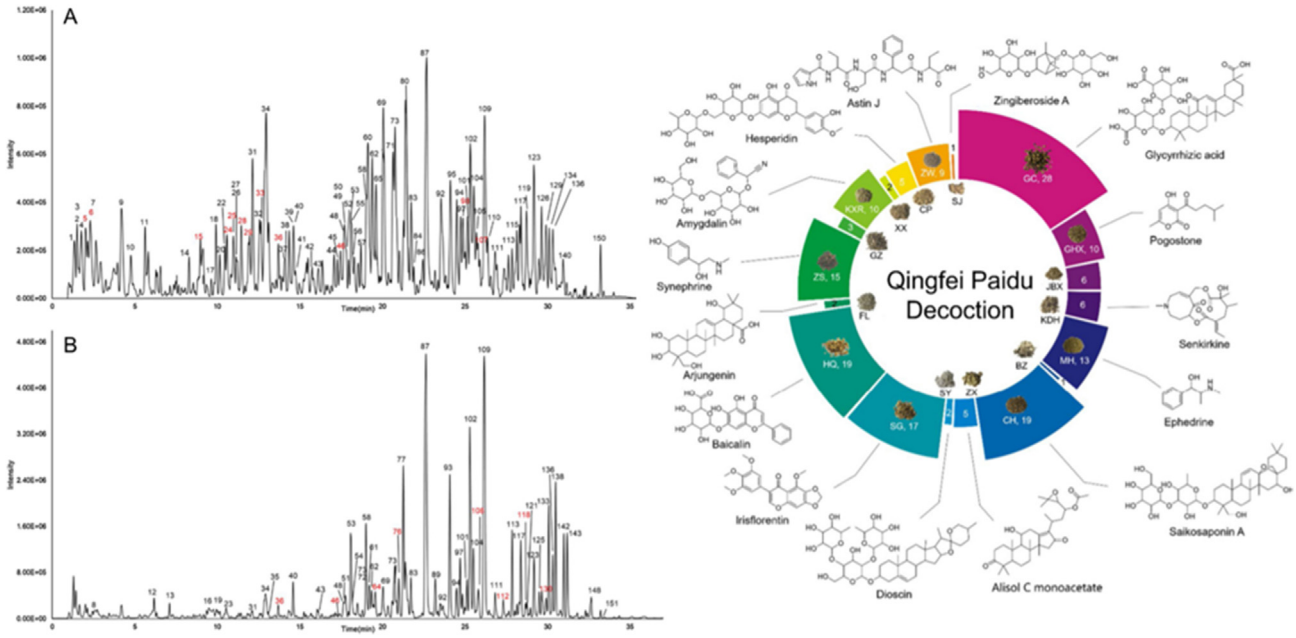

Fig. 8. Total ion chromatograms of Qingfei Paidu decoction: (A) ESI negative mode, (B) ESI positive mode and, on the right, the number of chemical constituents of each herb and representative constituent structures identified from Qingfei Paidu decoction. Source: Adapted from Yang et al. (2020) [85].

the viral main protease (M^{pro}). M^{pro} is a key enzyme for the treatment of coronavirus strains because it is fundamental in mediating viral replication and transcription [88]. Therefore, lots of efforts are being done to find M^{pro} inhibitors. Another protease involved with viral replication that has the attention of scientists is the papain-like protease (PL^{pro}), interesting as a target for drug development against SARS-CoV-2, as well as M^{pro} [88].

M^{pro} encoding by SARS-CoV-2 (SARS-CoV-2 M^{pro}) protease inhibitor was defined [89]. An evaluated inhibitor was GC376, with MS being used to assess the presence or absence of formation of a covalent adduct when incubating this inhibitor with SARS-CoV-2 M^{pro}. MS analysis was performed by direct infusion using a QTOF mass analyzer and ESI as ion source in the positive mode. Even for an excess of GC376 inhibitor, only a small portion of SARS-CoV-2 was covalently modified by it, indicating the necessity of enhanced inhibitors. Following a different workflow, focused on identifying drugs that target SARS-CoV-2 M^{pro} to discover potential antiviral compounds [90]. Analytical approaches in MS were used to measure the molecular mass of SARS-CoV-2 M^{pro} and to identify inhibitors with cited compounds such as carmofur, ebselen, and PX-12.

Some other compounds were identified with potential activity [91]. Boceprevir, GC-376, and calpain inhibitors II and XII were highlighted by the authors. MS was one of the devices used to characterize the mechanism of action. Native MS was used to determine the molecular mass of M^{pro} and to characterized, the binding of SARS-CoV-2 M^{pro} with the cited inhibitors. Finally, the functional unit of M^{pro} was characterized, with the dissociation constant being determined for SARS-CoV-2 M^{pro} [92].

Non-specific drugs were used to target critical viral proteins to identify new drug targets in conserved viral domains [93]. In this study, disulfiram and ebselen (Zn-ejector drugs) were studied, evaluating whether there was a covalent bond to cysteines in PL^{pro} and nsp10. MALDI-TOF-MS in positive mode was used to measure the molecular masses of PL^{pro} and nsp10 before and after adding these two drugs. After digesting these drugs treated with PL^{pro} with trypsin, they were further analyzed by LC-MS/MS (not specified).

A high-resolution ESI-QTOF-MS system to analyze piperazines with antiviral potential [94]. MS along with other techniques as nuclear magnetic resonance was used to determine the structure and absolute configuration of the molecules. The authors reported the identification of four new indolyl diketopiperazines, two new diketopiperazines, and 11 known diketopiperazines and intermediaries were isolated from the solid culture of Aspergillus Versicolor. Six of these compounds may be useful in fighting COVID-19 after additional studies.

With a different approach, have been reported that a therapeutic intervention that could be used is blocking the site of interaction of angiotensin-converting enzyme 2 (ACE2)/viral S-protein with antibodies or small molecules [95]. Thus, they used native MS in conjunction with limited load reduction to characterize ACE2/receptor-binding domain (RBD) complexes and evaluate the influence of heparin-related compounds on their stability. Native MS measurements used QTOF as a mass analyzer and nanoESI as an ion source. An interesting review article was written, presenting fragmentation schemes intrinsic to MS/MS, with the identification of product ions used in the SRM mode of herpes and influenza virus antiviral agents [96].

Finally, studies that targeted protein composition were also performed to therapeutic target discovery. The use of MS-based methods was demonstrated to study disturbances in protein abundance and phosphorylation during SARS-CoV-2 infection [97]. For this, the infection was studied in Vero E6 cells, which are susceptible to it. Kinases (drug target) and pathways altered by SARS-CoV-2 were identified, which may be used in trials with 87 drugs approved by FDA.

7. Conclusion

This review covers the main techniques, feasibilities, and limitations of MS in the analysis of biological fluids, drugs, and natural products to assist the diagnosis, prognosis, and search for agents against SARS-CoV-2. MS has become an essential tool in the detection, elucidation, and quantification of biomolecules and its

implications in the clinical area mainly due to its high precision, sensitivity, and wide dynamic range, in addition to its medium to high capabilities performance. Thus, its association with separation techniques has allowed the discovery of molecular signatures related to SARS-CoV-2 infection that can be extremely useful in screening metabolites of patients infected by the virus besides selecting the therapeutic approach and monitoring the patient.

We noticed that after the pandemic, the collaboration among scientists was amplified. We have observed a great amount of shared information and also a vast number of studies that put together scientists and physicians of several specialties involved in a unique goal, which is getting the most information as possible on this novel disease. No wonder why so much technology and alternatives have been produced over this last year. We hope that open communication between population, science, medicine, university, and private sector becomes a tendency going further.

Finally, about MS itself, we believe that this technique alone is not the definitive answer; its powerfulness improves when associated with chemometrics, bioinformatics, and microdevices development. In the future, portable equipment together with the automation of sampling and treatment could improve the analytical frequency providing more sensitive and faster analyses, not only for COVID-19 but differentiating it from other symptomatic alike diseases such as H1N1, dengue and zika fever, and other non-viral diseases.

There are still many challenges to overcome to implement MS as a main analytical instrument in clinical facilities, such as high cost, high maintenance, and the requirement for the specialized operator and laborious samples pre-treatment. However, facing the potential of the technique, we believe that medical specialized organizations are already considering this investment as worthy and are interested in acquiring an infrastructure that supports high-performance analytical equipment such as mass spectrometers. In the future, we believe these setups could help identify signs of changes in the human metabolic balance before the patient starts to manifest it physically, which could significantly change how medical therapies are performed. Furthermore, MS could get to a point of helping the hospitalized patients, by accessing the evolution of the disease and giving priorities to patients for the use of intense care unites, and hopefully, help to manage the COVID-19 pandemic.

CRediT authorship contribution statement

Nerilson M. Lima: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Bruno L.M. Fernandes:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Guilherme F. Alves:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Jéssica C.Q. de Souza:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Marcelo M. Siqueira:** Conceptualization, Data curation, Writing – original draft. **Maria Patrícia do Nascimento:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Olívia B.O. Moreira:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Alessandra Sussulini:** Writing – review & editing, Visualization. **Marcone A.L. de Oliveira:** Conceptualization, Resources, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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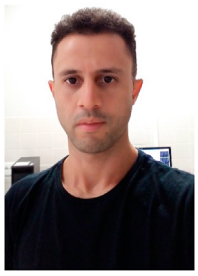

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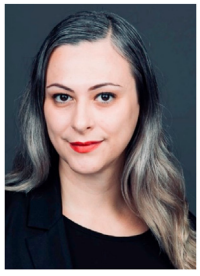

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