

## **Disease Heterogeneity and Differential Severities in COVID-19**

**Abstract:**

During the time of the ongoing worldwide pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), scientists are finding answers on the varying severities of the pandemic's novel variants. One of the more intriguing aspects of this virus occurs in the presentation and severity of symptoms in patients. How does the coronavirus (COVID-19) disease have this wide-range prognosis from asymptomatic to fatal? This thesis examines some promising areas of investigation regarding the innate immune system and inflammatory factors in the role of severe COVID-19 cases. Various experimental findings regarding the possible connection between inflammation factors of the human innate immune system and the severity of COVID-19 will be described.

**Keywords:**

Biology, COVID-19, Severe Acute Respiratory Syndrome Coronavirus 2, Innate Immune System, Inflammation, Interleukin-6, Tumor Necrosis Factor, Interferon Gamma, Lymphatic System, Neutrophils, Apoptosis, Asymptomatic, Proinflammatory Cytokines, C-Reactive Protein

Understanding the connections between COVID-19 and the immune system requires an overview of the various functions of the innate immune system. When it comes to pathogens such as viruses, the human body has multiple lines of defense. The lymphatic and immune system work together in response to pathogens that enter our bodies. The immune system is divided into the adaptive and innate immune system. The adaptive immune system is also referred to as the specific resistance, it is built up through exposure to pathogens. The innate immune system The lymphatic system is made up of vessels that are found through the body's tissues. It also consists of tissues and organs that produce immune cells. The lymphatic system is responsible for fluid recovery from the blood vessels, immunity with the aid of the immune cells found in the lymph nodes, and in lipid absorption in the small intestine. Different lymphocytes make up lymphatic tissues, some of which are T lymphocytes (T cells) and B lymphocytes (B cells). T cells develop in the thymus, which is a lymphatic organ found between the lungs. B cells once activated differentiate into plasma cells and eventually create antibodies. Antibodies are immunoglobulins that protect the body from pathogens through recognition and memory (Saladin). For the immune system to protect our body from pathogens, there are also other lines of defense.

External barriers like the skin and mucus membranes serve as the first line of defense. These barriers make part of the mechanism known as nonspecific resistance, which can also be known as the innate immune system. Nonspecific resistance also includes antimicrobial proteins which cause fever and inflammation. These external and antimicrobial barriers defend the body against a wide range of pathogens with limited specificity. Inflammation is one of the central aspects studied in the scientific literature regarding COVID-19 and therefore will be discussed in greater detail below. This is referred to as innate since we are all born with this type of

immunity; thus, it does not depend on prior exposure to a pathogen. Leukocytes make part of both the innate immune system and the adaptive immune system (Saladin).

There are five types of leukocytes: neutrophils, eosinophils, basophils, lymphocytes, monocytes. Each have their own roles in immunity and they can be found circulating in our blood.

Neutrophils are responsible for killing bacteria through phagocytosis and digestion. Eosinophils are known to phagocytize allergens and inflammatory chemicals. Basophils secrete histamine which dilates vessels thus increasing blood flow to an area and increasing inflammation. They also secrete an anticoagulant known as heparin. An anticoagulant can prevent clot formation by thrombocytes, also known as platelets. These platelets are components of the blood along with red blood cells (erythrocytes), white blood cells (leukocytes) and plasma. Lymphocytes in addition to the B and T cells described above have several forms like the natural killer cells (NK cells) which lyse host cells that have been infected with a virus or have become cancerous. The NK cells act as surveillance of the body's cells to ensure any of these abnormal cells are destroyed. During NK cell surveillance, some cells can be induced into apoptosis, where cells are signaled to "auto destruct" and lyse. Contrary to apoptosis, which is a controlled form of cell destruction, necrosis is an uncontrolled cell death. Necrosis usually occurs through cell swelling and inflammation (Nikinmaa). Lastly, monocytes are precursors to the macrophage which is another term for the rest of the active phagocytic cells, not including the leukocytes (Saladin). Along with the many types of cells in the immune system, there are other mechanisms with a large role in nonspecific resistance.

Inflammation is a nonspecific resistance pathway of the innate immune system. It is often characterized by swelling, heat, redness and pain. Inflammation is meant to limit the spread of pathogens, remove damaged tissue and initiate its repair. Inflammation can be brought about through different complement pathways. The classical pathway revolves around the antibody

binding to a foreign cell or virus and exposing the complement binding sites. The complement system is made up of around 20 Beta globulins that are activated by pathogens while circulating in the blood. In the classical pathway, complement proteins C1, C2, and C4 bind to a pathogen-antibody complex. In the alternative pathway for inflammation complement factors B, D, and P bind directly to the polysaccharides of the microbe without need of the antibodies. The complements then enhance inflammation by promoting secretion of inflammatory chemicals from the mast cells and basophils (Omar).

Some other inflammatory factors that act in conjunction with the complement system are the proinflammatory cytokines like interleukin-6 (IL-6), interleukin-1 (IL-1), Tumor necrosis factor (TNF), and interferon-gamma (IFN- $\gamma$ ).<sup>2</sup> TNF, IL-6 and IL-1 attract macrophages leading to accumulation at site of infection. These factors also upregulate adhesion molecules expression on inflammatory cells and thus allow for easier recruitment of many cells to an affected site. TNF, IL-6, and IL-1 all take part in the chronic inflammation phase. The TNF cytokine family is also known to induce apoptotic cell death. IFN- $\gamma$  works in antiproliferative and antitumor mechanisms. It also induces apoptosis as seen in the previous inflammation factors. All these inflammation factors are secreted by activated lymphocytes like CD4 T helper cells and NK cells after they bind to a virus (Castro et al.).

The last major player in the inflammation pathway is the C-reactive protein (CRP). Once the native form of this protein is at a site of inflammation or infection it dissociated into monomeric CRP. This acute inflammatory protein can increase through this dissociation almost 1,000-fold at infection or inflammation sites. More recent research has shown CRP to have an important role in host responses to infection though the complement pathway, apoptosis, and interestingly enough; the production of cytokines IL-6 and TNF (Sproston and Ashworth). CRP, IL-6, and TNF are all important factors which have been examined in relationship to COVID-19.

COVID-19 disease was first detected in November 2019. This disease is caused by an enveloped coronavirus called, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The mode of binding and entry is reliant on the angiotensin-converting enzyme 2 (ACE2) receptor found on the host cells. The SARS-CoV-2 has proteins on its surface called spike proteins which allow for virus “landing” and fusion of virus particle into host cell. ACE2 has a highly conserved expression because of its’ role in the vascular, renal, and myocardial function in animals (Bakhshandeh et al.). The SARS-CoV-2 virus acts through the conserved ACE2 receptor, giving it a high advantage in its replication, yet there is still much research to be done on the variability in COVID-19 severity.

There are many unknown factors that can cause COVID-19 heterogeneity. The host and environment are investigated as possible influences in disease severity and outcome. More specifically, a look into the human innate immune system’s inflammatory response has been observed in differing severities of the virus. This does not exclude possible adaptive immunity effects. For example, the higher severity disease effect on elderly population demonstrates lack of long-lasting pre-existing adaptive immunity by T or B cells against SARS-CoV-2. This could ultimately explain the milder responses in younger populations. Cross protection through adaptive immune responses against other coronaviruses can be partly responsible for mild cases in younger populations (Schultze and Aschenbrenner). This is only one recent study discussing the role of adaptive immunity. The innate immune response will be further discussed in this thesis.

Throughout the many steps of the innate immune system, there are varying hypotheses and studies on different cells and their role in severe COVID-19 cases. Heterogeneity of responses to the virus could be due to normal distributions of low, intermediate, and high responders in the interferon response in an infected cell. Through these different types of

responders, different magnitudes of cellular responses will be triggered, and very different overall effects will result. These differences could result in severe and mild cases of COVID-19 within hosts (Schultze and Aschenbrenner).

Other possible factors in heterogeneity include genetic molecules and their presence in certain organs in the body. The location of viral RNA was also studied, and it was found in high levels in ciliated and epithelial cells. It was also found in some macrophages. There was also a distinct difference between mild and severe cases of COVID-19 in terms of location and enrichment area. Viral RNA was found in single-cell transcriptomes of bronchoalveolar fluid in severe patient samples. This was not found for mild patients. Differences between the enrichment areas of viral RNA could also serve as a foundational difference within the variability of COVID-19 disease outcome. When studying the location and targets of the virus, some interesting findings were still unexplained. For example, the role of the innate immune cells as targets in SARS-CoV2 in other organs not related to the respiratory tract is still very unknown. There are more questions to be answered in terms of innate immune cells' role in SARS-CoV2 gaining access to organs like the retina or brain. Yet the virus still is found in both organs. Surprisingly, no clear distinctions were found between severe and mild cases in terms of enrichment in these types of organs (Schultze and Aschenbrenner).

In order to look for possible causes for severe and mild cases more specific studies will be explored in the following sections. To study the immune system cells during severe and mild COVID-19 cases many different approaches were taken. In some cases, different aspects of the innate immune system were observed. In some studies platelets were analyzed, while in other IL-6, CRP and even monocyte and lymphocyte counts were taken. All these measures show only one part of the complexity of this virus. One aspect of the variability in COVID-19 outcome deals with clot formation. Platelet hyperactivation has largely been found in severe COVID-19

cases. Exposure to the virus causes platelet aggregation, release of dense granules and upregulation of activation markers. It also aids in increasing release of coagulation factors and secretion of inflammatory cytokines (Schultze and Aschenbrenner).

Along with these inflammatory cytokines, there were also early transient waves of monocyte expression and IFN release during the beginning of infection. As the disease progressed in severe cases there were increasing levels of IFN alpha and IFN gamma. Along with these elevations, NK cells and neutrophils were also found in high numbers. In severe patients there were also decreased numbers of T cells and lymphocytes. A disease severity biomarker has also been established as an elevated ratio of neutrophil/lymphocyte numbers. Through the immune system there is a dysfunctional quantity of cells. In severe cases a term frequently used to describe the immunopathology of COVID-19 is a cytokine storm. A cytokine storm is defined as “all inflammatory conditions with elevated circulating cytokines that result in systemic inflammation as well as secondary organ dysfunction” (Schultze and Aschenbrenner). A major part of the cytokine storm is the inflammatory aspect of the innate immune system. The inflammatory response as discussed above has many components that work together to provide this general defensive response to pathogens.

The inflammatory response in relationship to COVID-19 was analyzed in hospital settings for most of the studies done. Through clinical retrospective studies of admitted patients, blood tests and even post-mortem analyses, differing levels of certain important cells and proteins were found in severe cases. In one study, plasma IL-1Ra levels were measured at three time points for 134 patients: during the onset which was at 0-3 days in intensive care unit (ICU), again at 7-10 days at ICU, and lastly at discharge. Plasma IL-1Ra levels were measured at three time points for 134 patients: during the onset 0-3 days in intensive care unit (ICU), 7-10 days at ICU, and at discharge. Plasma concentration of IL-6 was statistically higher in patients with

severe symptoms who were admitted to the ICU as compared to patients admitted to a conventional dependency ward (Bonnet et al.).

This high prevalence of interleukin-6 was also emphasized in other papers. An observational and retrospective study was done with severe and critical COVID-19 cases. Initial concentrations of IL-6, CRP, lymphocytes and neutrophils were collected retrospectively. These same measures were found in the course of the disease. It was found that the initial and peak concentrations of IL-6, CRP, and neutrophil count in the critical group were higher than those in the severe group. The initial lymphocyte count was lower though. There was also an increased rate of concentration for IL-6, CRP, and neutrophil count for the critical group as compared to the severe patients which were statistically significant. Through the results of this study, it could be concluded that the initial IL-6 concentration could be a good indicator for COVID-19 progression and severity (Wang et al.).

In another paper a post-mortem analysis was done, accumulation of inflammatory cells was found in COVID-19 non-survival cases. CRP levels were measured in this study. The virus can cause vascular damage that leads to an increase in plasma C-reactive protein (CRP) concentration. CRP activates blood coagulation, platelet adhesiveness and inflammation. Patients were stratified from a sample of 649 patients admitted to 10 hospitals in China based on severity of disease. They were separated into mild symptoms, moderate symptoms and severe cases. Moderate and severe cases were differentiated by oxygen index. Routine blood examinations were done as the progression of the disease continued. It was found that CRP levels in severe patients at admission were significantly higher than those in patients with moderate COVID-19. (An et al.)

The IFN system response is also a key factor in the inflammatory system as seen before in earlier papers. Natural killer cells (NK) and Tumor necrosis factor (TNF) play an important role in the IFN system and one paper looked at this connection. A longitudinal characterization of NK cells in different severity COVID-19 patients was done in a hospital setting in China. Both moderate and severe cases were observed. Transcriptional changes in NK cells were assessed in the blood by scRNA-seq analysis. NK cells showed a strong IFN alpha response with increased expression of IFN stimulated genes in early severe COVID-19. In early moderate cases, NK cells expressed TNF more readily. It was also found that in severe cases inflamed NK cells were more prominent than in moderate cases. It was then concluded that NK cell dysfunction caused by IFN alpha response is a hallmark for severe cases of COVID-19 (Krämer et al.). The IFN system is still being studied and there are more viable hypotheses being tested now in research.

Recently, more research has led to other possible reasons for the heterogeneity of this disease. More interestingly enough, the question on why some people are completely asymptomatic or just don't get the disease is posed. Human genetic differences may have a larger role than was originally deemed in this viral infection. For severe patients, proinflammatory genes were found to be upregulated in innate immune system cells. This high expression led to further complications. Then, followed by an accumulation of pathogenic inflammatory neutrophils and macrophages in the lung. Ultimately this leads to an excessive inflammatory and immune response leading to the common complications of COVID-19 infection: acute respiratory distress syndrome, pulmonary edema, apoptosis of epithelial cells. This inflammatory excess can lead to cell death due to elevated TNF and IFN gamma. This can drive apoptosis and necrosis (Schultze and Aschenbrenner).

Apart from the inflammatory factors, more recent research has shown a molecular basis for disease heterogeneity. The relevance of genetics in the study of this virus continues to grow and with doctors like Dr. Mayana Katz and Dr. Jean-Laurent Casanova the field expands with promising feats. In a recent New York Times article, these doctors explained some of their work with the virus and genetics. Doctor Mayana Katz believes genetics may have a role in the dissimilar reactions the population has to COVID-19. She collected samples from 100 couples where one person had an infected partner while the other did not get sick. Dr.Katz and her colleagues looked for combinations of genes that could cause this variation in responses. They found variants in genes that affected NK cell activity in the genome of infected people. The variants were consistent with significant differences between NK cell activity of healthy and infected people. Doctor Jean-Laurent Casanova also studies how healthy individuals could develop such a life-threatening disease. He investigates the “inborn errors of immunity”. His team has found a small percentage of severe COVID-19 patients who have mutations in interferon genes. This leads to a lack of a person’s ability in defense against pathogens. At least 15% also had misguided antibodies that would attack the interferon. Testing in advance for presence of these antibodies could be a way of understanding who is at risk for severe disease in the future. The research for this virus has also expanded to new territories like genome wide association studies. Genome wide association studies are being used to find links between genes and possible susceptibility or resistance to COVID-19 (Lamas). These consist of searching large amounts of genomic data of a population. These new methods could provide even more guidance as the world grapples with the complexity of the SARS-CoV-2 pandemic.

The SARS-CoV-2 pandemic has transformed our daily lives and scientists continue to search for more information on its complex and variable prognosis. The studies examined in this thesis present only a fraction of all the research done on COVID-19 disease. Based on the

collected studies, inflammation factors like IL-6, TNF and CRP all have shown significant correlations with high severity cases. These early findings in the inflammation pathway and COVID-19 explain the earliest forms of treatment for COVID-19. The early treatments targeted IL-6 and some of the more recent medication protocols also include specific targets in the inflammatory pathway. Taking the information gathered in research will lead to more effective medications and thus reduce the number of severe cases of COVID-19 as we continue to live through this pandemic.

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