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COMMENT

SARS-CoV-2 peptides/epitopes for specific and sensitive diagnosis

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The COVID-19 pandemic, caused by SARS-CoV-2, has presented an unprecedented challenge to global public health. The rapid, reliable, and affordable diagnosis of COVID-19 is essential for controlling the spread of the acute disease [1]. The nucleic acid test (NAT) is the gold standard for diagnosing COVID-19 due to its high sensitivity and accuracy [1]. Although NAT yields clinically actionable information, false-negative results often occur, especially when sample collection is not performed appropriately [2]. To complement NAT, serological testing is essential for the rapid detection and monitoring of mild or asymptomatic infections [1]. Serological tests provide valuable information for improving diagnosis, including the estimation of population exposure, disease severity, and clinical outcomes [1].

The selection of antigens/antibodies is the key to serological testing. Numerous serological studies have shown that the nucleoprotein (N) or spike (S) proteins, or parts thereof, are the preferred targets for diagnosing COVID-19 in infected or convalescent individuals [1]. However, the N or S proteins may cause false-positive results due to the cross-reactivity of antibodies generated through previous infection by other human coronaviruses [1]. In addition, the production of these protein antigens usually has relatively strict requirements to ensure quality and consistency, resulting in relatively high cost. The use of virus-specific peptides/epitopes for diagnosis may avoid most of the aforementioned issues. Compared to full-length protein antigen or antigen domain-based detection, there are several advantages of peptide/epitope-based detection. For example, peptide/epitope based detection methods have higher stability and lower cost, and they can be scaled up easily by chemical synthesis in a very short time [3].

The identification of virus-specific peptides/epitopes is the first step and the key for peptide-based diagnosis [4]. To date, there are a handful of reports on the diagnostic value of specific peptides/epitopes of SARS-CoV-2 by using different technologies [1, 5, 6]. The peptide microarray is the most commonly used platform for epitope analysis [1, 7]. By taking advantage of a peptide microarray with full spike protein coverage, Li et al. analyzed 2434 serum samples from COVID-19 patients, asymptomatic carriers and healthy individuals [1]. They identified eight peptides with high potential for diagnosing COVID-19, especially peptide S2–78 (aa 1148–1159 of S protein), which exhibited both high sensitivity (95.5%) and high specificity (96.7%), comparable to the diagnostic performance of the S1 protein when testing COVID-19 patients and individuals with asymptomatic infection. In addition, a panel of four selected peptides, S1–93 (aa 553–564), S1–97 (aa 577–588), S1–101 (aa 601–612) and S1–105 (aa 625–636), were combined to achieve the capability to avoid potential cross-reactivity with sera from patients infected by other coronaviruses. Musicò et al. utilized a high-density peptide microarray that displayed the entire SARS-CoV-2 proteome and identified an epitope of the N protein (aa 155–171) with good diagnostic performance for distinguishing COVID-19-positive individuals from healthy people [7]. By using this peptide, the study achieved 92% sensitivity and 100% specificity for IgG detection in COVID-19 samples, without any observed cross-reactivity to common human coronaviruses (HCoV-OC43, HKU1, NL63, and 229E). Additionally, IgM immunoreactivity demonstrated effectiveness in detecting COVID-19-positive samples collected within the first month after symptom onset.

Phage display-based strategies, specifically VirScan, are another robust tool for epitope analysis that employs a combination of parallel DNA synthesis and bacteriophage display to generate a standardized, synthetic representation of peptide epitopes [8]. Through deep serological profiling of 232 COVID-19 patients and 190 pre-COVID-19 controls using VirScan, Shrock et al. identified over 800 epitopes present in the SARS-CoV-2 proteome and developed a machine learning model to analyze the VirScan data. The final model could predict SARS-CoV-2 exposure history with 99% sensitivity and 98% specificity [5]. Zamecnik et al. utilized a focused SARS-CoV-2 T7 phage library containing 534 overlapping 38-aa peptides and immunoprecipitated it against COVID-19 patient sera to create a SARS-CoV-2-specific peptide microarray, namely, ReScan [9]. Subsequent testing revealed nine potential peptides for SARS-CoV-2 serological assays, with eight originating from the S and N proteins.

Apart from peptide microarray and VirScan, conventional approaches, such as ELISA, have also been utilized for screening virus-specific peptides/epitopes [10]. Amrun et al. identified four immunodominant peptides, namely, S14P5 (aa 553–570), S20P2 (aa 769–786), S21P2 (aa 809–826), and N4P5 (aa 153–170), from a SARS-CoV-2 peptide library containing S, N, envelope (E) and membrane (M) structural proteins by using pooled plasma samples from COVID-19 patients and peptide-based ELISA [10]. Moreover, the magnitude of IgG responses to S14P5, S21P2, and N4P5 was found to be strongly correlated with the severity of COVID-19.

In silico approaches to identify epitopes for detecting COVID-19 have been established [6, 11, 12]. Cai et al. computationally predicted a set of peptides, synthesized 20 of them, and tested them in a peptide-based magnetic chemiluminescence enzyme immunoassay (MCLIA) with serum samples from COVID-19 patients; both IgG and IgM were tested [11]. In particular, they

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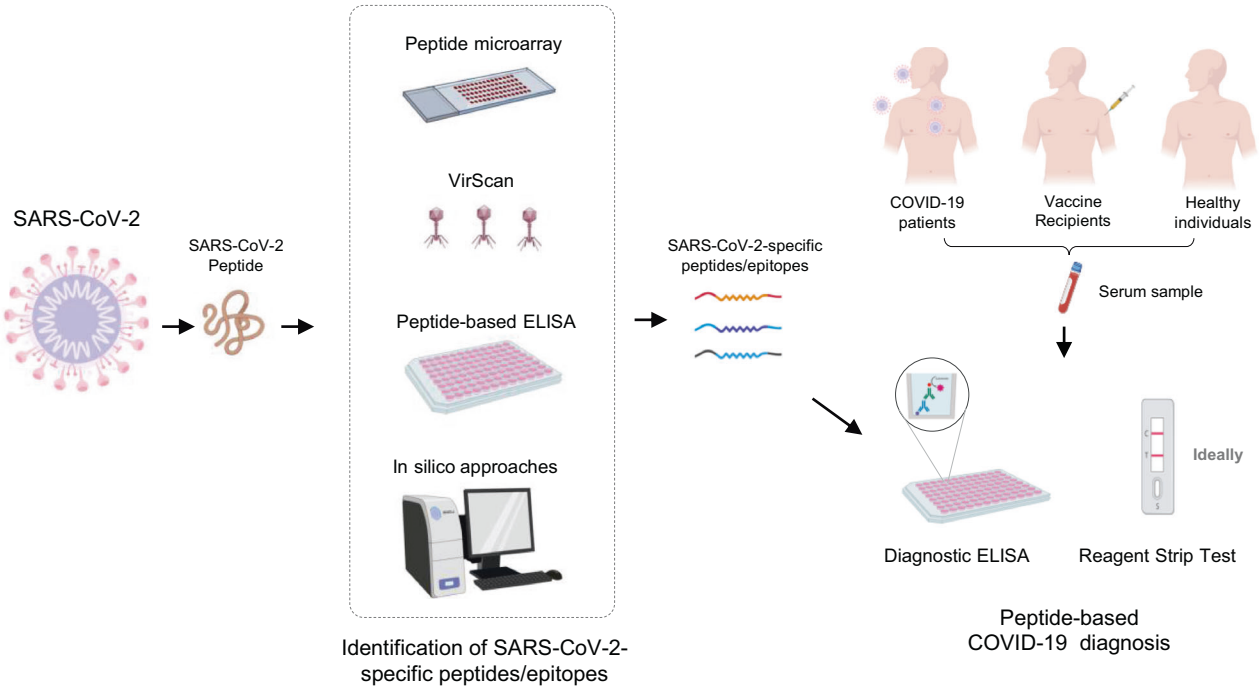

Fig. 1 SARS-CoV-2-specific peptides/epitopes for COVID-19 diagnosis

found that a peptide derived from the S1 protein possessed the best performance, and the positive detection rates for IgG and IgM were 71.4% and 57.2%, respectively. Vengesai et al. reported a general approach for profiling linear B-cell epitopes derived from SARS-CoV-2 using an in silico method and a peptide microarray [12]. They predicted immunogenic peptides that mimic linear B-cell epitopes using ABCpred and chose peptides with low sequence homology to human proteins and proteins from other human pathogens. A peptide microarray immunoassay showed that peptide QSM17284.1-76-89 (aa 76–89, derived from N protein), which has an acceptable diagnostic performance, was able to detect IgM against SARS-CoV-2 with an area under the curve (AUC) of 0.781. Lorenzo et al. utilized a comprehensive approach that combined immunoinformatics with Pepscan and identified 33 potential 16-mer antigenic peptides that were suitable for the diagnosis of SARS-CoV-2 [6]. The peptides located in the C-terminal region of the N protein exhibited the strongest reactions to IgA, IgM, and IgG. The observed differential reactivity among the different immunoglobulin isotypes within different regions of the S and N proteins, when combined, presented an advantageous opportunity for accurately diagnosing all infected patients.

Despite the advantages of epitopes [1], they also have limitations. For example, peptides/epitopes have much lower mass and surface accessibility than full-length proteins [3]. This makes it challenging to achieve performance as high as that of traditional ELISA or flow cytometry assays, resulting in limited applications of peptides in clinical practice. Therefore, a carrier or medium that can effectively carry peptides is crucial for improving diagnostic performance [13]. Zheng et al. developed a highly sensitive biosensor system utilizing CdSe-ZnS quantum dots (QDs) coupled with B-cell epitopes of SARS-CoV-2 for detection [14]. The biosensor system was able to identify the antibodies with a detection limit of 100 pM and showed more effective energy transfer between QDs and peptides than that of the corresponding proteins. Compared to traditional ELISA, the B-cell epitope-based QD biosensor exhibited higher sensitivity (92.3–98.1% positive rates in 207 COVID-19 patient sera) while requiring less time (5 min) and labor. Scussel et al. conducted a nanomagnetic peptide-based ELISA that employed superparamagnetic nanoparticles (SPMNPs) conjugated with peptides/epitopes of the S and N proteins for detecting COVID-19 [15]. Among the peptides tested, the p2pS mimotope exhibited optimal performance, achieving excellent sensitivity and specificity. Ou et al. developed an optimized ultrasensitive assay called UIM-COVID-19, which combined the single-molecule array platform (Simoa), receptor binding domain (RBD), and peptide S2-78 [1, 3]. The UIM-COVID-19 assay showed excellent differentiation ability between COVID-19 patients (convalescents) and healthy individuals or patients with other diseases, with AUC values ranging from 0.85 to 0.95. More importantly, due to the extremely high sensitivity, the authors were able to monitor the seroconversion time much earlier than with the traditional approaches.

In summary, a variety of studies, including studies from our group, suggest that peptides could play an important role in fighting against the COVID-19 pandemic, especially for diagnostics (Fig. 1). Further investigations are still needed to explore the full potential of peptide-based tests for COVID-19 diagnosis in clinical practice, especially for point-of-care tests.

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AUTHOR CONTRIBUTIONS

Conceptualization: SCT. Investigation: HWJ and YL. Writing: SCT and HWJ.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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