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Prediction of B lymphocytes epitopes which can lead to the development of a synthetic vaccine for the coronavirus COVID-19 (SARS-CoV-2)

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ABSTRACT

The outbreak of the novel coronavirus in Wuhan in the Hubei province of China (SARS CoV-2) that began in December 2019 presents a significant and urgent threat to global health. As of september 2021, this epidemic had spread to 170 countries with 219000000 confirmed cases, including 4550000 deaths. Coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Until nowadays, vaccination is is the only remedy to protect humans from this SARS CoV-2. In this study, we used an immuno-informatic approach to find B cell epitopes that will help build a universal vaccine. To this end, we first built a consensus sequence for the structural protein Spike (S protein). Parallel bioinformatic predictions identified a priori potential B cell epitopes for SARS-CoV-2. These predictions can facilitate effective vaccine design against this virus of high priority. In this study, The 475-487 epitope is the best it can be for continuous and discontinuous by the 5 predictive methods. Additional studies are necessary to confirm the addability of this epitope for possible use in the development of a SARS CoV-2 vaccine.

1. INTRODUCTION

The virus identified in China in January 2020 is a new coronavirus that causes severe acute respiratory syndrome coronavirus (SARS-CoV) called also COVID-19 (Coronavirus Disease) [1- 3]. It belongs to the family of coronaviruses, a group that has caused serious diseases in the past in humans noteworthy the Severe Acute Respiratory Syndrome (SARS) in 2002-2003 and Middle East Respiratory Syndrome (MERS) in 2012 which demonstrated the potential for transmission of new Vocs from animals to humans and from humans to humans [4]. With more than 170 000 cumulative cases observed worldwide as of march 15, with a lethality rate approximately of 3,7%, the World Health Organization (WHO) classified, on 11 March 2020, the outbreak as a pandemic [4]. The rapid emergence of the (SARS CoV 2) remind us that

CoVs are a severe global health threat so development of effective vaccines has become a high priority, this can only be done after mastering the genome of these new strains. Different ways to reduce transmission have been proposed as preventive measures to be implemented such as masks, hand hygiene practices, avoidance of public contact, case detection, contact tracing, and quarantines. To date, no specific antiviral treatment has proven effectiveness [5- 7] hence, only symptomatic treatment and supportive care are used. Molecular evolutionary analysis based on advanced bioinformatics technologies is a powerful tool to better understand not only the phylogeny of pathogens, but also their antigenicity [8- 12].

SarS-CoV2 is a positive-sense, single-stranded RNA virus. Its genome is about 30 kilobase pairs (kb) in length, consisting of 11 functional open reading frames (ORFs) that, in turn, encodes for all the enzymes required for viral RNA replication [4], [13]. The genome also encodes for the 3 structural proteins, including spike (S), envelope (E), membrane (M) and nucleocapsid (N), which are common to all coronaviruses (figure 1). The structural proteins are involved in various viral processes, including virus particle formation [14], [15]. Among the structural genes which vary in number and location, additional subgroup-specific accessory genes are found interspersed. Recent studies have shown that the proteins encoded by these genes could be modulators of pathogenicity in the natural host [16], [17].

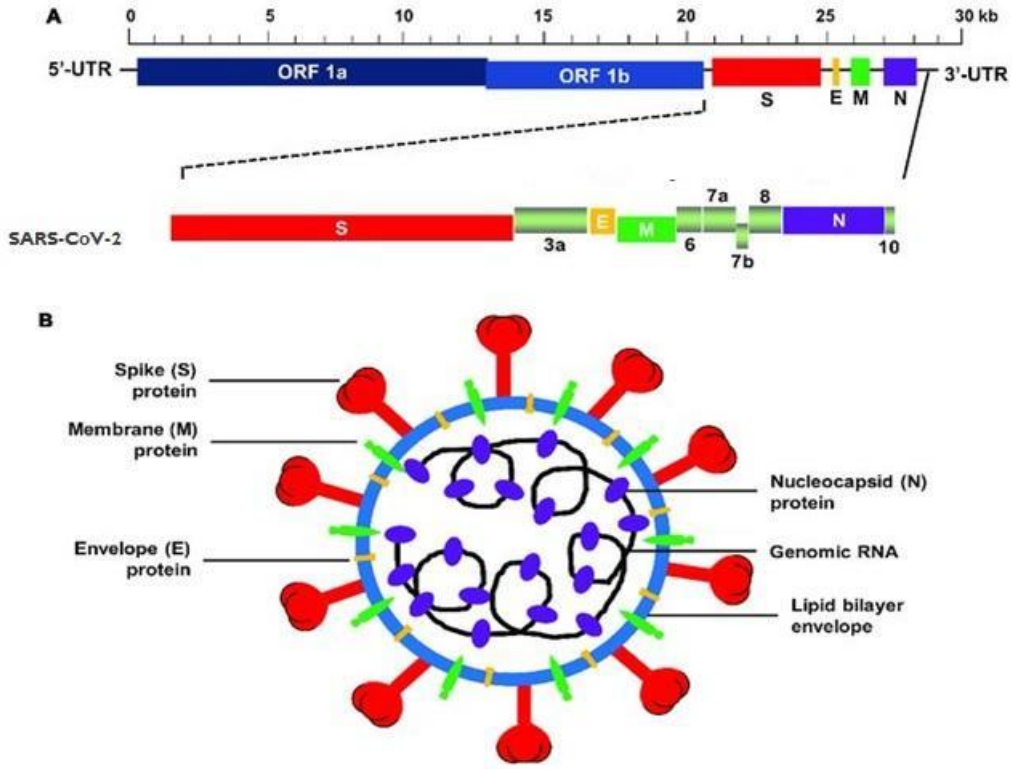

Figure 1: Schematic structure of SARS-CoV-2. (A) Schematic diagram of genomic organization of SARS-CoV-2. Structural proteins, including spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins, as well as non-structural proteins translated from ORF 1a and ORF 1b and accessory proteins, including 3a, 6, 7a, 7b, 8, and 10 are indicated. 5′-UTR and 3′-UTR, untranslated regions at the N- and C-terminal regions, respectively. Kb, kilobase pair. (B) Schematic structure of virion of 2019-nCoV and its major structural proteins [15].

Among the different proteins used by the coronavirus to replicate and invade cells, the spike protein is the major surface protein that it uses to bind to a receptor.

To enter cells, coronaviruses first recognize a host-cell-surface receptor for viral attachment and then fuse viral and host membranes for entry. Receptors play important roles in the membrane fusion process in addition to their roles in determining the viral attachment step [18].

The S protein of CoVs is a type I transmembrane glycoprotein displayed as an oligomer on the surface of the viral membrane. The precursor S protein contains a cleavage site at which the protein could be cleaved into two non-covalently associated subunits: the distal subunit S1 and the membrane-anchored subunit S2 [19]. The S1 subunit contains the cellular receptor-binding domain (RBD) [20], [13] while the S2 subunit contains a putative fusion peptide, transmembrane domain and two heptad repeat regions: the heptad repeat 1 and 2 (HR1 and HR2) (Figure 1) [21].

As other CoVs, the S protein of SARS-CoV2 contains S1 and S2 subunits, with S1 being responsible for receptor binding and S2 for membrane fusion [22]. SARS-CoV2 utilizes human dipeptidyl peptidase 4 (DPP4, also known as CD26) as its cellular receptor [23].

So we can prevent attachment and fusion, we will prevent entry but to target this protein, we need to know what it looks like.

The potential technological benefits of subunit vaccines over conventional whole virus vaccines have led us to think of using the epitopes of protein spike to develop an effective vaccine against this virus. The objective of this study is to determine epitopes for protein S of SARS-CoV-2 using immuno- computer methods by the stable part of gene S are exposed on the surface of the protein S, involved in the attachment phase and if they are capable of generating an immune reaction for B lymphocyte and T lymphocyte.

2. Methodology

In this work, we used modeling tools in order to study and determine the potential epitopes of S protein that can be used for the purpose of vaccine development against SarsCoV2.

2.1 Data set

In order to predict the epitopes of S protein, we have used the amino-acid sequence available in NCBI under accession [24] (QHD43416.1 and QII57161.1) and the crystal structure of protein number 6VSB in Protein data Bank [25].

2.2 Prediction of B epitopes

At the best of our knowledge, there are no studies at the moment on determination of b epitopes of SARS COV2, we have used the bioinformatics and immune-informatics tools in order to predict the potential epitopes in S protein.

We have used 3 different computational methods: HMM (Hidden Markov Model), ANN (artificial neural network) [26], and deep learning methods [27] through the use of server package epitope databases (IEDB) [28] by setting a threshold above 1.0, 1,2 and 0,5. The 3 methods were used in order to predict continuous and discontinuous B epitopes in S protein.

The results of the predictions by the 3 methods were filtered and checked. Only the common predicted epitope between the 3 methods were chosen in order to improve the predictive probability. We also used HMM and ANN to predict the discontinued epitopes and we used a filtration between the methods in order to keep only the best and common regions.

3. Results and Discussion

3.1 Prediction continues epitopes of S protein for B Cell

Prediction of the B cell epitope gave rise to 13 peptide sequences of S proteins: two predicted epitopes are located in two regions of active sites in the area of the protein analyzed (Tables 1 and Figure 2).

There was no formation of clusters of sequences (threshold identity 90%) for the 13 peptide sequences of protein S, which indicates that all the sequences obtained are different from each other. There was also no exact similarity (exact matches - 100%) when comparing the peptide sequences. The in silico prediction of immunogenicity using IEDB tools made it possible to pre-select mimotopes from the active site region of protein S as candidates. The preselected mimotopes should promote strong affinities with B cells.

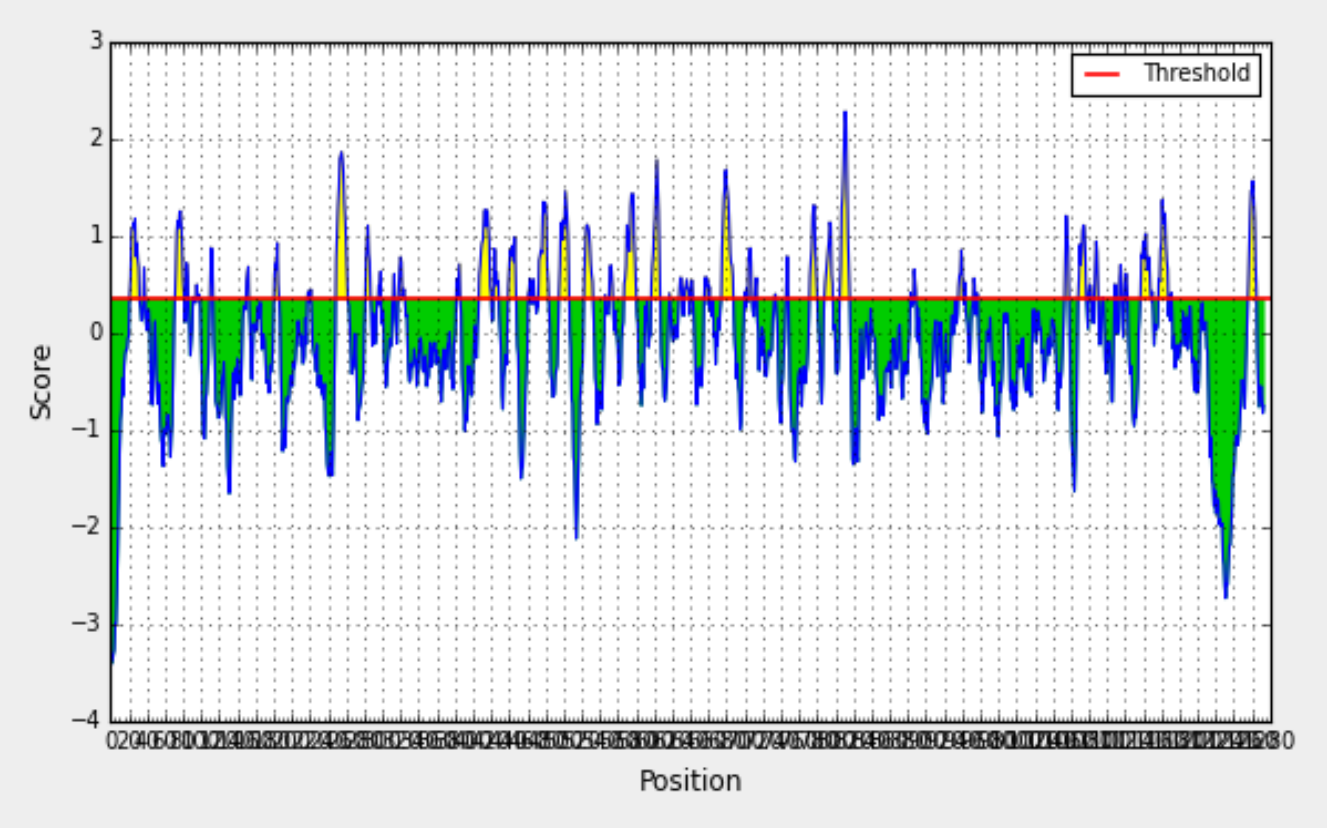

Figure 2a: Bepipred Linear Epitope Prediction Results

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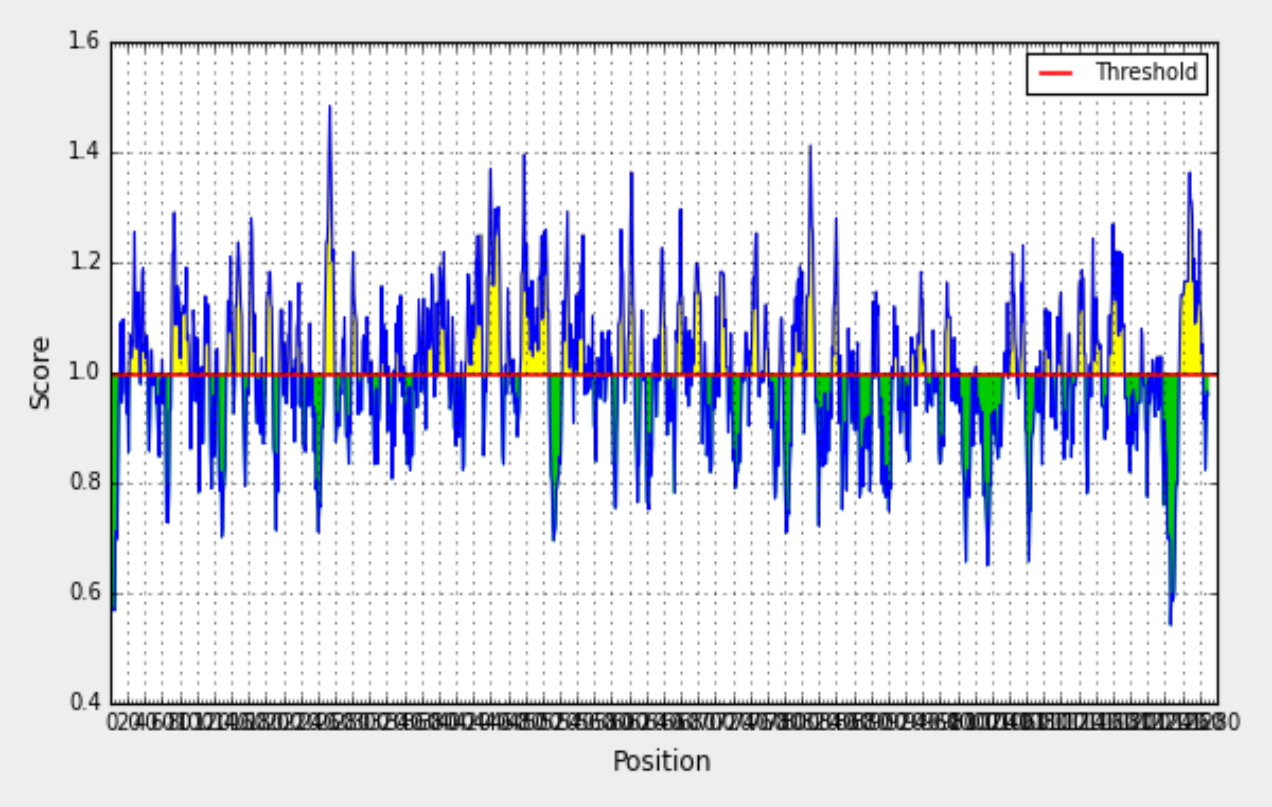

Figure 2b: Chou & Fasman Beta-Turn Prediction Results

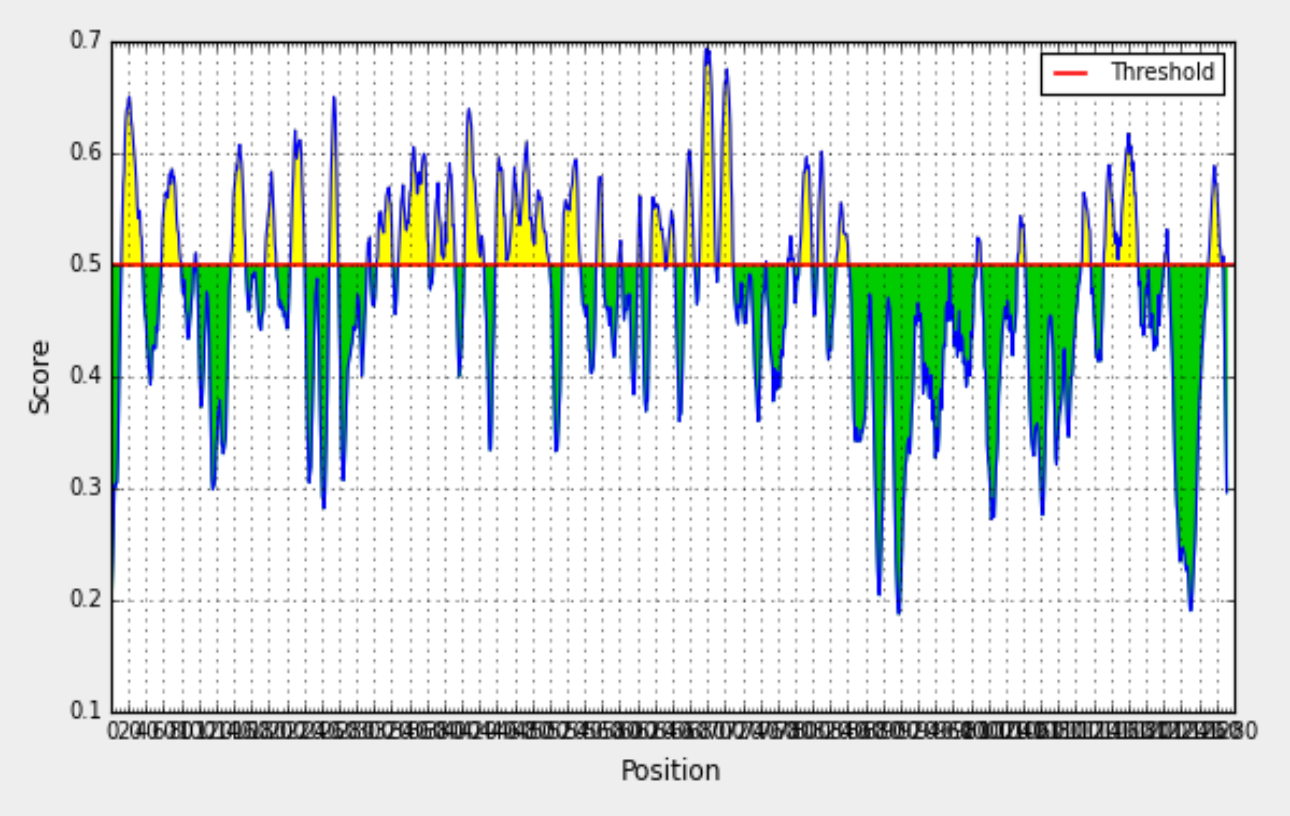

Figure 2c: Continues epitopes of S protein for B Cell.

Figure 2: (a)Bepipred Linear Epitope Prediction Results: The residues with scores above the threshold

(default value is 0.5) are predicted to be part of an epitope and colored in yellow on the graph (where Y-axes depicts residue scores and X-axes residue positions in the sequence) and marked with "E" in the output table. The Ê values of the scores are not affected by the selected threshold. The table below shows the relationship between selected thresholds and the sensitivity/specificity of the prediction method. (b) Chou & Fasman Beta-Turn Prediction Results: Chou and Fasman beta-turn prediction of the most antigenic protein, T2ASQ1. Notes: The x -axis and y -axis represent the position and score, respectively. The threshold is 1.001. The regions having beta turns in the protein are shown in yellow color, above the threshold value. (c) Continues epitopes of S protein for B Cell.

To be an epitope, the accessibility of the surface was also taken into consideration. The Emini surface accessibility scale was carried out, with an average score of 1,000, a minimum of 0.065 and a maximum of 6.664. The most surface accessible region was between 420 and 490 amino acid sequences. The graph is shown in Figure 2a.

Experimental evidence has shown that beta-turn and protein flexibility are also essential for the prediction of B cell epitopes. We therefore implemented the Chou-Fasman beta-turn prediction and the flexibility scale of Karplus-Schulz to estimate beta-turn and protein flexibility. The Chou-Fasman result gave an average score of 1.007, a minimum of 0.676, a maximum of 1.406, and the most recognized regions of the results generated were 446 to 467 amino acid sequences. The Karplus-Schulz result gave an average score of 0.993, a minimum of 0.867, and a maximum of 1.144. Figure 2b. Finally, hydrophilicity must be analyzed. Thus, the Parker hydrophilic prediction was therefore used to construct a hydrophilic scale based on the retention times of the peptides during high performance liquid chromatography (HPLC) on a column in reverse phase. The result revealed an average score of 0.952, a minimum of -5.157 and a maximum of 6.100. The most hydrophilic region was 443 to 481 amino acid sequences. The graph is shown in Figure 2c.

Table 1: The best continuous B epitopes predicted

Linear epitope	Start position	End position	peptide	Number of amino acids
1	13	37	SQCVNLTTRTQLPPAYTNSFTRGVY	25
2	59	81	FSNVTWFHAIHVSGTNGTKRFDN	23
3	97	98	KS	2
4	138	154	DPFLGVYYHKNNKSWME	17
5	177	189	MDLEGKQGNFKNL	13
6	206	221	KHTPINLVRDLPQGFS	16
7	250	260	TPGDSSSGWTA	11
8	293	296	LDPL	4
9	304	322	KSFTVEKGIYQTSNFRVQP	19
10	329	363	FPNITNLCPFGEVFNATRFASVYAWNRKRISNCVA	35
11	369	393	YNSASFSTFKCYGVSPTKLNDLCFT	25

12	404	426	GDEVRQIAPGQTGKIADYNYKLP	23
13	440	501	NLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQ AGSTPCNGVEGFNCYFPLQSYGFQPTN	62

3.2 Prediction discontinues epitopes of S protein for B Cell

For continuous and discontinuous B epitopes, linear epitopes were identified by the HMM and ANN methods, which showed that there are a number of linear regions. The position 475-487 was found to be the most immunogenic for SARS CoV2. The position is mainly part of the Receptor-Binding Domain that is involved in the binding between receptor ACE2 and S protein, which meaning the neutralization by antibodies will lead to block the RDB region and then lead to inhibit the internalization into cells. This opens the way to the limitation of the virus to the extracellular matrix, allowing the innate, macrophagic immune system to overcome the virus and destroy it. This region has shown the most important number of predictive epitopes and with higher probability. Other regions present also immunogenic power but they are not common between the 3 methods. It is widely recognized that knowledge about B-cell epitopes is important for the identification or design of therapeutic antibodies, and for gaining insights into efficacy synthetic vaccines [29], [30].

Despite of the fact that window-based techniques – both computational and experimental – have an undeniable appeal for identifying the location of B-cell epitopes, experimental techniques, such as X-ray crystallography and site-directed mutagenesis, are much more time consuming and expensive but of high accuracy.

SARS CoV 2 Spike glycoprotein and ACE2-FC region IgG1 both have a bonding and docking ability [31]. Then in this study we tried to determine the epitopes of the B cell contained in the spike protein in order to be able to use them to facilitate the development of a synthetic vaccine, especially since no cross-recognition of SARS-CoV monoclonal antibodies was observed during the study by [15]. Indeed, they observed no reactivity with SARS-CoV antibodies that recognize the SARS-CoV-2 spike receptor binding domain (RBD), despite the fact that SARS-CoV-2 retain the same capability to bind the ACE2 receptor of SARS-CoV [32]. Although the discontinuity of B cell epitopes is widely established, the degree of discontinuity is poorly understood. For example, what is the likelihood that one of the peptides of a single epitope of an antigen will span all the residues belonging to that epitope [32].

For B cell epitopes we used methods that predict linear epitopes (Bepipred Linear Epitope Prediction Results), and in the case of the spike glycoprotein where a reliable structure recently became available [15], the Discotope 2.0 [33] method that also predicts epitopes based on protein conformation and residue exposure. Two of the likely epitope regions defined on the basis of SARS-CoV data were independently confirmed by the Discotope prediction.

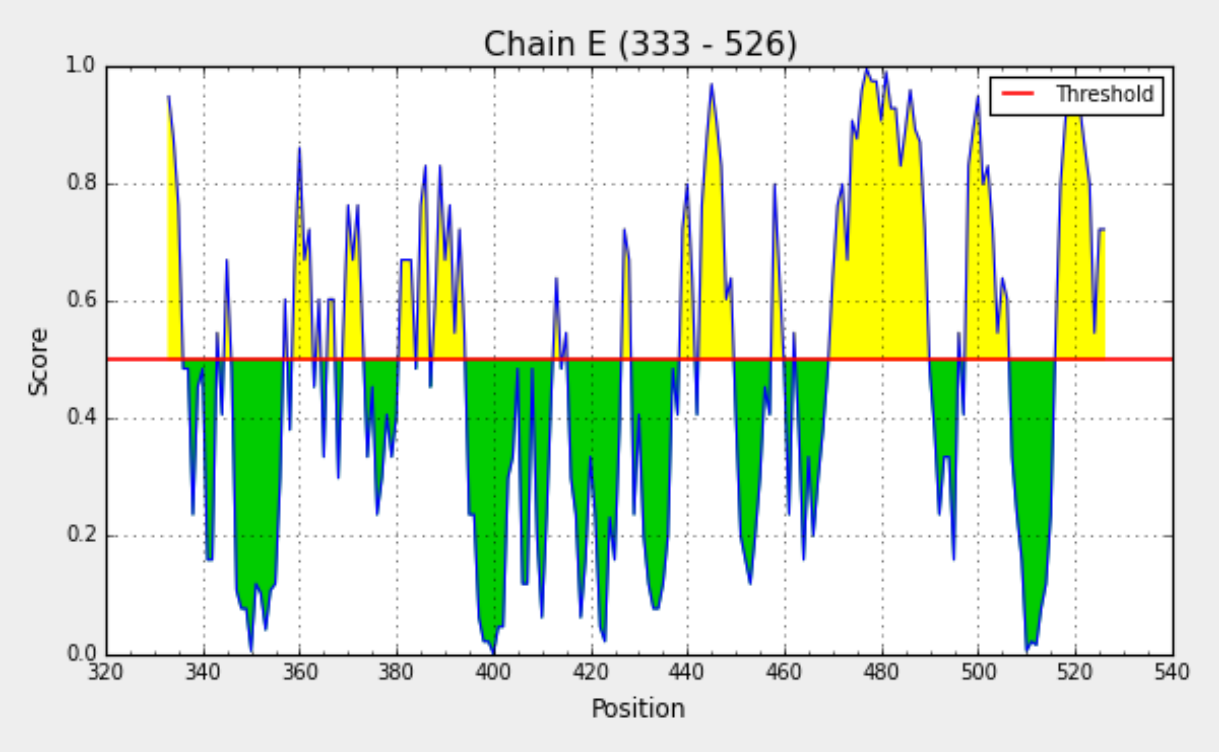

Figure 3: Discontinues epitopes of S protein for B Cell

Table 2: peptide sequences of predicted discontinuous epitopes

Epitopes	Start position	End position	Peptide	Number of amino acids	Score
1	469	490	STEIYQAGSTPCNGVEGFNCYF	22	0.838
2	516	526	ELLHAPATVCG	11	0.794
3	333	336	TNLC	4	0.768
4	439	450	NNLDSKVGGNYN	12	0.713
5	495	506	YGFQPTNGVGYQ	12	0.66
6	380	394	YGVSPTKLNDLCFTN	15	0.642
7	357	374	RISNCVADYSVLYNSASF	18	0.579
8	412	415	PGQT	4	0.531
9	343	346	NATR	4	0.527
10		429	PDDF	4	0.50

Our study shows that the longest continuous epitope is composed of 62 amino acids (Table 1) (NLDSKVGGNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTN), which is located between position 440 and 501 (figure 2) although the best discontinuous epitope is located between

position 469 and 490 (Figure 3) and contains 22 amino acids (Table 2) (STEIYQAGSTPCNGVEGFNCYF). The fact that the discontinuous and linear epitopes are almost superimposed and condensed in this region, offers the possibility for promising investigations for the development of antibodies that derive and aim at the inhibition of this virus. But it also offers therapeutic hope and can explain several things in terms of the human immune response to the virus. We know today, according to WHO, that 80% of people can recover without having medical intervention or taking medication which is very rare for this viral family. of which we know that SARS to kill around 10%, MERS go up to 35% while at the global level we are in 3.6% with a percentage lower than 1% for people lower than 50 years [3].

Our study not only allowed us to predict the immunogenetic regions but also, it could offer information which could be key in the explanation of several phenomena. These conclusions and results remain predictive and require both laboratory-scale confirmations for more knowledge. But until now our results are consistent with what is published at the laboratory level, whose inhibition of the RDB region can prevent the internalization of viruses and offer medical solutions.

4. CONCLUSION

This in silico study represents a major step forward in the development of a vaccine against sars-cov 2 virus that present a major risk to public health. However, the feasibility of such vaccine development must be verified by in vivo studies.

As a better way to use the results of this study, this work can be considred as a basis for in vivo studies.

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