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Review Article

Epitope-Based Peptide Vaccine Candidates Against COVID-19 by Immunoinformatics Approach: Review

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ABSTRACT

Vaccination as defined by the WHO is "the administration of agent-specific, but safe, antigenic components that in vaccinated individuals can induce protective immunity against the corresponding infectious agent". Regardless of their debated history, the standard vaccine approaches have been unsuccessful in providing vaccines for numerous infectious organisms. In the recent three decades, an enormous amount of immunological data was retrieved from clinical studies due to the advancement in human genome sequencing. These data are being deposited in databases and numerous scientific literatures. The development of several bioinformatics tools to analyze this rapidly increasing immunological databank has given rise to the field of immunoinformatics. This approach allows the selection of immunogenic residues from the pathogen genomes. The ideal residues could be industrialized as vaccine candidates to provide protective immune responses in the hosts. This methodology will significantly decrease the time and cost needed for vaccine development. This review focus on published articles that proposed vaccine candidates through immunoinformatics analysis. The reviewed six Published immunoinformatics studies, provided vaccine peptide candidates against SARS-COV-2, which is based on functional and non-functional immunogenic proteins like open reading frame, spike protein, envelope protein and membranous protein.All of which are designed by a unique strategy called reverse vaccinology. Spike protein was the most common used target with different suggested B and T cell peptides due to the difference in methodology between the papers.

Keywords: COVID-19 Vaccine candidates; Peptides; Immunoinformatics.

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1 Introduction

In December 2019, a cluster of pneumonia cases, caused by a newly identified β-coronavirus, occurred in Wuhan, China. However, there is no evidence so far that the origin of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was from the seafood market. Rather, bats are the natural reservoir of a wide variety of CoVs, including SARS-CoV-like and MERS-CoV-like viruses [1-4]. The world health organization (WHO) officially named the disease as coronavirus disease 2019 (COVID-19). COVID-19 has spread throughout China and merged almost all worldwide. On 30 January 2020, WHO officially declared the COVID-19 epidemic as a public health emergency of international concern [5, 6]. On 27/6/2020 the WHO reported a terrifying number of infections reaching 9 653 048 cases worldwide with 491 128 deaths.[7]

Identifying a "patient zero" is critical because it can help to curb further transmission; but in some infectious diseases such as COVID-19 the patients do not show signs of being sick quickly enough to make their condition known before they may have passed it on [8].

Interestingly, it was found that at least two different strains of SARS-CoV-2 had occurred a few months earlier before COVID-19 was officially reported [9]. A recently phyloepidemiologic analysis suggests that SARS-CoV-2 at the Huanan Seafood Market could have been imported from other places[10]. After sequencing and phylogenetic analysis, SARS-CoV-2 was considered as a member of β-CoVs [11, 12]. The CoVs family is a class of enveloped, positive-sense single-stranded RNA viruses having an extensive range of natural roots. These viruses can cause respiratory, enteric, hepatic, and neurologic diseases [13, 14]. The CoVs are genotypically and serologically divided into four subfamilies: α, β, γ, and δ-CoVs. Human CoV infections are caused by α- and β-CoVs[13]. Genome-wide phylogenetic analysis indicates that SARS-CoV-2 shares 79.5% and 50% sequence identity to SARS-CoV and MERS-CoV. However, there is 94.6% sequence identity between the seven conserved replicate domains in ORF1ab of SARS-CoV-2 and SARS-CoV[12].

The SARS-CoV-2 is not only causing respiratory problems, it may also damage the heart, kidneys, liver and other organs; in Wuhan 14 to 30% of COVID-19 patients have lost their kidney function and now require either dialysis or kidney transplants. The SARS-CoV-2 gains entry into humans by targeting the ACE2 receptor that found on lung cells, which destroy human lungs through cytokine storms, this leads to hyper-inflammation, forcing the immune cells to destroy healthy cells. This is why some COVID-19 patients need intensive care. The inflammatory chemicals released during COVID-19 infection cause the liver to produce proteins that defend the body from infections. However, these proteins can cause blood clotting, which can clog blood vessels in the heart and other organs; as a result, the organs are deprived of oxygen and nutrients which could ultimately lead to multi-organ failure and subsequent progression to acute lung injury, acute respiratory distress syndrome and often death. Although there are many vaccine candidates for COVID-19 [15-18], no effective treatment or vaccine has been approved so far [19-21].

It’s well known that most acute viral infections result in the development of protective immunity [22, 23] and as a consequence, some of the T-cells will provide immunological memory and protective immunity against these viruses, as well as for SARS-CoV-2 infection[24]. Nevertheless, the available data for human coronaviruses suggest the possibility that substantive adaptive immune

responses can fail to occur [25-27] and robust protective immunity can fail to develop[28]. A failure to develop protective immunity could occur due to a T cell and/orantibody response of deficient durability, with the neutralizing antibody response being dependent on the CD4+ T cell response [27, 29].

During these times of COVID-19 pandemic, understanding adaptive immunity to SARS-CoV-2 is vital for vaccine design. Using HLA-I and HLA-II predicted peptide, circulating SARS-CoV-2 specific CD8+ and CD4+ T-cells were identified in 70% and 100% of COVID-19 convalescent patients, respectively[30].

In the last two decades, vaccine design researches have been made easier, precise and rapid by applying immunoinformatics. This requires the knowledge of disease pathogenesis, the immune system dynamics, database search, sequence comparison, structural modeling as well as motif analysis [31-33]. By using this advanced bioinformatics tools and databases, various peptide-based vaccines could be designed where the peptides act as ligands [62-64]. This approach has been used frequently in Zika virus [34], Dengue virus[35], HIV [36]SARS-CoV-2[15], and so many more [37-41] proposing promising peptides for designing vaccines.

The SARS-CoV-2 genome size is about 30kb which encodes structural and non-structural proteins like other coronavirus strains; structural proteins include S protein (Spike), E protein (Envelope), M protein (Membrane), and N protein (Nucleocapsid)[42]**(Figure 1)**.In this work,six published immunoinformatics studies [15, 43-47] that provided vaccine candidates against SARS-COV-2 by reverse vaccinology are reviewed.

2 The main findings of the published studies

Accelerate researches for vaccine design is critical, immunoinformatics and docking analysis studies have minimized a lot of time, efforts and cost to facilitates the procedure of vaccine design by identifying the binding site on a protein structure and modeling the 3D structure of a complex from the free monomers[48, 49]. Other advantages are that experiments can be directed towards those residues and the functional effect of interaction may be studied. Furthermore, molecular docking analysis may be focused on a specific interface patch[50]; and that is what in common in these studies[15, 43-47]by proposing epitope-based peptide vaccine candidates against SARS-COV-2 by immunoinformatics approach:

Three papers curated by (Peele et al. , Bhattacharya et al, and focused heavily on Spike proteins S. in these papers, the COVID-19 S glycoprotein was considered as a good target for vaccine design because it forms a characteristic crown of the virus and protrudes from the viral envelope, which is why it is easily recognized by the immune system[51]. The protein sequence of spike glycoprotein was explored thoroughly using multiple immunoinformatics-based tools, to identify various epitopes for effective vaccine. These papers used IEDB database [52] to identify linear B-cell epitopes particularly by using BepiPred 2.0 prediction module [53]; while T-cell epitopes identification where conducted by multiple softwares such as, ProPred [54] and ProPred-1[55] for the selection of MHC-I and MHC-II binding epitopes respectively. within previously identified B-cell epitopes, The spike glycoprotein was analyzed for B-cell epitope identification in the IEDB database; they found 34linear B-cell epitopes with 29 epitopes against MHC-I and 8 epitopes

against MHC-II that can be possibly used for vaccine. Then they submitted the selected epitopes to VaxiJen2.0 server applying avirus as a target for evaluating the antigenic tendency [56]. Unfortunately, antigenic characterization in VaxiJen v.2.0 discarded 16 MHC-I epitopes out of 29 and 5 out of 8 MHC-II epitopes as these seemed to be non-antigenic in nature.Their results pinpoint 13 MHC-I (SQCVNLTTR, GVYYHKNNK, GKQGNFKNL, GIYQTSNFR, VSPTKLNDL, KIADYNYKL, KVGGNYNYL, EGFNCYFPL, GPKKSTNLV, SPRRARSVA, LGAENSVAY, FKNHTSPDV and DEDDSEPVL) and 3 MHC-II epitopes (IHVSGTNGT, VYYHKNNKS and FKNHTSPDV) within the spike glycoprotein of SARS-COV-2, and then they converted the antigenic epitopes into a single vaccine component, using(EAAAK)3 peptide linker. Finally, they run molecular docking between vaccine component and TLR-5 showed significant Atomic Contact Energy(ACE) value, which indicates spontaneous reactivity within the receptor-ligand complex.These epitopes are the ideal candidate to formulate a multi-epitope peptide vaccine; not only because of being selected from the linear B-cell epitopic region but also because of their antigenic properties was confirmed.

One of the advantages of this study, they used the PatchDock server[57], which generates docking complex with negative ACE value for analysis. The ACE value of the docking complex was -259.62, which indicates spontaneous reactivity between the vaccine component and TLR-5[58]. As proper protein-protein docking regulates the cellular functions, the docking between the vaccine component and TLR-5 will activate immune cascades for destroying the viral antigens[59]. Furthermore, they converted the antigenic epitopes into a single vaccine component, using (EAAAK) peptide linker. On the other hand, the limitation of this study, there is no population coverage analysis which that will draw attention due to the highly ongoing SARS-COV-2 mutations worldwide; but the key question,is these mutations have any actual functional effect on the pathogenicity of the virus. This is vital in our understanding of the infectious mechanisms and dictates the approach of vaccine development [60].To summarize, this immunoinformatics study predicted 13 MHC-I and 3 MHC-II epitopes within the spike glycoprotein of SARS-COV-2, which may be good candidate for a multi-epitope peptide vaccine against SARS-COV-2.

In the study done by Abdelmageed et al,[15] several techniques facilitating the combination of the immunoinformatics approach and comparative genomic approach were used in order to determine the potential peptides for designing T-cell epitope-based peptide vaccine using the envelope protein of COVID-19 as a target. The COVID-19 E protein is a small, integral membrane protein with critical functions such as pathogenesis, envelope formation, assembly and budding; alongside its interactions with other COVID-19 proteins and host cell proteins (release of infectious particles after budding) [61, 62]. It's well-known that the immune response of T-cell has the ability to a long-lasting memory response that B-cell, where the antigen can escape the antibody memory response[63]. based on this observation, and since SARS-CoV-2 specific T cells were found in patients recovered from these diseases,[64] a T epitope-based peptide vaccine will be more effective against SARS-CoV-2.The whole genome of COVID-19 was analyzed by comparative genomic approach to determine the potential antigenic target [65]. Artemis Comparative Tool (ACT) [66] was used to analyze human Coronavirus (HCov-HKU1) reference sequence vs. Wuhan-Hu-1 COVID-19.The result revealed extensive mutation among the tested genomes. A high rate of mutations in a terminal branch was observed, which are conformed in a recent study by European Bioinformatics Institute [67]; despite the high similarity between the genomes of the two strains.

The four proteins were then analyzed by Vaxigen software to test the probability of antigenic proteins. Protein E was found to be the most antigenic gene with the highest probability.The binding affinity to MHC molecules was then evaluated. The protein reference sequence was submitted to IEDB MHC predication tool; a number of MHC class I and II related peptides were found promising candidates. Among which the peptides YVYSRVKNL, SLVKPSFYV and LAILTALRL show high potentiality for vaccine design with adequate world population coverage. T cell epitope-based peptide vaccine was designed for COVID-19 using envelope protein as an immunogenic target.

It is well known that peptides recognized with a high number of HLA molecules are inducing immune response. Based on the above-mentioned results and taking into consideration the high binding affinity to both MHC classes I and II, conservancy and population coverage, three peptides are strongly proposed to formulate a new vaccine against SARS-CoV-2. Among the primarily selected epitopes, the obtained results showed very strong potential in the proposing epitopesYVYSRVKNL, SLVKPSFYV and LAILTALRL as a vaccine candidate due to its overall epitope conservancy, binding affinities and population coverage for the highest number of HLA molecules.

Sarkar et al.[44]conducted an immunoinformatics study to develop potential vaccines against the SARS-CoV-2, by reverse vaccinology [68] and immunoinformatics approach. Four protein sequences, Nucleocapsid Phosphoprotein (N), Membrane Glycoprotein (M), ORF3a Protein (ORF3a) and Surface Glycoprotein (S) were selected for the possible vaccine design. Only two proteins (N and S), were recognized as potent antigens (0.709, 0.534 respectively) and used in the next phases ofthe study. They used IEDB to generate epitopes, which revealed a good number of epitopes; however they selected ten epitopes only based on the antigenicity scores with higher binding affinity. The B-cell epitopes were also selected based on their antigenicity and length (more than 10 amino acids). Upon continual computational experimentation, three possible vaccine constructs were candidates; one vaccine construct was selected as the best vaccine based on molecular docking study which is supposed to effectively act against the SARS-CoV-2. Three vaccines were constructed by molecular docking using the selected epitopes which are supposed to be directed to fight against the SARS-CoV-2. To construct the vaccines, different linkers (EAAAK, GGGS, GPGPG and KK linkers) were used at their appropriate positions. PADRE sequence is an important sequence which was used in vaccine construction; the vaccine constructs with the best result in the molecular docking, was considered as the best vaccine construct. According to docking results, it was found that CV-1 was the best constructed vaccine. CV-1 showed the best and lowest scores in the docking as well as in the MM-GBSA study by HawkDock server[69].

In the work done *by Enayatkhani et al.* [16] Three proteins **(**N, ORF3a and M) were chosen and analyzed to predict the potential immunogenic B-cell and T-cell epitopes and then validated using various immunoinformatics servers and softwares. They used the Rankpep server [70] which covers almost all HLA to predicte different epitopes from N, ORF3a and M proteins sequence according to HLA I and HLA II alleles. Antigenic epitopes with high binding affinity scores were predicted that were selected for further analysis; while for the linear epitope prediction, the BepiPred server was employed. BepiPred analysis revealed several continuous predicted epitopes of N, ORF3aand M proteins. For cross-checking the predicted epitopes, the sequence of all three proteins was also

predicted by Kolaskar & Tongaonkar Antigenicity [71]. The linear B-cell epitopes that are both cellular and humoral immune responses are essential against coronaviruses infection. Finally, epitopes that were shared between B cell and T-cell were selected.

The final vaccine was designed based on the elicitation humoral and cellular immune responses. Due to the low immunogenicity of the epitope, they chose epitope-rich domains to generate a more diverse and robust response [72]. Based on *in silico* analysis, five epitopes-rich domains including highly scored and shared epitopes between T and B-cell epitopes were selected and joined to each other with a three AAA linker. Several evaluations for the candidate structural analysis and molecular docking and MD simulations study were done; which revealed stimulate both cellular and humoral immunity, NOM recombinant protein could be considered as a possible vaccine candidate against SARS-COV-2.

We checked each epitope of all studied proteins among other studies with its corresponding protein and between other proteins; we didn't find any overlapping (not even a partial) between epitopes; this could be due to each study was conducted by unique strategy.

To conclude, our review revealed that, there are six published immunoinformatics papers that proposed the most promising B and T-cells epitopes for vaccine candidates against SARS-CoV-2. SARS-CoV-2 gains entry into humans by targeting the ACE2 receptor that found on lung cells by S protein, which destroys human lungs through cytokine storms, this leads to hyper-inflammation, forcing the immune cells to destroy healthy cells[30]. Therefore, S protein is the main target of most vaccine studies with 31 T-cell epitopes and 21 B-cell epitopes. It's also clear that these studies proposed the most promising B and T-cells epitopes for vaccine candidates against SARS-CoV-2 are coming from S protein studies due to the same above mentioned reason. This result is in accordance with a recent review published on 07.06.2020, which recognized S protein as the most common tested protein in covid-19 vaccine studies.[73] N protein studies are the next with 6 T-cell epitopes and 19 B-cell epitopes; followed by E protein studies with 3 T-cell epitopes; while M with8 B-cell epitopes and ORF3a proteins studies with 1 T-cell epitope and 7 B-cell epitopes, **(Table 1-2**; **Figure 2).** Due to lack of information about SARS-CoV-2, these studies are vital for vaccine development. The most promising peptides have been gathered in this article which acts as a platform for vaccine design against SARS-COV-2 after *in vitro* and *in vivo* validation.

3 Summary of the peptides used in the reviewed papers

In Tilocca et al. work SLVKPSFYV, LTALRLCAY for MHC1 and KPSFYVY, SRVKNLNS for MHC2 were predicted as good candidates among other possible choices.

In Peele K. et al. work, the final vaccine construct was composed of425 amino acids including the 50S ribosomal protein adjuvant. Molecular docking with TLR-3 immune receptor and MD simulations confirmed stability of the binding pose of this vaccine. In the meanwhile, only one peptide (ITLCFTLKR) was found as probable vaccine candidate in the study done by Joshi et al. with 99.8% structural favorability in Ramachandran plot analysis. In addition to suitable range of IC50 and population coverage values.

In the work done by Sarkar et al. the Spike protein peptides SQCVNLTTRTQLPPAYTNSFTRGVY and FTISVTTEI were predicted to have the highest

binding affinity to the B-Cells and MHC I HLA-B1503 allele respectively. Furthermore, The Nucleocapsid peptides KTFPPTEPK and RWYFYYLGTGPEAGL were found to have the highest binding affinity to the MHC I HLA-A0202 allele.

In a similar work done by Vipul Kumar and Manoj Jena.[74] The constructed vaccine from all three SMN polyproteins was found to be a potent antigen with score 0.60, non-allergen with a score of -0.59, have a molecular weight of 38.8 KDa, PI of 9.92, and half-life inside the E.coli >10 hours.

In the Rakib al. study, the two following candidates' peptides (WTAGAAAYY and GAAAYYVGY) were chosen as best candidate for MHC1. While in Rehman et al. and colleagues' study, LFRKSN and SYGFQPT l were found to be highly B-cell antigenic peptides. while CVADYSVLY and FTNVYADSF were found to display an affinity for maximum number of MHC-I alleles, and YRLFRKSNL, VYAWNRKRI displayed affinity for maximum number of MHC-II alleles. The docking analysis revealed strong interactions of the chosen T cell epitopes with MHC-I and MHC-II alleles. As noticed from this work different approaches lead to the choose of different peptides for the same immunogenic targets.

Table 1: The most promising T-cells epitopes for vaccine candidates against COVID-19:

protein's name	T-cells Epitopes	References
S protein	SQCVNLTTR; GVYYHKNNK; GKQGNFKNL; GIYQTSNFR; VSPTKLNDL; KIADYNYKL; KVGGNYNYL; EGFNCYFPL; GPKKSTNLV; SPRRARSVA; LGAENSVAY; FKNHTSPDV; DEDDSEPVL; IHVSGTNGT; VYYHKNNKS; ; SVLNDILSR; GVLTESNKK; RLFRKSNLK; QIAPGQTGK; TSNFRVQPTESI; SNFRVQPTESIV; LLIVNNATNVVI; VVLSFELLHAPATVC; VVVLSFELLHAPATV; RVVVLSFELLHAPAT; GYQPYRVVVLSFELL; PYRVVVLSFELLHAP; QPYRVVVLSFELLHA; YRVVVLSFELLHAPA; TQLNRALTGIAVEQD; QLNRALTGIAVEQDK; WTAGAAAYY; GAAAYYVGY.	[44-46]
E protein	YVYSRVKNL; SLVKPSFYV; LLVTLAILTALRLCA	[15]
N protein	LIRQGTDYKHWP; RLNQLESKMSGK; LNQLESKMSGKG; LDRLNQLESKMSPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDL KFPRGQGVPINTNSSPDDQIGYYRRATRR; IRGGDGK; HWPQIAQFAPSASAFFGMSRIGMEVTPSGTWLTYTGAIKLDDK DPNFKDQVILLNKHIDAYKTFPPTEPKK	[16]
ORF-7A protein	ITLCFTLKR	[43]

Table 2: The most promising B-cells epitopes for vaccine candidates against COVID-19:

protein's name	B-cells Epitopes	References
S protein	LTPGDSSSGWTAG; VRQIAPGQTGKIAD; YQAGSTPCNGV; QTQTNSPRRARSV; ILPDPSKPSKRS; VVLSFELLHAPATVC; VVVLSFELLHAPATV; RVVVLSFELLHAPAT; GYQPYRVVVLSFELL; PYRVVVLSFELLHAP; QPYRVVVLSFELLHA; YRVVVLSFELLHAPA; TQLNRALTGIAVEQD; QLNRALTGIAVEQDK; WTAGAAAYY; GAAAYYVGY.	[44, 45]
M protein	NGTITVEELKKLLEQW; AN; PLLESE; KLGASQRVAGDS; LTWICLLQFA; LYIIKLIFLWLLWPVTLACFVLAAVY; AMACLVGLM; LSYFIASFR.	[16]
N protein	MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGA RSKQRRPQGLPNNTAS; RIRGGDGKMKDL; TGPEAGLPYGANK;GTTLPKGFYAEGSRGGSQASSRSSSRS RNSSRNSTPGSSRGTSPARMAGNGGD;SKMSGKGQQQQGQ TVTKKSAAEASKKPRQKRTATKAYN; KTFPPTEPKKDKKKKADETQALPQRQKKQQ; MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQR RPQGLPNNTAS; QHGKEDLKFPRGQGVPINTNSSPDDQIG; RIRGGDGKMKDL; QFAPS; EVTPSGTWL; KLDDKDPNFK; KTFPPTEPKKDKKKKADETQALPQRQKKQQ; WFTALTQH; GQGVPIN;QIGYYRR; KHWPQIAQFAPSASAFF; YTGAIKL; KDQVILLNKHIDAYKTF.	[16]
ORF3a protein	QGEIKDATPSDF; KIITLKKRWQL; QL; STQLSTDTGV; GWLIVGVALLAVFQS; SKGVHFVCNLLLLFVTVYSHLLLVAAG; YQLYST;	[16]

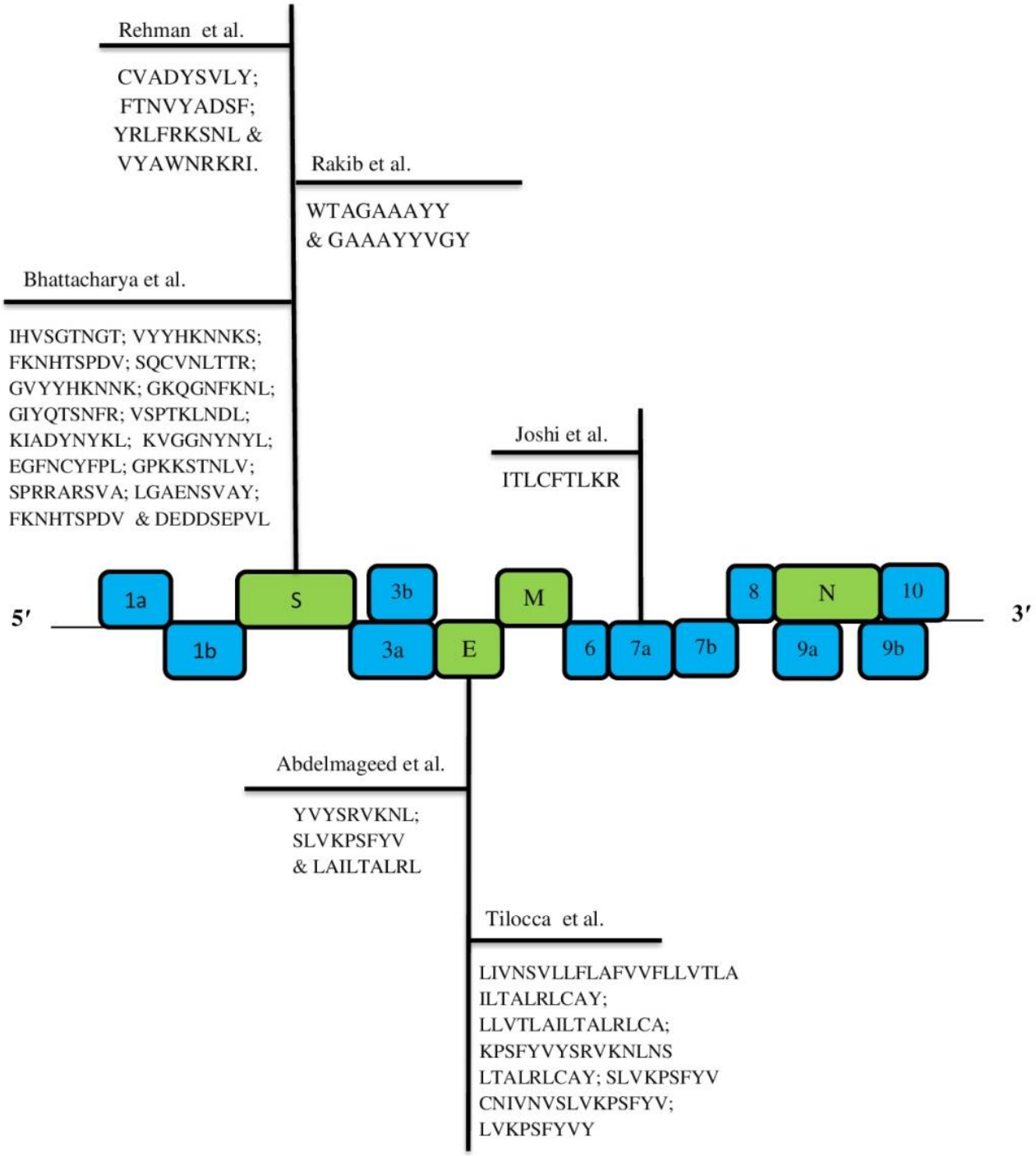

Figure 1:5′ and 3′ terminal sequences of the SARS-CoV-2genome. The gene order is 5′-replicase ORF1ab-spike protein S-envelope (E)-membrane (M)-N-3′. ORF3ab, ORF6, ORF7ab, ORF8, ORF9ab, and ORF10 are located at the predicted positions shown in the picture. 1a, 1b, 3a, 3b, 6, 7a, 7b, 8, 9a, 9b, 10 in the picture represent different ORF genes. It also shows the post promising peptides in each protein that has been studied.

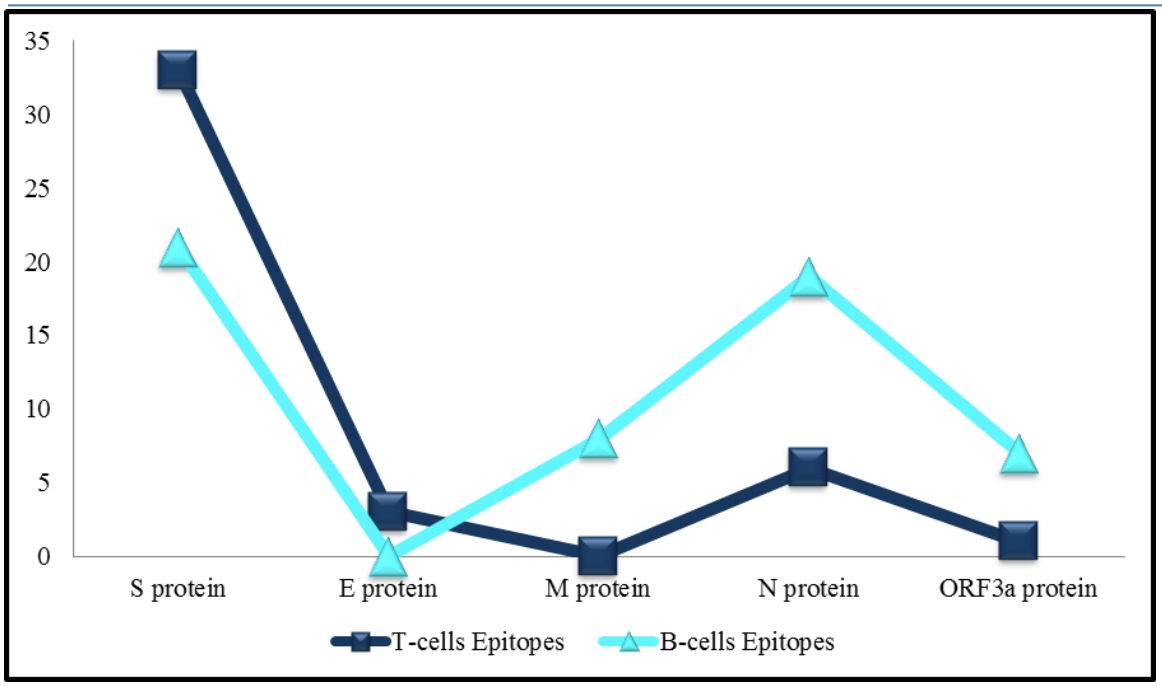

Figure 2: Statistical chart for the most promising B-cell and T-cells from the previous mentioned papers for vaccine candidates against SARS-COV-2

4 Conclusion

Up-to-date several vaccine candidates based on immunoinformatics studies have been suggested. In this review proposed vaccine candidates against SARS-COV-2 were predicted using following the methodology of reverse vaccinology. Different immunogenic targets were used in these papers ranging which is based on structural and nonstructural proteins. Furthermore, no overlap was noticed in the papers concentrating on the most common target (spike protein), probably due to the use of different bioinformatics tools for analysis. Spike protein seems like the best immunogenic target for COVID-19 vaccine design, which can be validated by the current and the future in *vitro* and *invivo* verification.

5 Declarations

5.1 Authors' Contributions:

All authors wrote and revised the paper. Illustrations were done by MIM. All authors read and approved the final manuscript.

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5.4 Competing Interests

The authors declare that they have no competing interests.

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