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Advances in rapid detection of SARS-CoV-2 by mass spectrometry

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ABSTRACT

COVID-19 has already been lasting for more than two years and it has been severely affecting the whole world. Still, detection of SARS-CoV-2 remains the frontline approach to combat the pandemic, and the reverse transcription polymerase chain reaction (RT-PCR)-based method is the well recognized detection method for the enormous analytical demands. However, the RT-PCR method typically takes a relatively long time, and can produce false positive and false negative results. Mass spectrometry (MS) is a very commonly used technique with extraordinary sensitivity, specificity and speed, and can produce qualitative and quantitative information of various analytes, which cannot be achieved by RT-PCR. Since the pandemic outbreak, various mass spectrometric approaches have been developed for rapid detection of SARS-CoV-2, including the LC-MS/MS approaches that could allow analysis of several hundred clinical samples per day with one MS system, MALDI-MS approaches that could directly analyze clinical samples for the detection, and efforts for the on-site detection with portable devices. In this review, these mass spectrometric approaches were summarized, and their pros and cons as well as further development were also discussed.

1. Introduction

Since its outbreak in Wuhan, China in December 2019, Coronavirus Disease 2019 (COVID-19) has affected more than 200 countries, and as of 31 January 2022, it has caused more than 364 million confirmed infections and more than 5.6 million deaths [1]. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was found to be the causative agent of COVID-19, which contains a single strand RNA that encodes four structural proteins, i.e., nucleocapsid (N), membrane (M), envelop (E) and spike (S) proteins, and 16 non-structural proteins. The continuous emergence of various SARS-CoV-2 variants, particularly those categorized as Variants of Concern (VOCs) by World Health Organization (WHO), have made the situation more complicated. For example, the Delta variant (B.1.617.2) identified firstly in India was estimated to be up to 117% more transmissible than the wild type SARS-CoV-2 [2], while the most prevalent variant currently, Omicron (B.1.1.529), could significantly elude immune response [3]. Fighting against the COVID-19 pandemic has become the overwhelming task all over the world. To this end, rapid detection of SARS-CoV-2 for large population testing is essential to effectively control and manage the disease.

Currently, detection of SARS-CoV-2 and its variants mainly relies on the reverse transcription polymerase chain reaction (RT-PCR) and serological approaches, such as enzyme-linked immunosorbent assay. RT-PCR, which offers high specificity and sensitivity, is the most well recognized and widely used method for detection of SARS-CoV-2 at the current stage [4]. However, this technique might also possess a number of drawbacks. For example, the quality of extracted viral RNA could significantly affect the detection sensitivity and accuracy, and therefore the extraction is required to be performed by experienced technicians. Besides, the high viscosity and high concentration of proteins and other interfering components from oral swab or sputum, which are the two most commonly collected samples, render further challenge to RNA extraction [5]. More importantly, false negative and false positive

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results of this approach are concerned [6,7]. Furthermore, the whole analysis usually takes several hours, and is typically performed in higher biosafety level (BSL) laboratories, which hinders the applicability of this approach. Serological approaches aim to detect viral antigens or antibodies. The most obvious advantage of this method is that portable size products are commercially available elsewhere, which enabled rapid and on-site detection carried by individuals. This approach could be complementary to RT-PCR, as the induced antibodies would retain in patients for a long period of time, therefore those mild and asymptomatic infected patients could also be identified. However, sensitivity and specificity of this method are limited [8]. Moreover, antibodies could take a few days to be induced after the onset of symptoms, thus this method is less applicable for detection of early stage infection [9].

Along with the wide utilization and ongoing development of the RT-PCR and serological approaches, mass spectrometry (MS) could be an attractive alternative for SARS-CoV-2 detection because of its advantages in speed, sensitivity and specificity, and ability to provide qualitative and quantitative information of various analytes (e.g., nucleic acids, proteins, peptides, organic molecules and elements), which cannot be achieved by the RT-PCR and serological approaches. Particularly, proteomics has been extensively used to investigate numerous biological mechanisms and important biomarkers of diseases [10]. The experimental workflow of proteomics has been well-developed, even for whole cell proteome and highly complex biological systems. Various studies have demonstrated the ability of MS in identification and prototyping of viruses and microorganisms by detecting their marker peptides via both liquid chromatography mass spectrometry (LC-MS) and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) [11–14]. Therefore, detection of SARS-CoV-2 based on MS-based proteomics approach could be attractive and highly feasible. On the other hand, metabolites, volatile organic compounds and lipid profiles of SARS-CoV-2 infected patients have received attention from researchers, because they could be indicative to SARS-CoV-2 infection [15,16]. Moreover, the speed, sensitivity and resolution of mass spectrometers have been being rapidly developed in recent years, facilitating the development of viral detection based on increasingly advanced instruments, and a number of methods involving the application of innovative sampling methods and portable mass spectrometers have been developed for potential on-site detection of SARS-CoV-2. In this review, the recent developments of different mass spectrometric approaches for rapid detection of SARS-CoV-2 based on various molecules, e.g., proteins, peptides, metabolites, volatile organic compounds and lipid profiles, are highlighted. Practical considerations necessary for the development of MS-based methods for detection of SARS-CoV-2 are also discussed.

2. Detection of SARS-CoV-2 based on targeted proteomics approaches

These approaches were typically based on liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) for the detection. The rationale of targeted proteomics-based methods is similar to the detection of specific RNA sequences for identification of SARS-CoV-2 in the RT-PCR method. Detection of specific sequences of viral proteins, which are translated from viral RNAs, could allow detection of SARS-CoV-2. The method development and virus detection strategy of targeted proteomics approaches are shown in Fig. 1. In brief, sample pretreatments and trypsin digestion are conducted for recombinant viral proteins or virus-like particles. Digested peptides are separated and detected by LC-MS/MS. Resulting spectral data are subjected to database searching for identification of peptides and proteins detected. Specific peptides are selected as targets for virus detection, and instrumental parameters for detection of these targeted peptides are optimized. After the method development, clinical samples are collected, and the same sample preparation procedure is performed. Targeted peptides selected in the method development are then determined by LC-MS/MS and the presence of virus could be indicated by the positive detection of the target peptides.

2.1. Establishment of target peptides

Initial efforts were made on the construction of a target peptide list for SARS-CoV-2 detection. Kapoor and Subba constructed a peptide list for SARS-CoV-2 proteins *in silico* [17]. Gouveia et al. conducted a shotgun proteomics study on SARS-CoV-2 infected Vero cells, and after considering several important factors for selection of target peptides, including signal properties (e.g., elution time and signal intensity), inter-species specificity, intra-species conservation and proneness to modifications, a list of 14 tryptic peptides from M, S and N proteins was constructed for the targeted analysis [18]. Criteria of choosing the detection targets might vary among different research work, particularly with/without the inclusion of peptides with missed cleavage. To achieve rapid detection, time for enzymatic digestion should be shorten, and therefore, digestion conditions must be optimized and peptides with no missed cleavage were typically preferred as the target peptides [18]. These preliminary studies provided useful insights and information for the further development of targeted proteomics methods in later studies. The MS-based proteomics approach was attempted by Nikolaev et al. at the early stage of the pandemic outbreak, who showed that peptides of N proteins could be detected from nasopharynx epithelial swabs of COVID-19-confirmed patients by nano-LC-MS/MS [19].

Peptides containing amino acids prone to modifications could significantly complicate the detection and quantification, and thus were not recommended to be selected as the detection targets [20]. For example, methionine and tryptophan were easily susceptible to oxidation *in vivo* [20,21] and cysteine could potentially form disulfide bonds [20,22], influencing the mass spectrometric detection of target peptides. Sequences with 6–12 amino acids were regarded as the optimal lengths for the detection and quantification. Viral peptide specificity was generally not considered, but conversation of sequence among VOCs was concerned, which could reduce the chance of false negative results, especially when samples were suspected to be infected by SARS-CoV-2 VOCs. These rationale of choosing target peptides are of particular importance for detection of variants of SARS-CoV-2. Target peptides that are highly conserved could be the general biomarkers of SARS-CoV-2, while those target peptides containing the mutated residues could be designated for detection of different SARS-CoV-2 variants.

2.2. Detection of target peptides

Multiple reaction monitoring (MRM), a technique with triple quadrupole mass spectrometer that can offer high sensitivity and specificity, is commonly used for detection and quantification of small molecules and proteins and its applications in detection of virus-associated proteins were previously reported [23,24]. Followed by their preliminary work [18], Gouveia et al. extended their research for development of a MRM-based method for rapid detection of specific peptides for SARS-CoV-2 in clinical samples [25]. This method was developed by using a mixture of nasopharyngeal swabs peptidome from healthy donors and SARS-CoV-2 viral peptidome and validated by SARS-CoV-2 infected patient swabs later. A serial dilution study revealed that the number and abundance of viral peptides detected decreased with the viral load,

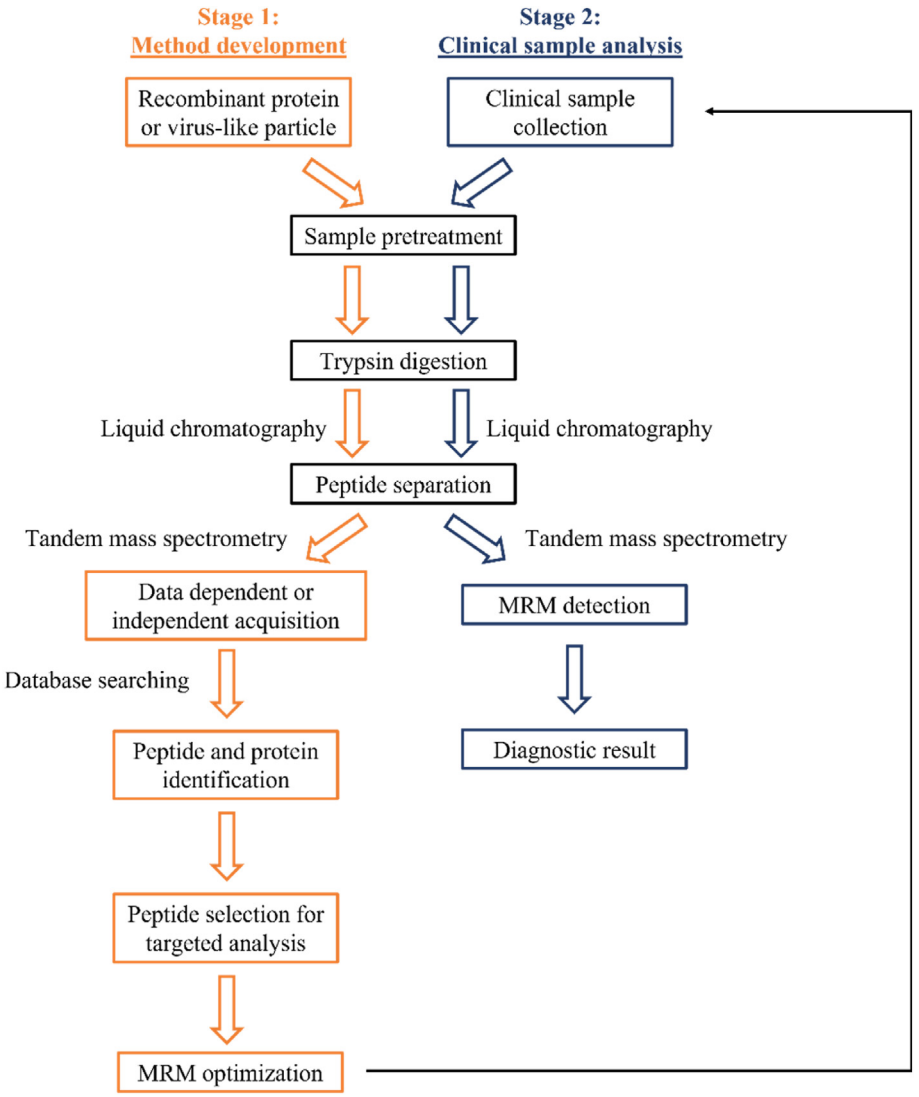

Fig. 1. Strategy for development of targeted proteomics approach for SARS-CoV-2 viral peptide detection and clinical sample analysis. Orange and blue colors in the flow chart indicate procedures in method development (stage 1) and clinical sample analysis (stage 2), respectively.

and three fast eluting peptides (eluted within 3 min [18]) from N proteins (ADETQALPQR, KADETQALPQR, and GFYAEGSR) were consistently found regardless of the viral load. In fact, KADETQALPQR and KKADETQALPQR, missed cleavage version of ADETQALPQR, were commonly resulted and observed. It was suggested that inclusion of the peptides with missed cleavages in data evaluation could be helpful for ensuring the reliability of results.

The advancement of mass spectrometers, particularly using orbitrap as mass analyzer, further elevates the analytical performances such as detection sensitivity and resolution. Parallel reaction monitoring (PRM), an orbitrap-based detection approach, has been demonstrated to allow virus identification by detection of target peptides [26]. It was recently demonstrated that targeted high-field asymmetric waveform ion mobility spectrometry (FAIMS) coupled with PRM could successfully detect N protein in SARS-CoV-2 positive nasopharyngeal swab samples [27]. N protein was enriched by an automated antibody capturing approach through an automatic liquid handler to enhance the sensitivity of detection, but more interferences were also introduced to the chromatographic separation. Therefore, introduction of FAIMS could provide an additional dimension for peptide separation which reduced matrix interference and further enhanced the detection sensitivity. Combining with the machine learning for data analysis, this method was shown to achieve a sensitivity of 98%, specificity of 100% and coefficient of variation (CV) of 7%. Limit of detection (LOD) could reach down to 2000 genome equivalents of SARS-CoV-2 virions, but the sample throughput was limited to 100 samples per day due to the inclusion of additional washing time and method blank injections for minimizing carryover problems after analyzing high viral load samples. Other than nasopharyngeal swabs, Chavan et al. manifested urine as the potential sample for COVID-19 diagnosis by LC-MS [28], although SARS-CoV-2 RNA was not commonly detected in urine by RT-PCR [29,30]. Similarly, biotinylated antibody was used to capture N protein, and streptavidin magnetic bead was utilized to recover the immunocomplex in this work. However, tryptic peptides of N protein could be detected in only one-third of COVID-19 positive urine samples used in this study. Nevertheless, the resulted N peptides were consistent to those target peptides selected in other studies (e.g., ADETQALPQR and its missed cleaved peptides) [18,25,31,32]. Moreover, study of

urine proteome difference between COVID-19 positive and negative patients might reveal other biomarkers for detection and prognosis. Apart from urine, gargle solution and saliva are samples that could be collected non-invasively for COVID-19 diagnosis. Ihling et al. initially demonstrated that viral proteins extracted from gargle solution of COVID-19 positive patients could be achieved by overnight acetone precipitation, trypsin digestion and finally LC-MS/MS detection [33]. Later on, this study was extended to saliva and the protocol was revised based on several practical considerations [21]. The overnight acetone precipitation was replaced by complete dissolution of sample solution in TRIZOL reagent, which was used as the protein denaturant for RNA preparation. This significantly reduced the sample preparation time, and samples could be split for a parallel RT-PCR analysis. The initial 3 h nanoflow LC separation was significantly reduced to 5 min with a normal flow ultra-high performance liquid chromatography (UHPLC) setup. Moreover, the later protocol utilized a triple quadrupole mass spectrometer that is of much lower cost and more readily available in clinical laboratories compared to orbitrap-based mass spectrometric instruments. As sample preparation could be performed in parallel, the daily sample throughput was depended on the time of instrumental analysis. In this case, 288 samples could be analyzed daily. This method allowed detection of as low as 500 amol/μL of N protein, which was sufficient to detect a sample with a cycle threshold (Ct) value of 22. Increase of the sample amount and conducting tryptic peptide enrichment were demonstrated to further improve the detection limit [21].

A research work focused on quantification of N and S viral peptides was reported by Pierce-Ruiz et al. [20]. Incorporation of isotope-labeled peptides as internal standards can greatly enhance the qualification (i.e., retention time alignment between the detected peptides and their corresponding isotope-labeled analogs was allowed for peak validation) and quantification accuracy of proteins [34]. Peptides selected as the targets were shown to be highly conserved among almost all common VOCs discovered. Notably, peptide ADETQALPQR was excluded from the final protocol because the yield of this peptide was lower than expected in the recombinant matrix. However, this peptide was suggested to be included as a detection target in many studies [18,25,31,32]. This method showed excellent precision that the relative standard deviation of S and N protein were only 3.67% and 5.11%, respectively, and the limit of quantitation (LOQ) could reach down to low nanomolar range [20].

Cardozo et al. established another promising assay targeting N protein peptides of SARS-CoV-2 from both oropharyngeal and nasopharyngeal swabs [22]. This method allowed analysis of 96 samples within 4 h, including 2 h trypsin digestion, which enabled more than 500 clinical samples to be analyzed per day. The essay sensitivity and specificity reached 84% and 97% respectively, and the decent analytical performance was attributed to enrichment of the viruses by magnetic bead-aided precipitation. Among the eight shortlisted peptides, two of them (IGMEVTPSGTWLTYTGAIK and DGIIWVATEGALNTPK) received particular attention because of their high Signal/Noise ratios for quantitative analysis by turbulent flow chromatography (TFC) coupled with triple quadrupole mass spectrometer. Quantification performance of this method was revealed by linearities and limits of quantitation of the two peptides, which were higher than 0.97 of R^2 value and in low ppb level, respectively. CVs of this analysis were lower than 20% which met the standard of clinical protein quantification assay. The study of analyte stability revealed that the target proteins in saline solutions could be stable for 30 days under room temperature, which was superior to the stability of RNA for PCR analysis that required an immediate −80°C storage after the sample collection. Moreover, a simple 5-min 90°C treatment was enough to inactivate the virus without affecting the detection of target peptides, which extended the applicability of this method to numerous low BSL laboratories.

2.3. Efforts for establishment of standardized protocols

With the development of various MS methods for detection of SARS-CoV-2 by different research groups, establishment of general and standardized protocols becomes highly desirable. Particular attention should be devoted to the Cov-MS consortium promptly established at the relatively early stage of the pandemic outbreak [32]. This consortium is composed of various academic research groups, industrial laboratories and major MS manufacturers. They committed to share their findings to the MS community so as to enhance the applicability and accessibility of the developed protocols, and ensure the validity of analytical parameters such as sensitivity, specificity and robustness. They also aimed to establish protocols that could be generally applied across different laboratories. Efforts from this working group led to the establishment of an MRM-based detection method consisted of 17 peptides as biomarkers from N and S proteins with 145 MRM transitions in total, and these peptides were detected even in the highly complex Universal Transport Medium-RT (UTM-RT). However, in contrast to selection criteria given by some studies [18,25], non-specific peptides were also included in this developed method because combination of peptides as detection targets could enhance the specificity [35], particularly that some peptides may arouse from microorganisms that only exist in SARS-CoV-2 infected samples [25]. Besides, detection based on limited targets may potentially cause false negative for SARS-CoV-2 VOCs. The potential of negative correlation of logarithmically (with base 2 in this study) transformed summed area under curve (LogSumAUC) and Ct value of samples was also demonstrated, although extra validation is required before the quantitative information is used clinically. Similar relationship was also presented in a study by Nikolaev et al., in which common logarithm was used instead of logarithm with base 2 [19]. The correlation coefficients of both studies were not sufficiently high, which could be attributed to the nature of samples that some free and isolated RNAs and proteins existed outside the virus particles. As a result, deviation in correlation between Ct value and LogSumAUC became explainable [19]. In addition to the protocol established by the consortium, this work also provided valuable information and guidance for development of novel MS detection method in the future. Particularly, improvements made by researchers could be highly peptide specific, i.e., some peptides may have more significant signal enhancement than others. MS from different vendors also showed different detection capability for different peptides. In short, this first generation assay could process 200 samples per day and be highly cost-effective (e.g., ~USD $5 per sample [32]). These methods only involved rapid and simple sample pretreatment and short period of analysis, enabling completion of one analysis within 30 min by MS instruments commonly available in clinical laboratories. It is expected that these methods will be further optimized to enhance the detection capability (e.g., sensitivity and accuracy) and throughput [32].

2.4. Untargeted proteomics approaches

Despite targeted SARS-CoV-2 specific peptide detection is straightforward, the applicability of untargeted proteomics approaches to identify SARS-CoV-2 were also explored. The untargeted approach could provide not only diagnostic results, but also valuable information regarding disease progression and therapeutic targets. Messner and teammates demonstrated a modified MS-based untargeted proteomics method, which involved a rapid semi-automation sample preparation procedure and short LC

gradient during the LC-MS/MS analysis [36]. This method, which was developed based on the ISO 13485 requirement, was demonstrated to allow detection of SARS-CoV-2 protein biomarkers in serum and blood, and some of them could even reveal the disease severity. The reproducibility of this method, i.e., CV of 7.3%, was satisfactory among the large scale untargeted proteomics studies.

3. Detection of SARS-CoV-2 by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)

In addition to the methods developed based on LC-MS/MS, MALDI-MS is another competent alternative detection technique, as it has been being widely applied in clinical laboratories for identification of pathogenic bacteria, fungi as well as virus, including human coronavirus [37]. Nachtigall and coworkers developed a MALDI-MS method for detection of SARS-CoV-2 in nasopharyngeal swab [38]. In this method, the same samples of nasopharyngeal swab solutions for the RT−PCR analysis were directly analyzed by MALDI-MS without sample preparation (an at least 20-min UV irradiation was used to prevent the contamination). Spectra were analyzed by a robust machine learning method, resulting in a high accuracy (93.9%) and acceptable false positive and false negative rates (7% and 5%, respectively) for 362 swab samples (in which 211 were shown positive to SARS-CoV-2), demonstrating its potential to be applied clinically. Similar study was performed by Rocca and coworker [39]. These studies demonstrated the simplicity and relatively lower cost of MALDI-MS, rendering it an attractive approach for COVID-19 diagnosis.

Serum peptidome profiling with MALDI-MS was proposed to be an alternative for COVID-19 detection, as serum is more static and less sensitive against external disturbance than nasopharyngeal swabs [40]. Samples were ready for MALDI-MS analysis after a simple dilution and matrix mixing, rendering the simplicity and high speed of analysis. Yan and coworkers demonstrated the differentiation of COVID-19 infected from non-COVID-19 infected individuals with similar symptoms, as well as classification of patients with various severity of infection, from their serum peptidome profiles. These considerations and data analysis based on eight machine learning methods were shown to be able to deliver 100% of SARS-CoV-2 specificity, 99% of accuracy and 98% of sensitivity [40]. Iles et al. developed a protocol aiming to detect SARS-CoV-2 specific components by direct MALDI-MS analysis of gargle solution and saliva [41]. Comprehensive study on the whole pipeline of analysis, including the starting model utilized (pseudo SARS-CoV-2 particle and saliva/gargle spiked with SARS-CoV-2-infected cell culture media), sample pretreatment process (e.g., UV inactivation of virus, prefiltering of samples followed by acetone precipitation, matrix selection and optimization), and selection of markers for virus detection, provided valuable information and references for the later development of MALDI-MS-based virus detection methods. It was suggested that the S1 protein peak was the most promising target for identification of SARS-CoV-2 with excellent specificity and sensitivity, while other components such as S2a, S2b, viral envelope proteins and light chains from antibodies could yield supporting information for virus infection. The method could be highly cost-effective, i.e., down to $1 − 2 USD for each sample [41], because of the simplicity and speed of MALDI-MS analysis. However, further validation with large cohort of clinical samples would be needed for this method.

Apart from viral proteins and peptides, MALDI-MS could also be applied in detection of virus with genomic approach. Recently, Rybicka and coworkers have established a MassARRAY® system, which was recognized and marked by European Conformity for in vitro Diagnostic Medical Devices (CE-IVD), combining the RT-PCR reaction with MALDI-MS detection of SARS-CoV-2 viral genes (Fig. 2) [42]. This method exhibited better accuracy than conventional RT-PCR, as viral gene could be detected by this method for RT-PCR negative samples, while some RT-PCR suspected samples could be clearly identified as positive [42]. The estimated LOD could reach down to the 400 copies/mL, demonstrating its superior sensitivity and ability to identify infected patients with low viral load.

4. Potential of MS for on-site detection of SARS-CoV-2

On-site detection could help to perform sample pre-screening, which could relieve the workload in testing laboratories, and promptly let the testing cohorts understand their health conditions. In recent decades, portable mass spectrometric instruments have become more popular and widely demonstrated to be applicable to various on-site analysis [43,44]. For example, a miniature dual linear ion-trap mass spectrometer coupled with ion mobility spectrometry was developed, which allowed unambiguous detection of analytes even in complex mixtures because the ion mobility device enabled an extra dimension of molecular separation [45]. Gas chromatography mass spectrometry (GC-MS) has been widely used for analyzing volatile organic compounds and the related field portable devices were commercially available [46,47]. Moreover, a nanoflow portable LC-MS was introduced and the reproducibility of analysis, particularly the analyte retention time, was comparable to the laboratory-based LC-MS and GC-MS [48,49]. Several studies also reported the applications of portable MS in clinics [50−52].

Along with the advancements in portable mass spectrometers, development of sample preparation methods suitable for on-site analysis was also highly important. For on-site detection, sample types should be promptly ready for analysis once they are collected. Other than samples commonly collected (e.g., nasopharynx swab), exhaled breath could be an alternative type of sample to be collected, as viral RNA of SARS-CoV-2 was found in breath samples although the detected viral load was less than in nasopharynx swabs [53], and it contains various volatile organic compounds and metabolites [54], the profiles of which could be indicative for SARS-CoV-2 infection. Recent studies showed that changes in volatile organic compound profiles were observed for both adults and children upon SARS-CoV-2 infection [55,56]. The non-invasive nature for collection of breath samples is another advantage, and the breath could be directly injected to portable GC-MS for analysis. A recent study applying portable GC-MS revealed that six compounds in exhaled breath, which included benzaldehyde, 1-propanol, 3,6-methylundecane, camphene, beta-cubebene, iodobenzene, were distinctive features for COVID-19 positive samples, with 68.5% of sensitivity and 85% of specificity [57]. It is reasonable to expect that marker compounds in exhaled breath could be of low concentrations, thus development of methods for simultaneous sample collection and enrichment would be highly desirable. Yuan et al. developed a novel solid phase microextraction (SPME)-based method, in which a SPME fiber was inserted into a face mask for trapping and concentrating organic compounds in exhaled breath [58]. Wearing face mask is a common measure to prevent SARS-CoV-2 infection, thus such sample collection could be readily performed and the sampling time could be extended without any extra efforts and requirements, rendering this sample collection method an attractive alternative [58]. Although authors utilized the direct analysis in real-time mass spectrometry (DART-MS), which is a laboratory-based technique in this stage, for instrumental analysis, SPME fiber is also well-compatible for portable GC-MS (Fig. 3) [59,60], indicating the high feasibility of executing this method on-site.

Apart from the above, some innovative handheld devices were also developed for mass spectrometric analysis in clinics. For

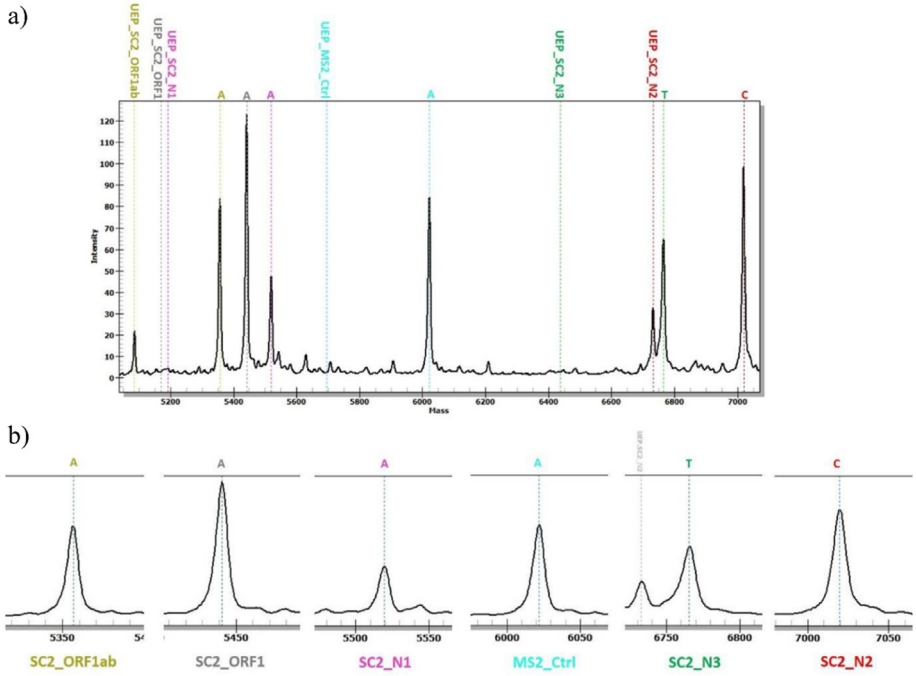

Fig. 2. (a) Targeted genes of SARS-CoV-2 were detected by MassARRAY® System in a SARS-CoV-2 positive sample. Extension primers and their respective analyte peaks were in the same color. (b) Amplicons were depicted in the zoomed spectra. (Adapted from Refs. [41,42]).

example, a handheld device modified based on the MasSpec Pen technology [61] integrated with ESI-MS was recently reported to successfully analyze lipid profiles in nasopharynx swabs [62]. It was demonstrated that the lipid profiles could allow identification of SARS-CoV-2 infected patients, as changing in lipid profiles was found upon SARS-CoV-2 infection [63,64]. The single-use swab sampling device applied in this approach consisted of three conduits (Fig. 4). The swab to be analyzed was inserted to the middle conduit, and chloroform/methanol was flowed to the device from another conduit so as to soak and extract the lipids in the swab for 10 s. Vacuum was then applied to transfer the solvent to the ESI ion source via the remaining conduit. Only 20—30 s was required for sample introduction and mass spectral acquisition, with less than about 45 s needed to complete the whole analysis. Statistical analysis was performed to investigate the collected lipid profiles and then the diagnostic results would be generated. This method successfully identified PCR-confirmed SARS-CoV-2 infected patients with symptoms from those PCR-negative individuals, no matter they were symptomatic or asymptomatic. The cross-validation accuracy, specificity and sensitivity were 78.4%, 77.21% and 81.8%, respectively, which were acceptable and might be further improved. Overall, this technique possessed the advantages of high sample throughput, simple operation, low solvent consumption, etc., and could be potentially further developed into a portable device for on-site analysis with a portable ESI-MS equipment.

5. Conclusions and perspectives

It is foreseeable that it is a long-lasting battle to combat COVID-19. Especially the emergence of different variants may significantly reduce the efficacies of the developed vaccines and drugs, rendering another round of time-consuming development of vaccines and medicines targeting these variants. This particularly highlights the importance of detection of SARS-CoV-2, as it acts as

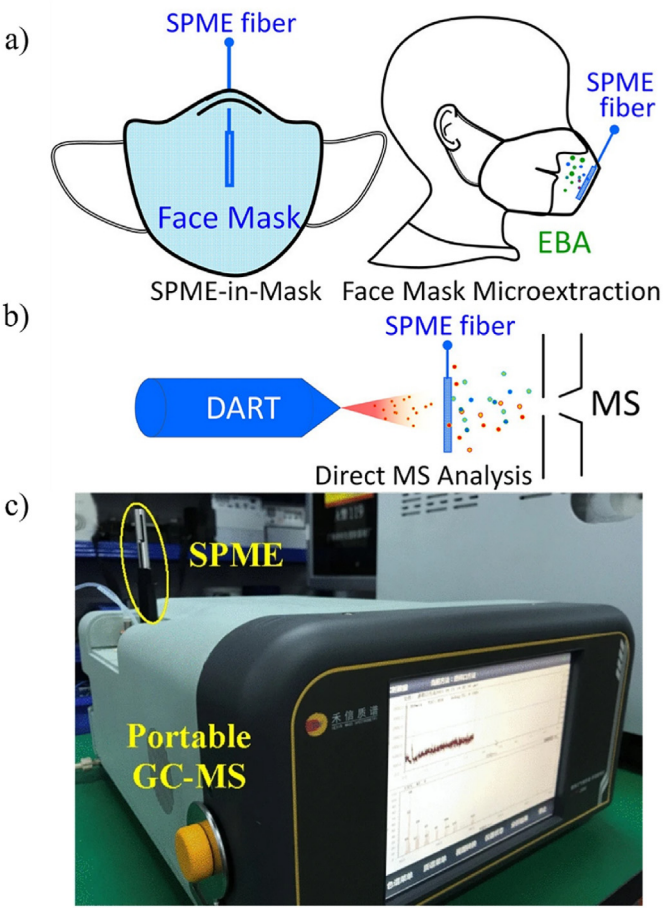

Fig. 3. a) Collection of exhaled breath aerosol (EBA) by a SPME fiber incorporated face mask and b) Direct analysis of the SPME fiber by DART-MS (Adapted from Refs. [58,60]). c) Analysis of the SPME fiber by portable GC-MS (Adapted from Ref. [60]).

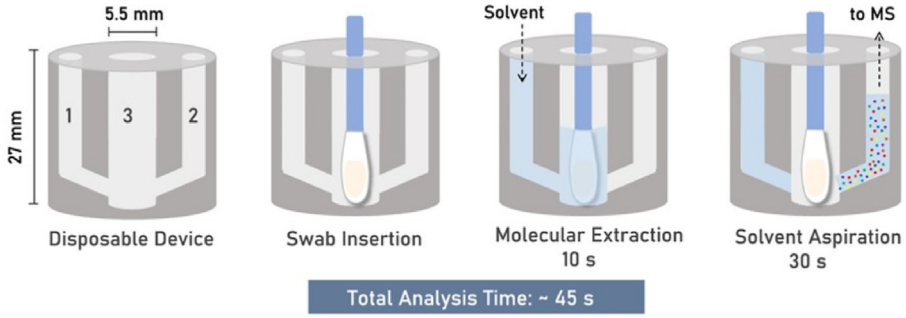

Fig. 4. The 3-conduit MS swab device for analysis. The nasopharyngeal is inserted to the middle conduit 3. The solvent is transported from conduit 1 to extract lipids from the swab for 10 s. The lipid-extracted solvent is delivered to the ESI-MS for data acquisition through conduit 2 for 30 s. The total analysis takes about 45 s only (Adapted from Ref. [62]).

the frontline approach to identify infected population and control the spread of the virus.

As discussed above, thanks to the efforts devoted by related researchers, various MS-based methods, particularly the targeted proteomic approaches and MALDI-MS approaches, have been developed for SARS-CoV-2 detection, with the achievable analytical performance including sample throughput of analysis of more than 500 clinical samples/day, CV or standard deviation of lower than 10%, detection limit down to 500 fM (corresponding to a Ct value of 22), etc. These studies demonstrated that MS could be a potential alternative applied in clinics for alleviating the huge workload of laboratories performing RT-PCR tests and delivering further accurate diagnostic results. MS also provided more dimensions of information, including but not limited to proteins, peptides, RNAs and metabolites, in different sample types such as nasopharynx swab, gargle, saliva serum, breath, etc. These methods could possibly overcome the technical drawbacks from other analytical methods, such as long analysis time as well as limited sensitivity and specificity, although further developments are required for their formal implementation in clinics.

In the road of the further development of related techniques, we recommend that the following areas could be considered: (1) Further optimization of various components in sample preparation and instrumental analysis in an effort to develop a standardized protocol. Steps and reagents used in sample pretreatment could be minimized so that the developed methods could be easily carried out in different laboratories; (2) Quantitative correlation between MS-based results and clinical parameters, e.g., Ct values, could be further investigated and validated; (3) The method development is recommended to be based on relatively low-price mass spectrometric instruments so that the developed method could be more easily accessible to different laboratories; (4) The developed method could be easily modified without extensive reinvestigation to detect new SARS-CoV-2 variants, as mutated target peptides would be utilized as the identifiers of SARS-CoV-2 variants. One limitation of MS comparing with PCR is no amplification performed during the instrumental analysis. Extra efforts can be put for the sample pretreatment, for example, performing the sample enrichment with target-specific beads to enhance the sample detection. Meanwhile, the sample throughput could still be increased by, for example, reducing the sample preparation time and instrumental analysis time, and parallel analysis of more samples. With the development of portable mass spectrometers as well as rapid and convenient sampling methods, on-site detection of SARS-CoV-2 may receive more attention in the future. To conclude, enormous endeavors have been made for developing methods for rapid detection of SARS-CoV-2 by MS, and they have been successfully applied in analysis of real-life clinical samples. With the advantages of high speed, sensitivity and specificity, MS could be potentially developed into an excellent method complementary to PCR for diagnosis of COVID-19 and significantly contribute to the combat against the COVID-19 pandemic.

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.

Data availability

No data was used for the research described in the article.

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