COVID-19: Captures Iron and Generates Reactive Oxygen Species to Damage the Human Immune System

Wenzhong Liu 1,2,* and Hualan Li 2

1 School of Computer Science and Engineering, Sichuan University of Science & Engineering, Zigong, 643002, China;
2 School of Life Science and Food Engineering, Yibin University, Yibin, 644000, China;
* Correspondence: Wenzhong Liu, liuwz@suse.edu.cn.

Abstract

At present, the novel coronavirus pneumonia has been widespread worldwide, and there is no specific medicine. In response to the emergency, we adopted bioinformatics methods to study the virus's pathogenic mechanism and found possible control methods. We speculated in previous studies that E protein was related to viral infectivity. This study adopted the domain search techniques to analyze the E protein. The results showed that the E protein could bind iron or heme. The iron and heme bound by the E protein came from the attacked hemoglobin and phagocytes. When E protein attached to heme, it synthesized oxygen and water into superoxide anions, hydrogen peroxide, and hydroxyl radicals. When the iron-bound E protein and the heme-bound E protein worked together, they converted superoxide anions and hydrogen peroxide into oxygen and water. These were the “ROS attack” and “ROS escape” of the virus. “ROS attack” damaged the tissues or cells exposed on the surface of the virus, and “ROS escape” decomposed the superoxide anion and hydrogen peroxide that attacked the virus. When NK cells exposed to infected cells, viruses that had not shed from the infected cells’ surface damaged them through “ROS attack”. Lymphocytes such as T cells and B cells, which could be close to the antigen of the virus surface, were also easily damaged or killed by the "ROS attack", resulting in a decrease in lymphocytes. When memory B cells exposed to the virus's surface antigen, “ROS attack” also damaged them, resulting in the patient's re-infection. The virus used the “ROS escape” to decompose hydrogen peroxide released by phagocytes into oxygen and water. The surrounding cells were replenished with oxygen, and the patient had a “happy hypoxia” state. When the phagocytes swallowed the virus, the E protein converted superoxide anions into oxygen and water. In this way, the virus parasitized in the vesicles of the phagocyte. While virus in the lysosome, the E protein generated ROS to damage nearby hydrolases. The virus parasitized the lysosome in this way. Excessive hydroxyl free radicals destroyed the membrane structure of the lysosome, causing the lysosome to release hydrolase, phagocytic cells autophagy and died. Therefore, the colonizing phagocytes of the virus was related to asymptomatic infection or retest-positive. In short, the virus inhibited the immune system through “ROS escape”, and damaged the immune system by “ROS attack”. The destruction instigated a strong cytokine storm and led to organ failure and complications. This theory is only used for academic discussion. We hoped this discovery would help prevent severe epidemics and save more lives.
Keywords: Novel Coronavirus; Envelope protein; Asymptomatic infection; Reinfection; lymphopenia; Macrophage; Cytokine Storm.

1. Background

By 2020, the novel coronavirus pneumonia quickly sweeps the world, and there will be no weakening trend. This may be closely related to the mysterious mechanism of the virus infecting the human body. Such as, asymptomatic infections have no symptoms such as dyspnea, lymphocyte counts, and chest CT images are familiar, but qRT-PCR specific for COVID-19 disease. Nucleic acid testing detects asymptomatic infections. The invasion of SARS-CoV-2 in asymptomatic patients only generates a specific mild immune response. The incubation stage is the approximate time from the first exposure to the virus to the onset of clinical symptoms or signs. Patients also develops the virus during this term. The typical asymptomatic transmission of cohabiting family members is as long as three weeks, which may cause severe COVID-19 pneumonia. 15-45% of SARS-CoV-2 infections are asymptomatic. Asymptomatic SARS-CoV-2 cases may increase at the population level and produce major public health problems.

Reinfection is more mysterious than asymptomatic infection. There have been sporadic reports of retest positive and reinfection. Reinfection is not equal to retested positive. After the initial infection, T cell immunity can be found in most recovered patients, like memory CD4+ T cells and memory CD8+T cells. For memory T cell responses, it includes spike proteins and nucleocapsid proteins, and membrane proteins. The interval between the reinfection and the last infection is 4-5 months, and the corresponding SARS-CoV-2 antibody is not detected during the reinfection period. Although the virus strain is different from the previous one, it cannot be ascertained if the spike protein's amino acid mutation causes reinfection. However, high-affinity IgG and high-titer neutralizing antibodies are found in the early stages of reinfection, resulting in a more robust antibody response. Yet, no IgM is found, and the lack of IgM response is compatible with reinfection.

SARS-CoV-2 infection can activate abnormal inflammation and immune response: IL-6, IL-1β, IL-8, IL-17, G-CSF, GM-CSF, IP10, MCP1, MIP1α and TNF, C-reactive protein And D-dimer increased. Very high pro-inflammatory cytokines produce a cytokine storm, causing local or systemic tissue damage—excessive dimer and cellulose levels for extensive capillary coagulation reactions. Inflammation and blood clotting occurs in many organs such as lungs, heart, kidneys, nervous system, bone marrow, and vessels.

The autopsy report indicates that the novel coronavirus pneumonia is a very destructive disease. It detects SARS-CoV-2 particles in the patient’s respiratory system, kidneys, and gastrointestinal tract. Many macrophages and monocytes infiltrate the lungs, and the amount of polykaryocyte cells is still large. There are likewise a few lymphocytes, eosinophils, and neutrophils. The lymphocytes are mostly positive CD4+T cells. And all manifested as diffuse alveolar damage, accompanied by fibrin membrane formation, and fibrin clumps in the alveoli. Hyaline membrane formation, fibrin exudate, epithelial damage, and diffuse-type II lung cell hyperplasia are found in the lungs. Inflammation and fibrin microthrombus appears in the tissues around the heart capillaries, liver sinuses, and renal tubules. Existing massive amounts of
literature show that viruses also damage the human immune system, directly or indirectly causing these severe diseases.

The immune problem of typical diseases is lymphopenia, but lymphocytes will rise during the recovery stage. The lymphopenia by SARS-CoV-2 contributes to being CD4+T cells, CD8+T cells, B cells, and natural killer cells, and the damage of CD8+T cells is more significant. There are many reasons for lymphopenia, such as IL-6, IL-10, or tumor necrosis factor (TNF). Dendritic cells and neutrophils also act indirectly to diminish the number of lymphocytes. Exaggerated activation of T cells or high-level expression of pro-apoptotic molecules may also induce depletion of T cells. However, the autopsy report reveals that the inflammatory cell infiltration comprised a mixture of CD4+T and CD8+T lymphocytes, mainly stands in the interstitial spaces and around the broader bronchioles and blood vessels. The surface of lymphocytes appears no programmed cell death protein-1 (PD-1) and PD-L1 protein. It detects no obvious virus infection in lymphocytes and mesenchymal cells. S protein did not combine with T lymphocytes.

It believes oxidative stress damage to lymphocytes may produce that lymphopenia. The autopsy report further proves that T-cell lymphocytes with a CD4:CD8 proportion of 1.7 infiltrated the interstitial myocardium. Hypertrophy in the myocardial fiber occurs---iron-catalyzed regulatory cell death induced by extreme peroxidation of fatty acids. Myocardial interstitial macrophage infiltration spreads, and there is no clear concerning harm to myocardial cells containing the left and right ventricles. So, myocarditis is lymphocytic inflammation, which collects many positive CD20-B cells, CD3+ T lymphocytes and various CD68+ macrophages without eosinophils, giant cells or granulomas. Besides, fibroblasts/macrophages in the heart are vital for motivating myocardial destruction.

Macrophages play a role in eliminating pathogens and promoting organ repair. However, macrophages are the prominent participants in the so-called cytokine storm and may damage tissues. The amount of megakaryocytes discovered in the lungs and heart is much more than usual. The deceased’s alveolar cavity with the pneumonia is filled with many macrophages scattered with neutrophils and lymphocytes. CD61+ megakaryocytes represent lung megakaryocytes, with obvious nuclear proliferation and atypicality, located in the alveolar capillaries. Neutrophils partially denature and trapped—many neutrophils in small blood vessels. Positive CD68-macrophages show the phenomena of intracytoplasmic phagocytosis, spherical eosinophilic hyaloplasm or hemophagocytic, and multinucleated giant cells. Several chemokines and inflammatory cytokines are secreted by alveolar macrophages, including IL-6, IL-10, and TNFα. Pulmonary inflammatory macrophages show strong interferon characteristics. Although interferon may have a protective effect in the previous stage of the disease, the continuous production of IFNγ may bring to extravagant macrophage activation problem.

The persistence of SARS-CoV-2 in monocytes may be short term. After the monocytes are infected by virus replication differentiate into tissue macrophages, the virus can replicate and produce progeny virions that can infect surrounding cells. Electron microscope observation discloses that there are SARS-CoV-2 particles in the cytoplasm of macrophages. Coronavirus-specific antibodies enhance the uptake of the virus by macrophages through combining with FcR. SARS-CoV-2 may enter alveolar macrophages through the interaction between S protein and ACE2 receptor. ACE2 is also expressed on the surface of lung macrophages. It detects CD68 and CD169 macrophages expressing ACE2 in the spleen’s marginal area and the
marginal sinuses of lymph nodes. These macrophages have the SARS-CoV-2 nucleoprotein antigen and act up-regulation of IL-6. S protein cooperates with monocytes/macrophages --- CD68. Alveolar macrophages widely express programmed death-ligand (PD-L1)29.

These signs show that CD169 macrophages, like a Trojan horse, make virus transmission, excessive inflammation, and activation-induced lymphocyte death36. Phagocytes infected with MERS-CoV can act as virus reservoirs and transport proteins to facilitate virus replication and spread. MERS-CoV replicates inside phagocytes and reduces the host’s innate immunity37. Besides, phagocytes can not kill Mycobacterium tuberculosis38, Brucella39, Leishmania40, and HIV41. For example, Leishmania parasites in phagocytic vacuoles. Therefore, the SARS-CoV-2 virus may also colonize phagocytes. Moreover, pathogens such as silica sand42,43 and Mycobacterium tuberculosis44,45 rupture lysosomes. After the phagocytes swallowed pathogens, lysosomes rupture too, producing the release of hydrolase. Hydrolase promote phagocytes’ inflammation and death. After the phagocytes swallow the debris and Mycobacterium tuberculosis, they are similarly inflamed and die. This vicious circle induces severe inflammation and fibrosis in neighboring tissues. SARS-CoV-2 pneumonia has similar tissue inflammation and fibrosis.

Although COVID-19 patients are also accompanied by nervous system inflammation, the evidence that the SARS-CoV-2 virus infects neurons is insufficient46. Patients with severe neurological diseases such as stroke only have mixed lymphocytes or mononuclear inflammatory infiltration in the meningeal space and cortical tissues. The patients have a neurovascular brain injury or microvascular dysfunction47. Cerebrospinal fluid (CSF) RT-PCR of patients with encephalitis and meningitis is negative for COVID-1948. Polymerase chain reaction (PCR) of cerebrospinal fluid of patients for acute disseminated encephalomyelitis is negative too48. In patients with neuromuscular diseases, cerebrospinal fluid analysis discloses that albumin cytology is dissociated. But the polymerase chain reaction test for COVID-19 is also negative49. Odor and taste disorders are characteristic symptoms of SARS-CoV-2 infection. Contrary to other infectious odor damage, the odor and taste loss of COVID-19 sounds to be characterized by a severe nose blocked; not any changes in the paranasal sinuses, such as ethmoid plate olfactory nerve, and meninges.No signs of acute or chronic ischemic events50. Only cerebrospinal fluid RT-PCR of patients with epilepsy is positive for COVID-1951.

These inflammations was also an oxidative stress injury because SARS-CoV-2 viruses might release reactive oxygen species (ROS). It noticed a strange coincidence that the most consumed T and B lymphocytes could contact the antigen of virus surface. According to immune theory, if the antigen (such as pathogen-specific protein) is on the surface of the pathogen, the pathogen can directly stimulate T cells. It entailed that oxidative stress broke these T and B lymphocytes’ cell membranes structure such as lipids and proteins when exposing to the virus. Lymphocytes rupture or apoptosis. Then the total number of lymphocytes in the body reduces. Sometime, NK cells did not require antigen stimulation to kill infected cells. When NK cells were exposed to the surface of infected cells, nearby unshed virus particles would release ROS, and NK cells were also destroyed by oxidative stress. Therefore, it believed that the virus released ROS to damage the human immune system.

We believed SARS-CoV-2 virus also damaged tissues nearby neurological system through oxidative stress action. If the phagocytes ruptured neared the odor and taste cells, the freed SARS-CoV-2 virus would attack the cells, generating oxidative stress damage to the cells, and odor and taste disorders will appear. If the infected phagocytes ruptured near the meningeal space,
the released virus could damage the cerebral cortex through ROS attack and induced brain inflammation. The oxidative stress damage to the immune and nervous system of patients with epilepsy was more serious. The infected phagocytes would rupture after penetrating the spinal cord tissue. The virus could invade the spinal fluid and then be carried into the cerebrospinal fluid through the cerebrospinal fluid circulation.

It is confusing: The E protein was not included in the report mentioned above on memory T cells' response. The heme theory believes that SARS-CoV-2 is an acidophilic anaerobic virus. SARS-CoV-2 suppresses heme metabolism by attacking hemoglobin and dissociating heme and hunting porphyrins. This attack provides an enormous amount of iron or heme. The E protein of SARS-CoV-2 is associated with highly infectious. E owns a heme iron linked sites and carries iron catalytic properties similar to the cytochrome C oxidase. If these sites had the iron catalytic activity of superoxide dismutase, catalase, peroxidase, it could offer reactive oxygen species such as O·-, H2O2, or 'OH. Peroxidase can produces hydroxyl radicals OH. OH to damage the structure of membranes, proteins, and nucleic acids. It also provoked peroxidation and rupture of lysosomal membranes. In this study, bioinformatics methods were applied to study the catalytic function of virus E protein. We employ the MEME tool to explore conserved domains between E protein and bacterial superoxide dismutase, catalase, peroxidase on a large scale and determine the iron link site of E protein with iron-catalyzed production of ROS. Then, we determined the iron catalytic region of E protein outside the membrane-binding area. The result showed the E protein had sites that captured iron and generated reactive oxygen species to damage the human immune system.

2. Method

2.1. Analysis method flow chart

It indicates the biological information analysis method flow of this research. First, we employed the localized MEME tool to search for several conserved domains of E protein enzymes. We later analyzed that E protein's heme linked site was on the viral membrane's outer surface. At last, it was a comprehensive study that summarizes the various enzymatic functions of E protein to catalyze the generation or decomposition of ROS.

2.2. Localized MEME tool to scan for conserved domains.

The analysis steps are:
1. We downloaded MEME from the official website and installed it in the virtual machine ubuntu operating system. The virtual machine was VM 15.2.
2. Download the E protein sequence of SARS-CoV-2 from NCBI official website.
3. Download the fasta format sequence of superoxide dismutase, catalase, peroxidase from Uniprot official website, subsequently. The search keyword was “bacteria” + enzyme name.
4. For each the sequences in all enzyme (each species), paired with the E protein sequence to generate fasta format files for MEME analysis.
5. For the files generated in 4, a batch of 50000 was used to create several batches. It was
considering the limited space of the virtual ubuntu system.

6. In ubuntu, search the conserved domains of E protein and enzymes (various species) with MEME tools in batches.

7. Collected the result files of conserved domains.

8. Searched and downloaded the 3D space files or domain functions of related enzymes from the NCBI official website and analyse the conserved domains’ role.

2.3. Transmembrane analysis

We directly compared the transmembrane sites of E Protein to the heme linked sites. Then it determined the heme linked sites were outside the membrane structure.

3. Results

3.1. E protein had active of the superoxide dismutase

It describes the superoxide dismutase (SOD) (EC) catalyzing superoxide radicals’ conversion to molecular oxygen in InterPro entry IPR019831. Its function is to destroy free radicals produced in cells. Fe/Mn SODs are ubiquitous enzymes responsible for most SOD activity in prokaryotes, fungi, cyanobacteria, and mitochondria. Fe/MnSOD is a homodimer or homotetramer. Fe/MnSOD is divided into two domains, an αN-terminal domain connected by a loop and an alpha/beta C-terminal domain.

| Domain    | Motif                                  | Count |
|-----------|----------------------------------------|-------|
| 1.Sod_Fe_N| ALRLCAYCC                              | 1     |
|           | CAYCCN                                 | 3     |
|           | CAYCCNI                                | 13    |
|           | CAYCCNIV                               | 1     |
|           | CAYCCNIVN                              | 18    |
|           | CAYCCNIVNVSLVKPSF                      | 1     |
|           | CCNIVN                                 | 1     |
|           | CCNIVNVSLVKP                           | 1     |
|           | ILTALRLCAYCCN                          | 1     |
|           | ILTALRLCAYCCNI                         | 1     |
|           | ILTALRLCAYCCNI                         | 1     |
|           | ILTALRLCAYCCNIVNVSLVKPSFYVY            | 1     |
|           | KPSFYVYSRVKNLN                         | 2     |
|           | LCAYCC                                 | 1     |
|           | LRLCAYCCNIVNV                         | 1     |
|           | MYSFVSEEFTLIVNSVLLFLAFVVFLLV          | 1     |
|           | TALRLCAYCC                             | 1     |
|           | TAILTALRLCAYCC                         | 2     |
|           | TAILTALRLCAYCCN                        | 5     |
We downloaded 184306 SOD enzyme sequences of various bacteria from the UniProt website. We adopted the local MEME tool to search for motif between each sequence and the E protein (E-VALUE<0.05). Then explored the conserved domain name corresponding to the
motif on the SOD sequence from the UniProt website through the web crawler method. At last, we searched for conserved domains containing “Fe”. Table 1 lists the conserved domains related to the SOD enzyme properties of E protein. “CAYCC” was the heme-binding motif found in heme theory. Table 1 shows that E protein has the N-terminal and C-terminal conserved domains of Fe/Mn-SOD. “CAYCC” and its left is Sod_Fe_N, “CAYCC” and it’s right is Sod_Fe_C. “CAYCC” also was the conservation motif of Ferritin, Ferric oxidoreductase, and Ferredoxin (2Fe-2S ferredoxin-type, 4Fe-4S ferredoxin-type). So, E protein had the catalytic regions of SOD enzyme at 1-69, including the transmembrane area and the outer part of the membrane: “MYSFVSEETGTLIVNSVLLFLAFVFLVTLAILTALRLCAYCCNIVNVSLVKPSFYVYYSRV KNLNSSR”.

We used the UniProt online ID mapping service to convert the PKB name into the ref-protein ID. We got five ref-proteins “F8FQ25 -> WP_013914333.1; H6NSN4 -> WP_013914333.1; A0A0K2HBM7 -> WP_012820698.1; A0A2A5K0Y4 -> WP_023485148.1; A0A2Z6BF72 -> WP_008879697.1”. For WP_013914333.1 and WP_023485148.1, we could not open the linked webpage of “Related Structures (Summary)” on the NCBI, and “Master data error” was prompted. The “Related Structures (Summary)” link of WP_012820698.1 and WP_008879697.1 corresponded to the file “2NYBA.cn3”. These two proteins’ motifs were “IEHWWNVVN”, comparable to the “CAYCCNIVN” of E protein. But neither of them was in the iron linked site in “2NYBA.cn3”. Because of the lack of structure files, it could not be determined that the last C of “CAYCCN” was the iron linked site of SOD enzyme.

The above analysis results revealed that the E protein had the catalytic role of SOD enzyme after binding iron. E protein catalyzed superoxide anion and water to generate oxygen and hydrogen peroxide.

3.2. E protein had active of the catalase

Hydrogen peroxide is a product of cell oxidative metabolism. It can be converted into hydroxyl free radicals through transition metals, which can damage various molecules in cells, leading to oxidative stress and cell death. Catalase (EC) is an antioxidant enzyme that catalyzes hydrogen peroxide conversion into the water and molecular oxygen. Most catalase enzymes are monofunctional heme-containing enzymes.

| Domain | Motif          | Count |
|--------|----------------|-------|
| 1. Catalase | AILTALRLCAYCC  | 1     |
|         | AILTALRLCAYCCNI | 1     |
|         | ALRLCAYCC       | 28    |
|         | ALRLCAYCCNI     | 13    |
|         | CAYCCN          | 2288  |
|         | CAYCCNI         | 762   |
|         | CAYCCNIV        | 506   |
|         | CAYCCNIVN       | 219   |
|         | CAYCCNIVNV      | 2     |
|         | CAYCCNIVNS      | 47    |
|         | CAYCCNIVNVS     | 3     |
| Sequence                | Count |
|-------------------------|-------|
| CAYCCNIVNVS'LVKPS       | 1     |
| CAYCCNIVNVS'LKP'SF     | 2     |
| CAYCCNIVNVS'LKP'SFY   | 3     |
| CAYCCNIVNVS'LKP'SFYVY  | 3     |
| CAYCCNIVNVS'LKP'SFYVY'SR | 1     |
| CAYCCNIVNVS'LKP'SFYVY'SRV | 11    |
| CAYCCNIVNVS'LKP'SFYVY'SRVK | 19    |
| CAYCCNIVNVS'LKP'SFYVY'SRVK | 209   |
| CAYCCNIVNVS'LKP'SFYVY'SRVK | 4     |
| CAYCCNIVNVS'LKP'SFYVY'SRVK | 10    |
| CAYCCNIVNVS'LKP'SFYVY'SRVK | 20    |
| CCNIVN                 | 5     |
| CCNIVNVS'LVK           | 1     |
| LTLR'LRLCAYCC          | 7     |
| LTLR'LRLCAYCCN         | 2     |
| LTLR'LRLCAYCCNIVNVS'LKP'SFYVY'SRVK | 1     |
| LTLR'LRLCAYCCNIVNVS'LKP'SFYVY'SRVKLN | 1889 |
| LTLR'LRLCAYCCNIVNVS'LKP'SFYVY'SRVKLN | 23 |
| LTLR'LRLCAYCCNIVNVS'LKP'SFYVY'SRVKLN | 1 |
| LTLR'LRLCAYCCNIVNVS'LKP'SFYVY'SRVKLN | 36 |
| LTLR'LRLCAYCCNIVNVS'LKP'SFYVY'SRVKLN | 249 |
| LTLR'LRLCAYCCNIVNVS'LKP'SFYVY'SRVKLN | 3 |
| LTLR'LRLCAYCCNIVNVS'LKP'SFYVY'SRVKLN | 305 |
| LTLR'LRLCAYCCNIVNVS'LKP'SFYVY'SRVKLN | 9 |
| LTLR'LRLCAYCCNIVNVS'LKP'SFYVY'SRVKLN | 25 |
| LTLR'LRLCAYCCNIVNVS'LKP'SFYVY'SRVKLN | 5 |
| LTLR'LRLCAYCCNIVNVS'LKP'SFYVY'SRVKLN | 2 |
| LTLR'LRLCAYCCNIVNVS'LKP'SFYVY'SRVKLN | 9 |
| LTLR'LRLCAYCCNIVNVS'LKP'SFYVY'SRVKLN | 44 |
| LTLR'LRLCAYCCNIVNVS'LKP'SFYVY'SRVKLN | 3 |
| LTLR'LRLCAYCCNIVNVS'LKP'SFYVY'SRVKLN | 3 |
| TALRLCAYCC             | 52    |
| TAILTALR'LRLCAYCCN     | 1     |
| TAILTALR'LRLCAYCCNIVN  | 1     |
| YCCNIV                 | 2     |
| YCCNIV                 | 1     |
| YCCNIV                 | 15    |
| 2. Catalase_C          | CAYCCNI | 3 |
We downloaded 73,983 catalase sequences of various bacteria from the UniProt website and used the local MEME tool to search for each sequence and the motif of E protein (E-VALUE<0.05). Then searched for the conserved domain corresponding to the motif on the hydrogen peroxide sequence from the UniProt website through a web crawler method. Searched for conserved domains containing “catalase”. Table 2 lists the main conserved domains related to the catalase properties of E protein. “CAYCC” is the heme-binding motif found in heme theory. Table 2 shows that E protein has catalase conserved domains: Catalase, Catalase_C, and Catalase-rel. Catalase is the core domain of heme catalase (InterPro entry IPR011614). Catalase-rel is a catalase-related immune response domain with an immunoreactive amphiphilic octapeptide recognized by T cells. Catalase_C is an extra C-terminal domain present in large catalase enzymes. It is related to the class I glutamine transferase domain, and the exact molecular function is still uncertain. “CAYCC”, and its C-end fragments belong to the catalase functional region. The catalase catalytic characteristic region of E protein is at positions 30-69, including the transmembrane area and the outer part of the membrane: “TLAILTALRLCAYCCNIVNVSLVKPS FYVYSRVKNLNSSR”.

The above analysis results showed that the E protein had catalase's catalytic function after binding to heme. Protein E catalyzed the decomposition of hydrogen peroxide into oxygen and water.

### 3.3. E protein had active of the peroxidase

Heme peroxidase is a heme-containing enzyme that uses hydrogen peroxide as an electron acceptor to catalyze many oxidation reactions. Heme peroxidase includes two superfamilies: one is found in bacteria, fungi and plants, and the other is found in animals. Among them, Lignin fungi peroxidase has a strong generating capacity of ·OH. In this study, we only selected conserved domains in fungal peroxidase.

We downloaded 554 peroxidase sequences of various fungi (keywords: Lignin fungi peroxidase) from the UniProt website. Then we used the local MEME tool to search for motifs between each sequence and the E protein (E-VALUE<0.05). So searched the conserved domain corresponding to the motif through a web crawler method on the UniProt website. Manually searched for conserved domains containing “PEROXIDASE”. Table 3 lists the conserved domains related to the peroxidase properties of E protein. “CAYCC” is the heme-binding motif found in heme theory. Table 3 shows that E protein has a peroxidase conserved domain: PEROXIDASE_4. “CAYCC” and its C-end fragments belong to the peroxidase functional region. We can see that the peroxidase catalytic characteristic region of E protein is the sequence of positions 38-64, including the outer membrane and a small part of the transmembrane region. “RLCAYCCNIVNVSLVKPSFYVYSRVKN”.

| Domain               | Motif  | Count |
|----------------------|--------|-------|
| PEROXIDASE_4         | CAYCCN | 1     |

Table 3. Domains of peroxidase (Lignin fungi) active of E protein
We used Uniprot to find the Ref protein name of the fungi peroxidase sequence and got three group protein names: R7T0H4->XP_007365204.1; R7SP50->XP_007370520.1; R7SH99->XP_007272084.1. The E protein corresponding to R7SH99 was “YCCNIVNVSLVKPSFYVYSRVKN”, which contained the heme linked site “YCC”. In the NCBI webpage, there was no “Related Structures (Summary)” content for these three proteins. After clicked “Conserved Domains (Concise)” in the NCBI webpage of these three proteins, and the introduction of “cd00692: Ligninase; Ligninase and other manganese-dependent fungal peroxidase” was displayed. It showed that the fungal PEROXIDASE_4 type domain was lignin peroxidase. The above analysis results showed that the E protein had the peroxidase enzyme’s catalytic role after binding to heme. E protein catalyzed the formation of hydrogen peroxide, even ·OH.

3.4 The heme linked sites were located on the outer surface of the viral membrane structure

The heme theory discovered the heme linked site “CAYCC” of E protein. We further investigated whether the sites where heme is outside the viral membrane. We downloaded the transmembrane domain (7K3G) of SARs-COV-2 E protein from the PDB database. Figure 1 shows that the transmembrane structure sequence is at the N-terminus of E protein: “ETGTLIVNSVLLFLAFVVFLLVTLAILTALR”, at positions 8-38. The heme linked site “CAYCC” is near the viral membrane structure’s outer side, at 40-44. The transmembrane structure of E proteins makes up a 5-mer ion channel. Maybe the enzyme catalytic properties of E protein are related to ion channels.
3.5 The virus's spear (ROS attack) and shield (ROS escape)

The virus's membrane structural proteins include surface glycoproteins (Spike protein), E protein, and M protein. The heme theory found that E protein had heme linked sites, and had Cytochrome c oxidase active. And the surface glycoprotein bound heme with poor stability, and M protein did not bind heme. Surface glycoprotein may aid E protein in capturing heme. The present study still supported that E protein had the heme-iron linked site. When E protein attached to iron, it had Fe-SOD enzyme activity. When it linked to heme, it had Cytochrome c oxidase, catalase, and peroxidase activity. E protein catalyzed superoxide anion and hydrogen peroxide into oxygen and water through Cytochrome c oxidase activity. E protein also catalyzed hydroxyl radicals from hydrogen peroxide through peroxidase activity. It catalyzed the hydrogen peroxide and oxygen from superoxide anion through Fe-SOD enzyme activity. E Protein catalyzed water and oxygen from hydrogen peroxide through catalase activity.
E protein-joined to iron and E protein-bound heme collaborated to catalyze the decomposition of superoxide anion and hydrogen peroxide into water and oxygen. The virus escape the immune system from attacking the virus by decomposing superoxide anions and hydrogen peroxide through the E protein. It was called the virus’s shield—"ROS escape". After E protein associated with heme, it catalyzed superoxide anions, hydrogen peroxide, and hydroxyl radicals from oxygen and water. The virus attacked nearby tissues by the superoxide anion, hydrogen peroxide, and hydroxyl free radicals. It was named the spear of the virus ---"ROS attack". Hydroxyl free radical was the primary tool for viruses to invade tissues because it more significantly damaged lipids, proteins, and nucleic acids. Then, the E protein that bound to heme damaged the immune system.

4. Discussion

4.1 The iron and heme required for the E-catalysis came from hemoglobin or phagocytes

The iron needed for the iron catalysis of the E protein may occur from two significant pathways. One is the iron or heme dissociated by the virus protein attacking hemoglobin. The second is iron or heme that macrophages engulf. The heme theory ORF3 and other viral proteins attacked hemoglobin, dissociating heme into iron and porphyrin. This attack produced a large amount of dissociated iron and heme. SARs-COV-2 virus in the blood could captured these released iron and heme to achieve “ROS escape” or “ROS attack”. There were also many iron or heme particles in phagocytes. In elevated systemic iron levels or inflammation, elevated hepcidin acts on the macrophage iron transporter channel, resulting in iron retention in the macrophages. The autopsy reports also explain that phagocytes have numerous hemophagocytic phenomena. So, this phagocytosis will even swallow many iron or heme particles.

4.2 Iron on the balance between virus infection and “ROS attack”

The heme theory discovered that the N protein also had heme iron-binding sites, and virus replication may take iron catalysis to start it. When the virus replicated in large numbers, the demand for iron increased, and then the intensity of attacking red blood cells also increased. When the uptake of iron by the virus reached saturation, the attack on hemoglobin became weaker. Virus infection and ROS attacks may also have a trade-off relationship. When the iron intake was too much, the virus impaired the cells with ACE and CD147 receptors through a ROS attack. Otherwise, the virus infected these cells to raise the virus replication. Study evidence shows that red blood cell membranes of patients are harmed by denaturation, injuring structural proteins. When the virus got in less iron, it may infect red blood cells through the CD147 receptor. Otherwise, the virus may hurt the red blood cell membrane through ROS, inducing the red blood cell to perish. Phagocytes will strengthen the phagocytosis for red blood cells with impaired functions. It may also explain that some patients appeared not to show excessive iron levels in the red blood cell.
4.3 The virus colonizes phagocyted and caused asymptomatic infection.

Asymptomatic infection was related to the viruses' haven—mononuclear phagocytes. If phagocytes engulfed pathogens, it produced numerous superoxide anions through the respiratory outbreak mechanism. Superoxide anions killed aerobic bacteria. The E proteins had activities through the iron catalytic process. They converted superoxide anions into H$_2$O$_2$, and then decomposes H$_2$O$_2$ into water and oxygen to prevent the virus from being oxidized. So the virus can live in the vesicle.

The E protein got peroxidase activity through the iron catalytic system and contributed ·OH. When the ·OH concentration approached a certain threshold, the lysosomal membrane ruptured. Then the hydrolase was released into the cytoplasm. It led to autophagy death of phagocytes, releasing virus, iron or heme particles. When the ·OH accumulation was low, SARS-CoV-2 could inhibit the activity of hydrolytic enzymes near itself. The lysosome neither digested the virus nor ruptured, achieving the parasitism of virus.

Therefore, mononuclear phagocytes scattered in the body may be the central sheltering place for viruses. There is an interesting coincidence here for time. The life cycle of macrophages is more than several weeks. Because of physical differences, the life span of macrophages in some people may be longer. The life cycle is consistent with the time of isolation. The time for quarantine measures is based on the statistical data. A 14-day quarantine measure is performed for people who have been in contact with patients with the novel coronavirus. It will confirm infected persons positively by nucleic acid test before the end of quarantine measure. If travelers return from an epidemic area abroad, they will be quarantined for 14 days in the city of arrival. Then they will be sequestered for 14 days when they return to their town of residence too. Some people with recessive infections have a negative nucleic acid test in the first stage of quarantine measure. The nucleic acid test will be positive during the second stage of the quarantine measure. There is also a minimal number of negative cases in both quarantine phases, and they only develop into positive after the two quarantine phases.

4.4 Oxidative stress damage of memory B cells caused re-infection

B cells’ differentiation process is divided into five stages: pre-B cells, immature B cells, mature B cells, activated B cells, and plasma cells. The differentiation of pre-B cells and immature B cells is antigen-independent, and the differentiation process takes place in the bone marrow. The antigen-dependent stage means that mature B cells are stimulated after antigen stimulation and continue to differentiate into plasma cells that synthesize and secrete antibodies. The differentiation at this stage is mainly carried out in the peripheral immune organs.

In the early stages of onset, the frequency of CD27+ and CD38+ plasma cells in COVID-19 deceased patients was lower than that in surviving patients. Memory B cells include CD27+, CD27- and Ag-specific memory B cells, among which CD27+ type memory B cells play a significant role in secondary immunity. CD27 is a vital surface antigen molecular marker for memory B cells. Memory B cells express different membrane immunoglobulins (mlg), which are antigen receptors (BCR). CD27+ expresses mlgG and mlgM, but CD27- Express mlgG. mlg can recognize antigens, get the first signal of antigen stimulation, stimulate B cells to differentiate into plasma cells through a cascade reaction, and secrete antibodies again. Therefore, losing IgM
antibodies in reinfections was linked to the abnormal loss of CD27+ memory B cells. When CD27+ memory B cells were exposed to the surface of the virus in the direction of mIgM, they were easily damaged and die due to "ROS attack".

4.5 "Happy hypoxia" is a symptom that attacked hemoglobin and impaired immune cell

Former patients experienced a weird stage called “happy hypoxia”. At this stage, the patient’s blood oxygen saturation was only 70%. In theory, the patient should have difficulty breathing and movement. But these people could move, talk, and laugh. However, the patient’s condition deteriorated.

When red blood cells transported oxygen to the capillaries, the oxygen molecules fall off from the hemoglobin. Then they passed through the red blood cell membrane through osmosis and then entered the capillary blood vessels. At last, they joined the nearby tissue cells from the capillary blood vessels through osmosis. Blood oxygen saturation was the ratio of oxygenated hemoglobin to all hemoglobin. According to the heme theory, proteins such as ORF3 attacked hemoglobin, which reduced the amount of hemoglobin that carried oxygen and decreased the patient’s blood oxygen saturation.

If the amount of oxygen-carrying hemoglobin decreased, the tissue cells should appear hypoxic. Therefore, patients should not be “happy”. The hypoxia of cells caused shock, and thus it should not be a kind of nerve paralysis. The heme theory speculated that the attacked hemoglobin released oxygen molecules into the blood. This was an important factor for the "happiness" phenomenon, and another reason may be related to the immune response.

After activation, phagocytes released many H2O2 to the outside of the cell to kill the pathogen. The SARS-COV-2 virus catalyzed the decomposition of these H2O2 to yield oxygen. When this catalysis occurred in the blood, the dissolved oxygen in the blood was higher. When this catalysis occurs near the cells, the oxygen entered the tissue cells through osmosis. It concealed the problem of cellular hypoxia induced by the decrease in the amount of oxygenated hemoglobin. The virus also expanded infection in a patient’s “happy” state.

Specific T cells, B cells, and NK cells were hurt by the virus’s “ROS attack” when exposed to the virus’s surface. The ROS attack was an oxygen-consuming process. The virus could steal the oxygen molecules inside or outside the red blood vessels to produce ·OH. However, after viral proteins attacked hemoglobin, oxygen-carrying hemoglobin was lowered, and there were few exogenous oxygen molecules. So, the virus could decompose H2O2 released by phagocytes to produce ·OH. This ·OH hurt membrane structures of T cells, B cells, and NK cells, leading to these cells’ damage or death. It exacerbated inflammation and made immune cells to secrete more cytokines.

5. Conclusion

The novel coronavirus pneumonia is a deadly disease, and the current global pandemic is inseparable from its high contagion. Understanding the novel coronavirus’s critical damage mechanism to the immune system is of great significance to contain the epidemic, cure the disease,
and save lives. We speculated that the E protein of the SARs-COV-2 virus had an essential relationship with the disease’s infectiousness in the previous study of heme theory. This study used domain search techniques to analyze the E protein of the SARs-CoV-2 virus.

The results showed that the E protein of the SARs-COV-2 virus had the conserved domains of Cytochrome c oxidase, Fe-SOD enzyme, catalase, and peroxidase. The heme-binding sites were on the outer surface of the viral membrane structure. When E protein bound to iron, it had Fe-SOD enzyme activity. When E protein-bound heme, it had Cytochrome c oxidase (found in heme theory), catalase, and peroxidase activity. When E protein bound to heme, oxygen and water were set as a starting point to synthesize superoxide anion, hydrogen peroxide, and hydroxyl radicals. It named the “ROS attack” of the virus. When an iron-bound E protein and a heme-bound E protein could cooperate, they converted superoxide anion and hydrogen peroxide into oxygen and water. It was called “ROS escape” of the virus. “ROS attacks” damaged the tissues or cells exposed on the virus’s surface, and “ROS escape” dissolved the superoxide anion and hydrogen peroxide that attacks the virus.

The iron and heme bound by E protein came from attacked hemoglobin and phagocytes. Viral proteins attacked hemoglobin and dissociated hemoglobin to shed large amounts of iron and heme. E protein of the virus could bind these iron and heme. Attacking hemoglobin also enhanced the red blood cell phagocytic behavior of phagocytes. Phagocytes also swallowed iron, heme, and red blood cells. After entering phagocytes, the virus directly got iron and heme from vesicles or lysosomes. After the phagocyte ruptures, the virus also captured the overflowing iron and heme.

Lymphopenia was associated with “ROS attack”. When NK cells were exposed to infected cells, the virus that had not shed from the infected cells’ surface attacked them through ROS. NK cells were damaged or died because of the attack on the cell membrane structure. While Lymphocytes such as T cells and B cells neared the virus surface antigen, they were also injured or die by the virus’s ROS attacking. It is worth noting that the E protein also has a Catalase-rel domain related to T cell immunity, which overlaps with the heme binding site, and its specific function is unclear. Memory B cells were also damaged or break down for the virus’s ROS attacking when exposing to the virus surface antigen. Therefore, the abnormal deficiency of memory B cells was an essential factor in patients’ re-infection.

The virus used the “ROS escape” method to decompose the H₂O₂ released by the phagocytes into oxygen and water to avoid external killing. In this process, the surrounding cells were supplemented by oxygen molecules unexpectedly, causing the patient to appear "happy hypoxic state." When phagocytes swallowed the virus, the E protein converted superoxide anions into oxygen. Then it transferred the intermediate product hydrogen peroxide into oxygen and water. So the virus parasitized in the vesicles of phagocytes in this way. After the vesicles fused with lysosomes, the E protein produced ROS. The hydrolase neared to the virus was damaged by oxidative stress and lost its activity. Viruses parasitized in the lysosomes of phagocytes in this way. While the hydroxyl radical exceeded a specific number, it damaged the lysosomal membrane. Lysosome ruptured to release hydrolase, and phagocytes died by autophagy. After the surrounding phagocytes swallowed cell fragments and virus particles, death similar to autophagy occurred again. This vicious circle caused inflammation and fibrosis of surrounding tissues or cells. Therefore, the virus parasitizing the vesicles or lysosomes of phagocytes was related to asymptomatic infection or retest positive.

In short, viruses applied ”ROS attack" to damage multiple organs and tissues in the body,
such as damaging immune (example, bone marrow, spleen) and nerves (like the spinal cord, brain) organs. The attack provoked a strong cytokine storm and caused organ failure and complications. We hope that this discovery will help prevent the severe epidemic and save more lives.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Availability of data and material**

The datasets and results supporting the conclusions of this article are available at https://pan.baidu.com/s/1YBe6TXLTCcN9AH9ZMbnkQ, code: ordn. Or: https://mega.nz/folder/sjoEkQ6D#JK4AXi9KIQLBIUwXmKVQGg

**Competing interests**

The authors declare that they have no competing interests.

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Author details

1 School of Computer Science and Engineering, Sichuan University of Science & Engineering, Zigong, 643002, China.
2 School of Life Science and Food Engineering, Yibin University, Yibin, 644000, China.

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