Immune response to SARS-Cov-2 infection in at risk populations

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Contents

1. Abastract

Key words: SARS-CoV-2, Immune reaction, Vaccination

This paper concerns itself with the topic of SARS-CoV-2, the immune system, their relationship and the risk factors that might be involved and affect the eventual prognosis of a person having the disease.

With a wide range of symptomatology and a pathophysiology that is still not completely clear. The only medical treatment currently available today is Dexamethasone which is given to hospitalized patients with respiratory difficulties and how exactly does dexamethasone act in this context is not completely clear.

For this reason it is important to try and elucidate how exactly SARS-CoV-2 acts and interacts with the immune system, in order to perhaps find a new pharmacological target for treatment or, at least, to further clarify the cellular processes and interactions occurring when a patient is infected with SARS-CoV-2.

In this work ideas that not only concerning the cellular biology will be explored but also ones related to the population level such as mass immunization and the difference between passive and natural immunity, T cell Assay and IGRA use for the purpose of screening individuals at risk, different cytokine and interferon profiles and their connection with different disease prognoses.

It is crucial for knowledge to be known and further taught to medical professionals and even to the population to some extent so that more people will know what the possible consequences of the disease are and what are the chances of different people with different risk factors to have a clinical picture with a certain progression and prognosis.

Sažetak

Ključne riječi: SARS-CoV-2, Imunološka reakcija, Cijepljenje

Ovaj rad bavi se temom SARS-CoV-2, imunološkim sustavom, njihovim međusobnim odnosom i čimbenicima rizika koji bi mogli biti uključeni i utjecati na konačnu prognozu oboljele osobe.

Zbog širokog raspona različitih simptoma i fiziologije, ova poveznica još nije u potpunosti razjašnjena. Trenutno jedini raspoloživ tretman je deksametazon, koji se daje hospitaliziranim

pacijentima s respiratornim poteškoćama. Zasad još nije razjašnjeno kako deksametazon djeluje u tom kontekstu.
Upravo zbog tog razloga važno je da pokušamo razjasniti kako SARS-CoV-2 djeluje i stupa u interakciju sa imunološkim sustavom, kako bi se pronašao novi farmakološki cilj za liječenje, ili, barem, kako bi se dodatno razjasnili stanični procesi i međudjelovanja koja se događaju kada je pacijent zaražen virusom SARS-CoV-2.
U ovom će se radu istraživati ideje koje se ne odnose samo na staničnu biologiju, već i ideje koje se odnose na razinu populacije, kao na primjer masovna imunizacija, te razlika između pasivnog i prirodnog imuniteta, 'Test T stanica' i 'IGRA' u svrhu probira osoba sa rizikom, različiti profili citokina i interferona, te njihova povezanost sa različitim prognozama bolesti. Ključno je da se ovakvo znanje podučava medicinskim stručnjacima, pa čak, u određenoj mjeri, i općoj populaciji, kako bi više ljudi bilo upoznato sa mogućim posljedicama navedene bolesti, te da bi znali kakve su vjerojatnosti da će ljudi sa određenim čimbenicima rizika imati određenu kliničku sliku, razvoj bolesti, te u konačnici i prognozu.

2. Introduction

SARS-CoV-2 is a virus that belongs to the family Coronaviridae and realm Riboviria. It is one of the species of the of several other coronaviruses, most of which cause nothing more than the common cold e.g. HCoV-OC43, HCoV-HKU1, HCoV-229E, and HCoV-NL63[i]. However, two other species have become notorious after the pandemics they caused in the past - Middle East respiratory syndrome (MERS) and SARS-CoV-2's predecessor Severe Acute Respiratory Syndrome coronavirus 1 (SARS-CoV-1)
The disease itself has, after an incubation period which is rapid with COVID-19: ~5–6 days versus 2–11 days in SARS-CoV1 infections.
The virus can be readily isolated during the first week[ii] of symptoms. However, hardly any isolates can be obtained from samples taken after day eight despite persistent high viral loads.
The disease has a wide spectrum of severity. From a common cold, which tends to cause mild Upper Respiratory Tract Infection (URT) symptoms and occasional gastrointestinal involvement on one side of the clinical spectrum all the way to severe and systemic'flu'-like symptoms that can progress to acute respiratory distress (ARDS), pneumonia, renal failure, and death.

The most common symptoms that present in the vast majority of patient presentations are fever, cough, and dyspnea. Further symptoms e.g. muscle ache, confusion, headache, sore throat, rhinorrhoea, chest pain, diarrhoea, nausea and vomiting are present more rarely as a general rule.[iii]

2.1 Epidemiology

Case Fatality Rate (CFR) seems to be elevated among those with pre-existing comorbid conditions e.g. cardiovascular disease, diabetes, chronic respiratory disease, hypertension and cancer.

One must also consider any risk factors that may increase the severity of the illness in a given patient. The most important one being the patient's age. Compared to those aged 30–59 years, those aged below 30 and above 59 years were 0.6 and 5.1 times more likely to die after developing symptoms[iv]

The risk of the infection itself becoming symptomatic increases with age. In general, older age is associated with greater COVID-19 morbidity, admittance to the intensive care unit, progression to ARDS, higher fevers and greater mortality rates.[v] Some evidence support the hypothesis that it is due to a lower capacity of $CD4^+$ and $CD8^+$ T-cells to produce Interferon γ (IFNγ) and Interleukin 2 (IL2), as well as an impairment in T-cell activation from dendritic cells in patients with acute COVID19 (Coronavirus Disease 2019) over the age of 55. All these could compromise the function of the adaptive immune response to fulfill its anti-viral role.[vi]

2.2 Pathogenesis

SARS-CoV-2 is enveloped, positive-sense and single-stranded of the RNA type. the virus's genome shares 50% of its sequences with MERS-CoV. Its genome comprises 14 open reading frames, two-thirds of which encode 16 Non-Structural Proteins (NSP 1–16) that constitute the Replicase Complex (important for viral replication). The remaining one-third encodes nine accessory proteins (ORF) and four structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N), of which Spike mediates SARS-CoV penetration into the host cells. However, the S gene of SARS-CoV-2 is highly variable and shares less than 75% nucleotide identity. Spike also has a receptor-binding domain

(RBD) that mediates the direct contact with the corresponding cellular receptor, Angiotensin-Converting Enzyme 2 (ACE2), and an S1/S2 cleavage site that is cleaved by cellular Cathepsin L (not Cathepsin B, which will be discussed further later) and the Transmembrane Protease Serine 2 (TMPRSS2) – two very important elements in the pathogenesis.

TMPRSS2 facilitates the viral entry to the membrane, whereas cathepsin L activates the viral Spike in endosomes and can compensate for entry into cells that lack the needed TMPRSS2. Once the genome is in the cytosol, ORF1a and ORF1b are translated into viral Replicase proteins, which are then cleaved into individual NSPs (via the host's and viral proteases). These form the RNA-dependent RNA polymerase. The replicase components then rearrange in the endoplasmic reticulum (ER) into double membrane vesicles that which in turn facilitate viral replication of both the genomic and Sub-Genomic RNAs (sgRNA); these sgRNAs are then translated into accessory and viral structural proteins and facilitate viral particle formation.[vi]

3. Immune Response

The body's reaction to the virus and seroconversion rate varies. When considering IgM production, the results ranged between 11% and 71% in the early stage (1-7 days after symptom onset), between 36% and 87% in the intermediate stage (8-14 days), and between 56% and 97% after 14 days. IgG detection also ranged considerably with between 4% and 57% in the early stage, between 54% and 88% in the intermediate stage, and between 91% and 100% after 14 days.

When considering the duration and tenacity of the immune response to the virus, one naturally has to check the IgG titers over time after the illness itself ended. In one analysis of multiple studies it was found that when following up on titer of healed patients, with the latest follow up done 60-65 days after the initial symptom presentation, all patients had positive IgG titers, albeit quantitatively different.[vii]

Another analysis of multiple studies checked both the antibody response both qualitatively (seropositivity) and quantitatively (titer levels) across different antibodies.

Qualitatively, the anti–SARS-COV-2 antibodies (IgM/IgG) positive rates peaked at the 2nd week. Likewise, the peak value of positive rate in N-IgG was observed at the 2nd week, but the RBD-IgG positive rate peaked at the 5th week. The positive rates of RBD-IgM and the N-IgM peaked at the 2nd week and then began to decline rapidly. Later on, at the convalescence period, no obvious decline in the positive rate of the total antibody was witnessed with any of the IgM classes (IgM, N-IgM and RBD-IgM) or the IgG classes (IgG, N-IgG, and RBD-IgG). The first decline witnessed was with the IgM classes that had dropped to a 25% seropositivity at week 27.

Quantitatively, the titer of all classes of IgG and IgM all peaked at the week 4 to 5. From here the IgM class started to decrease, albeit at different rates. During the Convalescent period the N and RBD-IgM waned off quickly. Disparately, the average value of IgM and the N-IgM titers decreased below the threshold value before the week 11, and RBD -IgM at week 16-17. On the contrary, all IgG classes titers remained in high amounts when compared not only to the cut-off value, but also to their own peak value.[viii]

3.1 Interferon Profile and its Relation to Disease Severity

The severity of the disease is known to be dependent on the immune system profile. However, opinions differ on whether the severity and mortality of the disease is more likely in patients who have a so called “hyper-inflammatory” profile vs. a “hypo-inflammatory” profile.

Pro-inflammatory cytokines e.g. IL1β, IL6, IL8, and TNFα associated with Cytokine Storm have been shown to be increased in the plasma of moderate and severe COVID-19 patients. In addition, during follow-up on patients with favorable outcome, plasma IL6 levels decreased between the moment of admission to the hospital and the last observation carried. This suggests that such pro-inflammatory cytokines, particularly IL6, predispose towards a stronger immune reaction against the virus at the cost of tissue injury.

When looking at Interferons, on the other hand, results show that after in vitro stimulation of immune cells from diseased patients, IFNα levels lower than 2.1 pg/ml and IFNγ levels lower than 15 IU/mL at admission to the hospital were associated with more complications during hospitalization.

Interferons act as a key link between the innate and the adaptive immune response. Type I IFNs (IFN α and β) are secreted by Plasmacytoid Dendritic Cells, while type II IFNs (IFNγ) are predominantly produced by Natural Killer Cells (NK Cells) and in minor proportion by T cells and macrophages. Both IFNs are involved in numerous important antiviral precaution measures the body takes such as inducing apoptosis of infected cells and activating macrophages, NK cells and T lymphocytes.
Different pathways could contribute to the decreased amount of IFNs in severe COVID19 patients, from concealed viral production invisible to PPARs to direct synthesis of structural and nonstructural viral proteins that antagonize IFN signaling.
This gives the impression that a low level of these molecules, resulting in a lack of some or all of their important antiviral functions, increases the risk of complications.[ix]

Upon trying to decipher these results the relationship becomes clear and even stronger by the knowledge that an in vitro treatment with IFNα restored IFNγ secretion in COVID19 patients while the secretion of pro-inflammatory cytokines IL6 and IL1β remained stable or decreased, respectively. The connection between IFNα and IFNγ is thought to be that innate cells produce less IFNα, and consequently NK cells produce less IFNγ.
Moreover, IFNγ level was also confirmed to be an independent factor for disease complications.
This shows that these cytokines, if decided to be used in some way as part of a treatment in the future, do not need to be individually concerned with when formulating a drug. Since it seems that the use of only IFNα may affect the rest of the cytokine profile in a physiological manner.
As a last note on this matter, it has been shown that the virus induces an aberrant IFNα response in cultured cells, characterized by a delayed antiviral response which may provide a window for virus replication and an improper recruitment of inflammatory monocyte macrophage populations.[x]

With all of the above mentioned and with the knowledge that a low IFNγ is positively correlated with disease severity, it is perhaps worth studying whether a mass use of QuantiFERON (Interferon-gamma release assays, or IGRA) may help clinicians provide adjusted treatment and medical care in accordance with the patient's IFN profile instead of the patient's symptomatology.

One must mention the possibility of using the IGRA assay as a mass screening tool for the population's immunity. For this one must know if the IGRA results correlate with the antibody titer, in both quality and quantity, as the latter is a well-known and accepted mesure for immune response and strength of immunity. Results seem to go both ways, as a study on healthcare workers in a nursing home showed. The health care workers were checked by both tests at three time points: before 1st dose, before 2nd dose and after 2nd dose of the BNT162b2 mRNA COVID19 vaccine. It was found that even though there was a very high concordance between antibody and the IGRA assay in the ability to detect immune response to COVID19 there was a relatively low quantitative correlation. This means that the presence of a patient's immunity is detectable in both tests, however, the level of immunity, which changes with time, is insufficiently reflected by the IGRA assay.[xi]

3.2 GM-CSF, CXCL10 and IL10 profile and its Relation to Disease Severity and Prognosis

In complete contrast with previous evidence showing that disease severity is positively correlated with a low IFN in the serum and by that supporting a hypo-inflammatory or an Immunosuppressive picture leading to increased severity of disease, it has been shown that an elevated amount of GM-CSF (Granulocyte-Macrophage Colony-Stimulating Factor), CXCL10 and IL10 were associated with a higher mechanical ventilation (MV) time when comparing between patients with Community Acquired Pneumonia (CAP) and patients with COVID19 pneumonia. However it is important to note that only GM-CSF was independently associated with a longer duration of MV with no adjustments. IL10 and CXCL10 were independently associated with a longer duration of MV only when adjusted for respiratory severity.

GM-CSF is secreted by epithelial cells from injured tissue or leukocytes, to induce survival, proliferation and/or differentiation of myeloid cells, playing a critical role in regulating antimicrobial defense. However, an aberrant production of GM-CSF may result in excessive inflammation and tissue damage, mainly by macrophage M1 polarization and overactivation. CXCL10 is a pro-inflammatory Th1-chemokine driving migration to the site of infection of Th-1 T-cells, monocytes and neutrophils that express its receptor. Production of CXCL10 has already been shown to be increased in SARS-CoV-1 in the past. Furthermore, Plasma

concentrations of CXCL10 were recently reported to predict disease progression in COVID19.[xii]

The above-mentioned can perhaps point at a possible direction of treatment. Blocking GM-CSF (Lenzilumab) and CXCL10 (Eldelumab/MDX-1100) may represent an attractive therapy option likely to dampen the dysregulated immune response that could be driving the duration of MV.

The role of IL10, either beneficial or deleterious remains a difficult issue since it is the only anti-inflammatory cytokine implicated here and therefore does not fit the picture. However, further research is warranted.

Perhaps the dots can somehow be connected now on the debate over the hyper/hypo-inflammatory profile and its relation to disease severity: Perhaps a low IFN profile combined with a high pro-inflammatory IL, CXCL10 and GM-CSF profile may contribute to disease severity.

3.3 Past Exposure to the Virus and the Matter of Natural Immunity vs. Immunity Conferred by Vaccination

Another factor to be considered when considering susceptibility is, ironically enough, past exposure, especially in the context of vaccination.

It was reported that subjects who were sick with COVID19 reached an "immune plateau" after the first dose of BNT162b2 (Pfizer/BioNTech) mRNA vaccine, whereas those not previously sick needed a second dose of the vaccine in order to reach their plateau. Furthermore, the plateaus were not equal, The COVID19 naive group required a second vaccination to obtain titers equal to or higher than the cut-off titer. Furthermore, even after the second vaccination, COVID19 naive participants had lower neutralizing antibody titers compared to COVID19 recovered participants.

The antibodies induced by the vaccine did manage to cross-neutralize the variants B.1.1.7 (alpha variant) and B.1.351 (beta variant), but the neutralizing capacity and Fc-mediated functionality against B.1.351 was consistently lower than to the homologous virus.

These results may imply that the vaccine is in some way limited in its capacity to confer protection both qualitatively (expressed by antibody titers) and by the variation in capacity to neutralize different variants.[xiii]

Could this perhaps be due to the single a single antigen being used in vaccination? As natural infection causes a polyclonal T-cell activation towards various viral antigens (many of which not yet discovered) – perhaps this wider and more extensive approach can provide better immunity.

An assay containing multiple viral peptides, thus containing multiple immunogenic antigens, combined with a T cell proliferation assay and IGRA, is being investigated with the purpose of assessing the immune response to the virus.

The potential here is, on the one hand, for conferring immunity and on the other hand to check each patient's level of immunity.

After the patient's serum is incubated with the peptides - one could measure the degree of T cell proliferation and the level of IFNγ (which, as mentioned above, is inversely correlated with disease severity). Both of these pieces of information could not only provide valuable information about each individual patient's state of immunity and capacity to fight the virus but also, potentially, to use as an extracorporeal tool for vaccination using polyclonal T cell activation.[xiv]

4. COVID19 and its Relation to Other Diseases

4.1 Smoking and COPD

Since the virus causes initially a respiratory disease, since the way of entry is mostly through the respiratory system and since the route of infection is via respiratory droplets, it is not unlikely that other respiratory diseases can affect variables such as prognosis, morbidity and mortality from the disease.

When discussing smoking, specifically, an increased expression of ACE2, the receptor for the virus's Spike protein, a study that analysed human data sets that pertains to lung tissue from a cohort of smokers undergoing thoracic surgery for transplantation, lung resection, or nodule resection vs. a pathologically normal lung tissue found that the lung samples from patients who reported smoking with the greatest number of pack years expressed the highest levels of ACE2. And since one might also want to control for age and sex - ACE2 expression was equivalent between men and women and between young (<29 years) and elderly individuals (>70 years), which suggests that the increased morbidity of men and older patients with COVID19 is unlikely to result from inherent differences in the basal level of ACE2 expression in the lung. On a more positive note, in a cohort of patients comprising either current smokers or former smokers who had refrained from smoking for at least 12 months, quitting smoking was associated with a 40% decrease in ACE2 expression, demonstrating some extent of reversibility.

Furthermore, Cathepsin B expression, however not Cathepsin L, was consistently increased in mice and humans exposed to cigarette smoke. A meta-analysis found that across the trachea, large airways, and small airways, Cathepsin B was in the top 11% of genes dysregulated in the respiratory tract of cigarette smokers (ACE2 was also included in that list). This is relevant because in this way the virus can utilize an alternative pathway in TMPRSS2-negative cells.[xv]

Chronic Obstructive Pulmonary Disease's (COPD) relationship with smoking is obvious and unnecessary to elaborate on. Which is why it is not unreasonable to assume that the above mentioned cellular changes are also to be expected in COPD patients (at least in those in which the COPD was caused by smoking). However, it would also be wise to mention the other changes found specifically in COPD.

It has been shown that although the number of alveolar macrophages is increased in patients with COPD, their phagocytic ability, compared with that in smokers without COPD, is diminished.[xvi] In addition, Polymeric immunoglobulin receptor (pIgR), a receptor important for IgA transcytosis and secretion, was found to be decreased in COPD patients on immunohistochemical staining of human broncho-epithelial cells (HBEC)[xvii]. The action of plgR is done by means of transcytosis of the receptor. The dimeric IgA/pIgR complex is transcytosed across the epithelial cell on the apical pole, where a proteolytic cleavage releases dimeric IgA bound to the main part of the extracellular domain of the pIgR, to form the secretory IgA. Therefore, a decrease in plgR results in a decrease in IgA, and this decrease in IgA, an antibody type well known for its protective role in the immune response of the respiratory system, can also contribute to the increased susceptibility and morbidity from the disease due to lack of humoural protection in the lumen of the respiratory system.

4.2 Diabetes Mellitus

Diabetes Mellitus is a complex disorder with many elements that have the potential to influence the prognosis of the disease. Therefore it is important to a look at each pathophysiological aspect of Diabetes and see how it relates to the disease caused by the virus. However, before one dwells into that complexity it is important to first make sure that a relationship does, indeed, exist between Diabetes and the virus. It was shown that the prognosis is grimmer in Diabetic patients, with higher admission rates to hospitals, development of severe pneumonia and higher mortality rates, when compared to patients without comorbidities.[xviii]

Which disease elements might contribute to this worsening of prognosis?

When one takes a look at hyperglycemia, the main characteristic of all types of Diabetes, which even when occurs in a short term manner, still compromises the immune system on various fronts. Hyperglycemia activates protein kinase C, which in turn inhibits neutrophil migration, phagocytosis, superoxide production and microbial killing. Furthermore, also pertaining to neutrophil activity, neutrophil extracellular traps are reduced in concentration, Toll-Like Receptor (TLR) expression is induced and neutrophil apoptosis is inhibited[xix] (which is crtical for the deployment of the extracellular traps). Since neutrophils are the very

first responders to any type of infection in the body, this reduced function may contribute to a much harsher initial infection, which in turn, lead to a more severe late disease and consequently a worse prognosis.

Furthermore, IL6, one of the cytokines mentioned above with a clear connection to disease progression and prognosis, was found to be increased in those with Diabetes[xx] (in addition to other pro-inflammatory molecules and various Acute Phase Reactants, which would cause a hyper-inflammatory state).

When inspecting the influence of hyperglycemia on the humoral immune system, it has been shown that hyperglycemia causes direct glycosylation of proteins and can alter the structure of the Complement System; these changes, in turn inhibit immunoglobulin mediated opsonization of the pathogens and complement fixation which in turn decreases phagocytosis[xix].

However, what of those patients who have their glycemic state under control? Those who maintain their disease poorly would undoubtedly have a worse prognosis. But what of those that manage their disease well?

Here, there are evidence that the virus actually invades the beta cells of the pancreas, which would theoretically cause the worsening of the Diabetes. This is unlike the previous example where Diabetes was influencing the state of COVID19 prognosis. Here, on the other hand, SARS-CoV-2 actually influences the course of the Diabetes. Showing a bidirectional relationship.

This sounds counterintuitive, since the receptor for the virus, ACE2, is thought not to be present in the pancreas. However, by detection of mRNA corresponding to the ACE2 protein[xxi], it has been found that this mRNA is also produced in many organs but the bronchus e.g. ileum, jejunum, duodenum, testis, lung, pulmonary blood vessels, prostate , pancreas and more.

This shines an entire new light on the matter of COVID19 pathogenesis, previously thought to occur almost exclusively in the bronchus. However, these results imply that the virus can invade many other types of tissues where the disease cause other types of damage.

But what of these results? One can now ponder what effect the virus might have on each of the above mentioned tissues. When one however mentions the pancreas, especially in the context of a diabetic patient. It is not unlikely that a severe worsening and exacerbation would occur in response to viral infection of the beta cells. For example, in a study[xxii] conducted on 658 hospitalized patients with confirmed COVID19, the majority of whom had elevated levels of D-dimer, C-reactive protein (CRP) and IL6 and other pro-inflammatory

markers, it was shown that COVID19 infection caused ketosis or ketoacidosis and induced diabetic ketoacidosis (DKA) in patients with diabetes. DKA occurs with increased ketone production in a combination with decreased ketone utilization. It is is a potentially fatal metabolic complication attributable to uncontrolled blood glucose more common in people with type 1 diabetes, albeit also possible in type 2 diabetes in combination with viral infection.
In the current study, 42 patients with COVID19 had ketosis, including 27 who did not have diabetes. Meanwhile, five patients with COVID19 showed ketoacidosis, 3 of whom had diabetes and 2 that did not have diabetes, which suggests that COVID-19 might accelerate lipolysis and induce ketosis, with further resultant development to ketoacidosis.

To conclude this section, one can observe that the immunological profile of the diabetic patient, expressed by the increased pro-inflammatory markers, specifically IL6, not only worsens the prognosis on a statistical level, but such immunological profile changes can also be shown to influence the cellular and humoral immunity. In addition, the prognosis itself changes significantly once one takes into account the increased danger of getting DKA and its related risks.

4.3 Asthma

A well known obstructive pulmonary disease. Asthma's pathophysiology is based on a type I Hypersensitivity mechanism. It is characterized by inflammation in the respiratory mucosa from the trachea to terminal bronchioles, but with a predominance in the bronchi. The pattern of inflammation in asthma is characteristic of allergic diseases, with similar inflammatory cells seen in the nasal mucosa in rhinitis, those being mostly Mast cells, Eosinophils, and IgE secreting Plasma cells.[xxiii]
It would seem reasonable to assume a that a connection between Asthma and COVID19 severity exists a-priori since it has been a well known fact that chronic Asthma patients have a higher severity of other respiratory diseases of viral origins e.g. Influenza. However, it was actually shown by a meta-analysis that compiled and compared the results of 14 studies (after starting with 457 studies and excluding 443 of them by various unfulfilled criteria) that

involved a total of 17694 participants that asthmatic patients had neither, in fact, a higher risk of becoming seriously ill nor had a higher mortality rate of COVID-19 disease[xxiv]. These results come against one's expectations that Asthma should, in fact, worsen disease severity and prognosis.

It was shown, again, that other variables the asthmatic patients (but not necessarily risk factors per se) were more connected with COVID19 than the Asthma itself. Patients with asthma who also had a diagnosis of COVID19 were older, and had a higher prevalence of hypertension, dyslipidaemia, diabetes, obesity and smoking habits than asthmatic individuals without COVID-19. By contrast, atopy-related factors such as rhinitis or eczema were significantly more frequent in patients without COVID19. The higher prevalence of hypertension, dyslipidaemia, diabetes and obesity was further confirmed in those patients requiring hospital admission, as compared with those who only required outpatient management.

There is the matter, of course, of management of Asthma, which involves several pharmacological therapies. Perhaps, the regular use of Inhaled Corticosteroids (ICS) or biologic therapy, is, in some way, connected with disease severity or disease prognosis. It is not unreasonable to go down this route since ICS do not only reduce airway congestion and bronchospasm but also cause localized airway immunosuppression. Biologic therapy e.g. Omalizumab (anti-IgE antibody), Mepolizumab (anti-IL5 antibody) are also, by definition, immunosuppressants (albeit working on a different branch of the immune system and on a different T-cell lineage).

Pertaining to biologic therapy, despite increased severity and comorbidity of symptoms in the ear, nose and throat level, the need for COVID19 related hospital admission in patients on biologic therapy with asthma was relatively marginal - only 0.23%. Of note, one patient undergoing treatment with biologics died; he was a 52-year-old male with high blood pressure, diabetes mellitus and dyslipidaemia.[xxv] When dealing with ICS, the proportion of patients with asthma using inhaled ICS was significantly lower in individuals requiring hospital admission, 48.3% compared to 61.5%[xxv]. however, except for this piece of data, not much else was found that could possibly point a blaming finger on ICS as a possible risk factor for increased COVID19 severity or mortality.

One point that should be mentioned with some caution, due to its current status as only a hypothesis: in one study ACE2 and TMPRSS2 expression was actually lower in patients taking ICS than in patients not taking ICS[xxvi]. This result is should, of course, be replicated and reviewed. However, it should reassure the reader that the initial intuition about the

relationship between ICS, biologic therapy and COVID19 – is not as straight forward as one expected.

4.4 Malignant Disease

As mentioned above, the immune system profile has great significance when it comes to prognosis. Unsurprisingly then one would expect to find a worse prognosis when comparing the average cancer patient with the average immunocompetent patient. This results not only from the weakened state of the immune system but also from the cancer itself[xxvii], which successfully escapes the immune system regardless of its level of activity. The mechanism of this immune evasion involves several factors that helps evade the immunosurveillance and to also promote immune destruction. Regulatory T cells, myeloid suppressor cells, inhibitory cytokines and immune checkpoints are the major components of the immune system acting in concert with cancer cells and causing the subversion of anti-tumor immunity. [xxviii]
Upon examining the numbers, one sees that even before dealing with the prognosis, the susceptibility for nosocomial infections is highly increased in cancer patients and especially to respiratory pathogens and severe pneumonia and this is not only due to their immunosuppressive state due to the malignancy and antitumour therapy but also due to their prolonged hospital stays. In one study it was actually found that within 14 days, antitumour therapies were significantly associated with the occurrence of severe clinical events in COVID19 infection[xxix].
This comes as no surprise of course. when taking a closer look at the types of cancer involved, the results here are also hardly surprising, since COVID19 is a respiratory virus. Lung cancer was the most frequent type of cancer found, followed by esophageal cancer, breast cancer and Laryngocarcinoma[xxix].

How, however, can one deal with such a difficult case of immunosuppression in combination with tumor cell proliferation? Here are also several interesting angles. Several anti-cancer drugs might actually act in the benefit of the patient and fight against both the cancer and the virus.

For example, IFN-α2b, in one study, was given to all patients that had COVID19. It is important to note that while all patients received various prophylactic antibiotics, there was no case of proven or suspected bacterial infection. It was shown that none of the patients developed respiratory distress requiring prolonged oxygen supplementation or intubation, neither did any of them, consequently need intensive care treatment.[xxx] When one adds these results to the fact that IFN-α2b is also beneficial to AIDS-related Kaposi sarcoma, hairy cell leukemia, and melanoma[xxxi] - one can now begin to realize the dual purpose of these anti-cancer drugs not only for the cancer but also as anti-viral medications.

Lastly, another anti-cancer drug, Acalabrutinib, a selective Bruton Tyrosine Kinase (BTK) inhibitor, has been shown to actually improve prognosis in severe COVID19 patients. In a 14 day treatment course oxygenation level was improved in the majority of patients, CRP and IL6 levels quickly lowered, as did lymphopenia[xxxii]. At the end of Acalabrutinib treatment, 72.7% patients in the supplemental oxygen cohort had been discharged on room air and 50% of patients in the mechanical ventilation had been successfully extubated. Considering the fact that the original indication for Acalabrutinib is actually Mantle Cell Lymphoma[xxxiii], this shows potential in treating those patients that also have COVID19. The mechanism of action here is not entirely clear but seems to be connected to the drug's effect on the BTK of macrophages. BTK is activated by TLR of macrophages which in turn activate Nuclear Factor-kB (NF-kB), which trigger the production of multiple pro-inflammatory cytokines and chemokines. BTK inhibitors may prove effective in reducing excessive inflammation profile characteristic in severe COVID19 patients[xxxiv] by interfering with this pro-inflammatory pathway that begins in the macrophages' TLR – thus reducing pro-inflammatory signaling.

5. A Closer Look into certain Cell Types and their Relation to the Virus and the Prognosis and Clinical Picture

5.1 Natural Killer Cells

Called by many the CD8+ killer T cell equivalent of the innate immune system and for a good reason. There are many mechanisms by which NK cells operate in order to protect against viruses (and intracellular pathogens in general).

These are of the lymphocytes lineage and are produced from the same common lymphoid progenitor that gives rise to T cells and B cells. However these cells are part of the innate immune system, and by that they are functional without prior activation. Their function is mediated mostly by two types of receptors - Inhibitory and Activating receptors. Inhibitory receptors reduce NK cell activity by binding MHC type I molecules (the expression of which is reduced in stressed or infected cells) whereas the activating receptors recognize the molecules expressed/upregulated on stressed or infected cells. The eventual executed program of the NK cell occurs after integration of all signals received from all the inhibitory and activating signals from the plasma membrane.

In addition, these cells secrete cytokines such as IFNγ (which activate macrophages to destroy ingested microbes).[xxxv] Through all these mechanisms the NK cell provide early defense against intracellular microbial infections.

The mechanism of NK cytotoxicity is similar to that of CD8+ cytotoxic T cells where intracellular granules containing cytotoxic proteins are released via exocytosis. Perforin, a protein which facilitates the entry of other proteins called Granzymes, cytotoxic enzymes, into the cell which in turn results in apoptosis.[xxxvi]

It is important to note that a immunohistochemical difference exists between the effector (so called Cytotoxic) NK cells and the Cytokine producing NK cells. CD56-bright cells are the ones producing the cytokines and thr CD56-dim cells function as effector cells.

When speaking of NK cell phenotype compared with healthy controls, patients with COVID19 showed fewer Immature NK cells (CD56-bright,CD16-neg) and Maturing NK cells (CD56-dim, CD16-bright, NKG2A+) with a parallel increase in the CD56-dim, CD16-bright NK subset. Moreover the CD56-dim, CD16 bright NK cells were found to be higher in patients who had eventually died than in survivors.

In addition, there was an increase in PD1 expression, a well known immune checkpoint, on CD56-dim NK cells in sick patients in comparison with healthy controls.

When one takes a closer look at the proteins involved in NK function, the same study found elevated levels of sFAS, Granzyme B and Perforin in COVID19 patients in comparison with controls. However, lower levels of sFAS-ligand and Granulosyn were found, which makes it harder to draw conclusions from these data. [xxxvii]

To conclude, it seems as though the NK cell profile in COVID19 shows an increase in NK cell activation, which comes as no surprise since this cell type is an important branch of the intracellular defense of the innate immune system. On the other hand, the elevated PD1 levels in CD56-dim implies an exhausted NK cell phenotype, although as of today little data is available about the exact effect expression of immune checkpoints on NK cells' cell surface. Perhaps targeting PD1 on the surface of these cells could be a potential target for future therapies.

5.2 Neutrophils

A part of the innate immune system, the most abundant white blood cell in the blood and is the most immediate responder in any inflammatory process.

Originating from the bone marrow's hematopoietic stem cell (HSC) and has a few main functions. The first, as a phagocyte, is to phagocytose any infectious pathogen it encounters (especially those that are opsonized) and necrotic cell products.[xxxviii] The second is a unique feature of neutrophils – Extracellular Traps (NETs)[xxxix], by which this cell type can protect against extracellular pathogens. This is a form of "programmed cell death" in which the nuclei and chromatin of the neutrophil, attached to granules containing antimicrobial substances such as Defensins and Cathelicidins are ejected and harm any cells or tissues (even healthy ones).

The relationship between neutrophils and SARS-CoV-2 is still not completely clear and further research is required in order to further elucidate the mechanisms involved in the pathophysiology. However, an elevated level of neutrophils was observed in the nasopharyngeal epithelium of infected individuals and later on in the distal parts of the lung. Furthermore, blood levels of several neutrophil markers such as LCN2, HGF have been identified as significant indicators and markers of critical illness and mortality[xl].

In severe cases of COVID19 a so-called "Emergency Myelopoiesis" was documented, with an increased release of pre-Neutrophils and pro-Neutrophils (done via flow cytometry) characterized by an CD10-lo, CD101-negative immunophenotype[xli]. This is reminiscent of

the so-called “Left Shift” one sees in septic patients. In addition to this Emergency Myelopoiesis, the genetic transcriptional programing resembled those of immunosuppressed individuals and controls or in mild cases of the disease[xlii]. The causal relationship is in this case is however not certain. Is it the dysregulated neutrophil programming and Emergency Myelopoiesis that contribute to the worse prognosis or is the severe case of the disease characterized, by some yet unknown mechanism, in this faulty cellular activity and increased immature cell count? Further research on the exact causal relation between the two is warranted.

When one inquires further into the cellular mechanisms involved, one finds further evidence of cellular dysfunction. This perhaps can contribute more to the understanding of the pathophysiology. Elevated levels of CD63, whose expression is tightly linked to increased levels of degranulation and granule production, was documented. In addition elevated levels of NETs were reported COVID19 patients in comparison with healthy individuals, a finding that also positively correlates with disease severity. The mechanism is, according to one study, again involves the ACE2 receptor. The virus can induce NETs formation via activation of ACE2 Serine Protease Axis. This in turn, via the direct action of NETs on lung epithelium, causes the apoptosis of in some yet unknown mechanism.[xliii] Further evidence supporting this relation is the fact that serum of severe COVID19 patients was shown to activate neutrophils and induce NETs formation[xliv].

As a final note on this subject, with the current knowledge on the matter neutrophils are being considered as a possible target in COVID19 treatment. For example, Navarixin, an antagonist of the CXCR2 (a neutrophil receptor that induces NETs formation upon binding IL8) has showed potential in in ameliorating the symptoms of the disease in patients with concurrent COPD, asthma, bronchiectasis and other pulmonary diseases[xlv]

5.3 Macrophages

Like neutrophils and originating from the same HSC. This phagocyte also serves a crucial role in innate immunity. Originating from blood circulating monocytes, these differentiate into macrophages upon their arrival to tissues. There are the second responders to any inflammatory incident after neutrophils although some constitutive level of these cells always exists in tissues.

This cell type has several functions: phagocytosis of pathogens and their killing via Reactive Oxygen Species (ROS) and proteolytic enzymes inside their phagolysosome, Phagocytosis of cell debris, neutrophil remnants and more, cytokine secretion for endothelial activation with the end goal of further monocyte recruitment (this contributes to the positive feedback mechanism with which the immune system works by in general), angiogenesis, tissue repair and more.[xlvi]

According to immunohistochemical markers macrophages can be separated into Classical activation (CD14+CD16-), Nonclassical (CD14dimCD16+) and Intermediate (CD14+CD16+) and according to their location within the lung can be separated into Interstitial Macrophages (IM) which reside between the alveolar epithelium and the endothelial epithelium and Alveolar Macrophages (AM) which reside in the alveolar lumen itself in close contact with the epithelial cells.

The virus infects the lung - that is obvious - but what takes place there exactly? Cell tropism investigation discovered that on ex-vivo organs of human lung tissue the cells that contained the virus were type 1 alveolar cells, type 2 alveolar epithelial cell, AM cells, bronchiolar epithelium, submucosal glands and hyaline membranes. Furthermore, CD163 staining showed viral colonization of macrophages.[xlvii]

Interestingly enough, monocytes from patients that previously had the disease showed an increased glycolytic activity[xlviii] and an increase in lipid droplet accumulation (the lipid droplet accumulation is specifically connected to an increase in inflammatory mediators production). [xlix] Both of these metabolic alteration, in fact, promote viral replication.

Another difference in monocyte and macrophage profile was found between patients that needed ICU treatment and those who did not: it seems as though the cytokine profile present in the severe cases showed a higher Intermediate (CD14+CD16+) macrophage level with an increased level of IL6. This matches well the histopathological picture of a large inflammatory infiltrate seen in the lungs of COVID19 patients. Moreover,
it seems that perhaps this is one of the mechanisms involved in the lung dysfunctionality seen in the severe cases of COVID19 patients.[l]

On the other hand another study found an increased level of Classical (CD14+CD16-) macrophages, up to 95% as a matter of fact, with the Nonclassical (CD14dimCD16+) and Intermediate (CD14+CD16+) macrophage being significantly lower in severe cases and with the Intermediate (CD14+CD16+) macrophage level being a higher percentage in comparison with controls only in moderate disease.[li] This implies that there are different monocyte profiles in different disease severities.

From the findings above, the mechanism and reasons behind various monocyte profiles are still unclear. Further research is definitely warranted.
As a last note, in order to show the potential of the knowledge about this cell type and the importance and significance of more research to be done on this matter: An agent that inhibits DGAT1, an important enzyme in lipid droplet formation, was actually shown to reduce SARS-CoV-2 replication and pro-inflammatory mediators. [lii] Perhaps this mechanism could be used as a target for future treatments.

6. Immune Checkpoints PD1 and CTLA4

Immune checkpoints have been known for many years as a decisive part of the immune process. These cell surface receptors, upon binding to their corresponding ligands, decrease the activity of that immune cell to which it is bound to. It is not completely known how the expression of these cell surface receptors are regulated but the function of these would naturally come into action as an opposing force to the positive feedback immune mechanisms, which at the beginning of the inflammatory process cause a fast and efficient amplification process for many of the cellular processes involved in the immune response against a pathogen.
CTLA4 (cytotoxic T-lymphocyte-associated protein 4), expressed mostly on T cells and is a of the CD28 family and as such, it binds B7 found on many APCs such as macrophages, dendritic cells and activated B cells. Its affinity to B7, however, is much higher than that of CD28. By binding B7, an important co-stimulatory molecule, it delivers inhibitory signals and dampens the immune response.[liii]
In a similar fashion, PD1 (Programmed Cell Death Protein 1), expressed mostly on activated T cells, binds PD1L and PD2L on the cell surface of dendritic cells, macrophages, B cells, endothelial cells and tumor cells[liv]. Upon binding to its ligand, it down regulates the immune response by suppressing T cell activity and enhancing self-tolerance. It also increases rates of cell apoptosis of many T cells but not of T regulatory cells (Tregs), whose rate of apoptosis it decreases.[lv]

When taking a closer look into the role of these immune checkpoint and COVID19, one sees that the data does not point to one direction or pattern in particular. In one case it was shown that CD8_ (PD-1,−CTLA-4−, TIGIT−) T cells, defined as "non-exhausted", were found to be much lower in the group that had Severe disease[lvi]. This points towards the immunosuppressive portrayal of the severe disease since an increase in these "exhausted" CD8+ T cells would be able to act better against the virus. In addition to that, another study found that the expression of many immune checkpoint was also increased in severe and critical cases of COVID19, with the exception of PDL2[lvii].
This goes hand in hand with the results already presented in the past that an increase in activity of the PD1-PD1L/PD2L is present in many other respiratory infections caused by viruses, resulting in a decreased ability to produce important mediators such as IFNγ, TNFa and a loss of degranulation and cytotoxic abilities[lviii]. Perhaps this indicates that the COVID19 virus is nothing special in regards to its pathophysiology and that there is a general pathophysiological pattern involved in viral immunity that is leading to this pattern of immune checkpoint activity.

7. Memory T Cells and the Matter of Heterologous Immunity

Memory T cells are one of most discussed and crucial cell types in the context of COVID19 and for a good reason.
Unlike their naive counterparts these cells fulfill certain roles in antiviral protection that, albeit similar to the regular CD4 and CD8 cells, are crucial for patient's immunity in the long run. These provide a more rapid response upon re-infection and include the T Follicular Helper (Tfh) cells that can activate B cells. They are also present in barrier tissues, which in in the case of SARS-CoV-2, is the lung. Resident Memory T (Trm) cells are one of those types of cells. It is important to note that even though they play a crucial role in antiviral protection upon re-infection – the quantity of these cells is not represented in blood counts since they do not exchange their population. This makes it hard to monitor their activity[lix].
The mechanism by which memory cells work, in general, and the mechanism of Trm, specifically, is not completely understood albeit much is already known about it. The

importance of such cells is however crucial for antiviral protection not only quantitively (cell count) but also qualitatively.

This variation in quality could perhaps be illustrated by the concept of Heterologous Immunity, which in the context of SARS-CoV-2, could be defined as "Memory T cells whose corresponding antigen only cross reacts with SARS-CoV-2 antigens but was not originally created via an encounter with SARS-CoV-2". This would mean that the strength of immunity should be lower. This sparked much interest when CD4+ and CD8+ T cells reactive to SARS-CoV-2 proteins were discovered in naive individuals, or in blood samples taken years before the pandemic had even happened.

This has sparked much thought in the context of "Immunodominance" meaning the tendency of the immune system to be skewed to only a few pathogenic epitopes even though the pathogen itself has many more. Differences in the patterns of immunodominance have been described in individuals that had COVID19 vs. those with who were not, with those that were not exposed showing higher immunity to NSP peptide whereas those exposed to SARS-CoV-2 showed a higher immunity to N and M proteins.

It is also important to note that this new concept could perhaps be an obstacle in the quest of conferring strong immunity to the population, especially with to the new technology involving the T cell Assay discussed above relying on polyclonal T cell activation.

In conclusion, the significance of Heterologous immunity is not yet known but should definitely be taken into account when discussing SARS-CoV-2 immunity since with the concept of Immunodominance one can not only speak in the classic quantitative terms of antiviral immunity but also in qualitative terms related to specific pathogenic epitopes[lix]

8. Conclusion

SARS-CoV-2, the virus that causes the viral disease that has occupied a large extent of our lives in the past 2 years, causes a systemic disease with a wide range of symptomatology and manifestations ranging from a typical URT with cough all the way to severe respiratory symptoms and even ARDS, organ failure and death.

The progression of the disease is dependent on many immunological factors. With evidence going both ways as to whether a hyper-active or a hypo-active immune profile contributes to a worse prognosis. On the one hand a decreased level IFNα and IFNγ shows a worse prognosis, supporting the hypoactive picture and on the other, pro-inflammatory cytokines such as IL1β, IL6, IL8 were shown to be increased in severe disease, thus supporting the hyperactive hypothesis. Perhaps it is not a clear black and white picture but a more complex one with the many knobs of the immune system deviating from their correct setting in different ways and directions. It is nevertheless very useful to look into various immune profiles with the aim of targeting treatments.

It is also important to mention the significance of immunization that, even though required an extra dose to reach their "Immune Plateau", still clearly showed evidence of improved prognosis and reduced symptomatology and morbidity.

Educating this knowledge and further communicating of these ideas is yet another crucial part, not in the treatment of the disease, but in its overall prevention.

This work attempts to not only show the complexity of the SARS-CoV-2 and the disease it causes on its cellular level but also on its larger, physiological level. On both levels evidence in different directions were found, which again goes to show that more research of SARS-CoV-2 is warranted in the future for the understanding of both the virus, the immune system and the relation between them - especially in the context of prognosis and possible risk factors.

Perhaps with the help of information included in this work and with further advances that will be made in the future we will be able to better handle COVID19 cases and be better able to predict the prognosis of different people according to not only their background diseases, but also Immune Profiles that can integrate the many elements and branches of the immune system, with the goal of better targeting of treatment and better overall patient care.

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10. Biography

Barak Kesner was born in Israel in 11/05/1992.

He started medical school in 2016, received the Dean's Award on 2019 on the 3rd year. He is to graduate on the summer of 2022.

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