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REVIEW

SARS-CoV-2 infection: molecular mechanisms of severe outcomes to suggest therapeutics

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ABSTRACT

Introduction: The year 2020 was defined by the 29,903 base pairs of RNA that codes for the SARS-CoV-2 genome. SARS-CoV-2 infects humans to cause COVID-19, spreading from patient-to-patient yet impacts patients very divergently.

Areas covered: Within this review, we address the known molecular mechanisms and supporting data for COVID-19 clinical course and pathology, clinical risk factors and molecular signatures, therapeutics of severe COVID-19, and reinfection/vaccination. Literature and published datasets were reviewed using PubMed, Google Scholar, and NCBI SRA tools. The combination of exaggerated cytokine signaling, pneumonia, NETosis, pyroptosis, thrombocytopathy, endotheliopathy, multiple organ dysfunction syndrome (MODS), and acute respiratory distress syndrome (ARDS) create a positive feedback loop of severe damage in patients with COVID-19 that impacts the entire body and may persist for months following infection. Understanding the molecular pathways of severe COVID-19 opens the door for novel therapeutic design. We summarize the current insights into pathology, risk factors, secondary infections, genetics, omics, and drugs being tested to treat severe COVID-19.

Expert opinion: A growing level of support suggests the need for stronger integration of biomarkers and precision medicine to guide treatment strategies of severe COVID-19, where each patient has unique outcomes and thus require guided treatment.

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1. Introduction

The impact of 29,903 base pairs of RNA is staggering in lives, costs, and disruptions to nearly every person in the world [1]. As of March 2021, the >120,000,000 worldwide cases have had >2,700,000 deaths [2] for the Coronavirus Disease of 2019 (COVID-19). These lethality rates exceed that of the four common human coronaviruses (HKU1, NL63, OC43, and 229E), and the spread of the virus exceeds that of known human pathogenic coronaviruses (SARS-CoV and MERS-CoV) [3]. SARS-CoV -2 likely emerged from China in 2019, thought to arise from bat species of coronaviruses [4]. Like SARS-CoV-2 and SARS-CoV, the bat is a pool for potential human pathogenic coronaviruses, leaving the possibility that future pandemics could be mitigated through our understanding of how the virus results in pathogenicity and lethality. In this review, we lay out the clinical pathology and risk factors for severe COVID-19 followed by dissecting the unique biological mechanisms of the pathogen and host that give rise to adverse immune responses and pathologies. These insights on the mechanisms can give us a more robust understanding of treatment options of infectious agents.

2. COVID-19 clinical course and pathology

Early in the pandemic, SARS-CoV-2 was shown to spread person-to-person including transmission from asymptomatic individuals [5]. The primary route of transmission, respiratory droplet, yields a high transmission rate with many of the phenotypes associated with respiratory dysfunction [6]. The viral load is highest in nasal swabs, relative to throat swabs, with elevation around the presentation of symptoms and little difference between severity of infection [7]. Studies within close communities, namely within nursing homes, point to initial spread of virus by presymptomatic individuals before the elevation of symptoms [8]. While the virus has been seen in additional specimens including feces and blood [9], these sources of transmission have minimally been connected to pathology outside of some gastrointestinal symptoms.

Patients with COVID-19 can be divided into asymptomatic, mild, severe, and lethal. Understanding and integration of medical information in asymptomatic patients has been challenging, as these patients tend to not be tested or they develop symptoms after the initial asymptomatic designation [10]. Asymptomatic patients with COVID-19 shed virus for 15 to 26 days with decreased immune response as noted by IgG, neutralizing antibody, and cytokine

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Article highlights

- In some individuals COVID-19 causes severe lung infections associated with pneumonia.
- Severe COVID-19 is associated with lymphopenia and systemic immune activation.
- Immune activation can result in neutrophil extracellular traps and platelet activation associated with coagulation, endotheliopathy, and vascular dysfunction.
- Alterations of the immune system from genetics to secondary infections can yield elevated risk of immune pathology for COVID-19.
- Many of these immune activations can be targeted with therapies on the market, with testing underway to validate their usage in Patients with COVID-19 .
- Ongoing strategies are needed to mitigate vaccine evasion by SARS-CoV-2 variants.

profiles relative to symptomatic patients [11]. In general, asymptomatic patients are younger and do show signs of infection within computed tomography (CT) scans [12]. In children, the largest predicted group of asymptomatic patients, the clinical course is often mild with very few severe or lethal cases [13,14]. A small fraction of children progress on to developing a delayed but severe exaggerated immune response leading to a syndrome characterized by systemic vascular dysfunction and acute lung injury, described as Multi-systemic Inflammatory Syndrome (MIS-C) [15].

The respiratory infections that are caused by this virus range from mild to severe, and some patients even develop critical multi-organ failure with advanced lung disease. Throughout this pandemic, it has become clear that the immune system and other molecular pathways play a critical role in the outcomes of patients with mild or severe infections. Mild symptoms in COVID-19 include dry cough, fever, diarrhea, headache, muscle or joint pain, expectoration, and fatigue [16–18]. In the early phase of asymptomatic, mild symptom, or even more severe patients before requiring hospitalization there is a window for potential intervention in the early SARS-CoV-2 infection. This is particularly true for patients, through contact tracing, that seek testing and confirmation of infection before the onset of symptoms. Risk factors such as age, obesity, or other health factors can often dictate the need for intervention in individuals with early infection detection [19–21]. Several treatments for these patients have been suggested including anti-viral compounds/drugs, inhaled corticosteroids (such as inhalers), and supplemental oxygen [22]. Controlled, randomized trials of these interventions are nearly impossible and thus remain tough to assess their successes in preventing hospitalizations and severe COVID-19 [23]. Yet these front-line treatments in patients with contact tracing or routine testing are critically important in battling COVID-19 [20,24].

Patients hospitalized with COVID-19 typically have comorbidities and present with shortness of breath, hypoxia and evidence of viral pneumonia [25]. There is a slight elevation in hospitalizations for males relative to females [26,27]. Severe patients with COVID-19 are often those annotated as needing mechanical ventilation or more advanced therapy from the ICU. Patients with severe SARS-CoV-2 infections have increased proinflammatory macrophages, neutrophils, and cytokines that can create a cascade-like cytokine storm effect that leads to acute respiratory distress syndrome (ARDS), fibrosis of the lung, myocardial inflammation, pathological thrombi, renal complications, and other signs of multi-organ failure (Figure 1) [28]. Laboratory findings of these patients show lymphopenia (CD4 and CD8 cells), prolonged prothrombin time, and elevated lactate dehydrogenase C-reactive protein, creatinine kinase, D-Dimer, and plasma cytokines (IL2, IL6, IL10, GSCF, IP10, MCP1, MIP1A, TNFα) [29]. The elevation of these factors tend to be higher in patients with lethality [30]. Many of these factors point to a systemic immune complication.

2.1. Pneumonia and ARDS

The most common feature of severe COVID-19 is pneumonia. CT scans have shown that even asymptomatic patients can have subclinical inflammatory changes in their lungs, but the severity of changes is higher in severe patients [31]. The impact in the lungs is often bilateral, diffuse nonlocalized presenting similar to other non-COVID-19 bronchopneumonia cases [32]. Damage often is found in the middle, lower, and posterior regions of the lung presenting as ground-glass opacification on CT with a subset developing fibrotic streaks [33,34]. This presentation commonly occurs six to eleven days after initial symptoms [35,36]. The severe pneumonia cases present with increased linear opacities, and bronchial wall thickening with pleural effusion and peripheral immune cell activation [37]. Observation of chest imaging can stratify patients into two clusters, those with nodal viral associated pneumonia without changes to pulmonary compliance versus those with decreased pulmonary compliance and ARDS where damage is often exacerbated by ventilator use [38]. Unlike most ARDS, which occurs within days of symptoms, the onset in COVID-19 is more delayed, averaging 8–12 days [39]. This suggests that ARDS is likely the result of excessive inflammation and connected to Multiple Organ Dysfunction Syndrome (MODS) [40].

2.2. Lymphopenia

Lymphocyte levels in the blood of patients with COVID-19 have been shown to be an indicator of prognosis, with lower lymphocyte levels leading to worse outcomes [41]. Similar to SARS-CoV-2, both MERS-CoV and SARS-CoV were reported to upregulate apoptosis of T lymphocytes [42] with patients being shown to have higher levels of pro-apoptotic factors including plasma Fas-Ligand and cleaved intracellular caspases within both CD4+ and CD8 + T lymphocytes [43].

Another likely mechanism of lymphopenia is pyroptosis, which is a programmed inflammatory cell death driven by inflammasome production of IL1β. Compared to apoptosis, pyroptosis results in the spilling of cellular contents into the extracellular fluid, which can be followed with elevated blood markers like lactate dehydrogenase [44]. Severe patients with COVID-19 have a significant increase in lactate dehydrogenase levels indicating cellular damage; however, the IL1β level was undetectable in both moderate and severe cases [45]. Widespread pyroptosis of lymphocytes is often accompanied by an increased IL1β level in the blood, indicating amplified inflammasome activity [46].

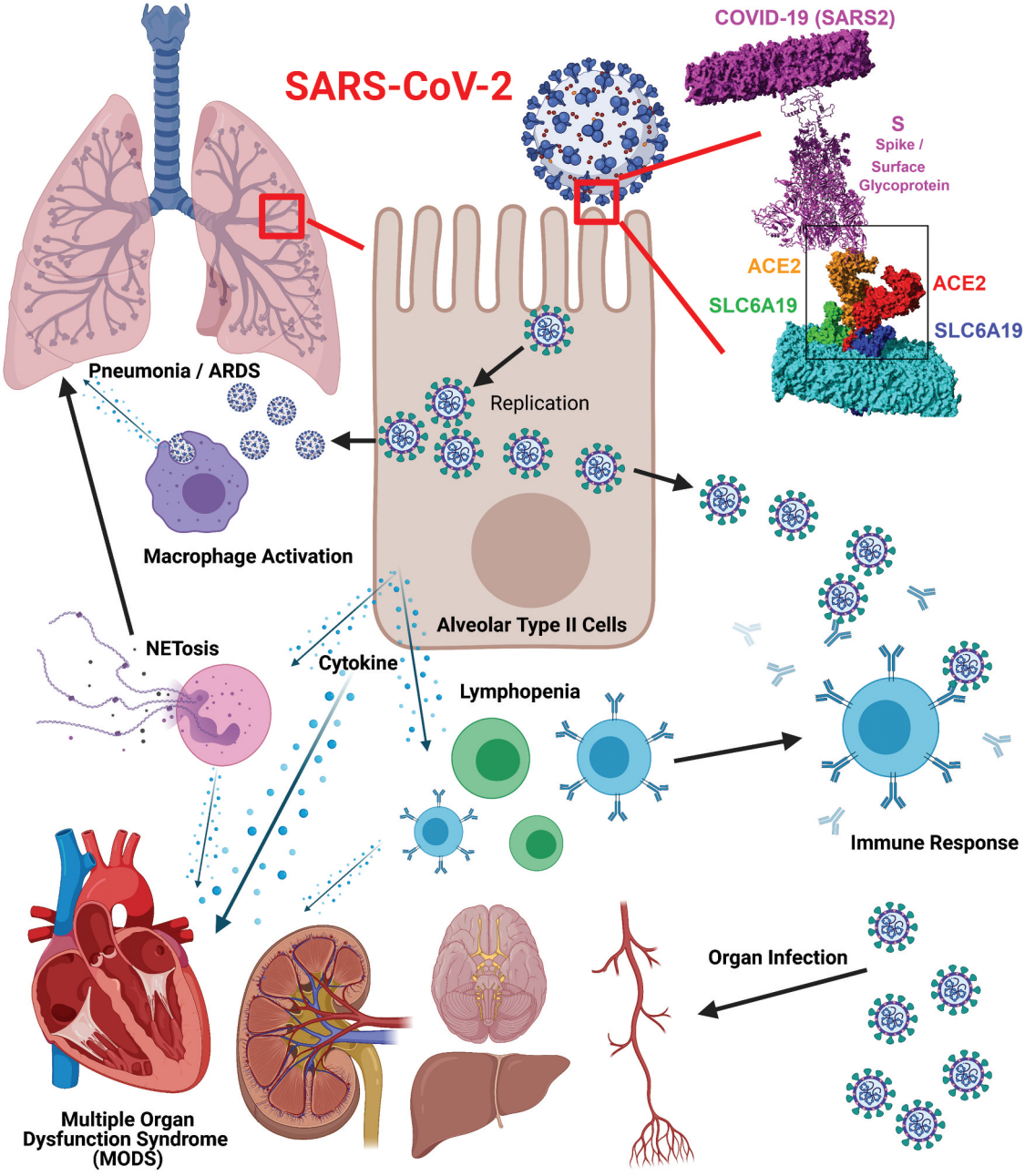

Figure 1. **Schematic of SARS-CoV-2 entry to cytokine storm elevated multiple organ dysfunction syndrome (MODS)**. Figure was generated in BioRender with additional modified images from our previous work [157] .

Another possible mechanism of lymphopenia is T cell exhaustion, where persistent activation of T cells drives a non-responsive immune cell state [47]. Interestingly, CD8 + T cells obtained from severe patients with COVID-19 had upregulation of specific T cell exhaustion markers including CCL4 and GZMB [48], indicating a degree of exhaustion and resultant lymphopenia. The CD8 + T cells most likely mediate the tissue damage at the onset of the disease course, and then persistent viral antigen results in exhaustion contributing to the lymphopenia. The exact mechanisms as to how SARS-CoV-2 induces lymphopenia is still being fleshed out; however, viral-induced upregulation of pro-apoptotic factors in the plasma, pyroptosis, and T-cell exhaustion likely play roles in the pathogenesis.

2.3. Systemic immune activation

The hyper-immune response is thought to be a contributor responsible for severe COVID-19 disease manifestation. The observation of robust signatures, both cytokine elevation and immune cells, in the blood of severe patients with COVID-19 suggests modulation of innate and acquired immune responses. The innate immune response is triggered by various damage-associated molecular patterns and pathogen-associated molecular patterns that get released after a viral infected cell dies. The innate immune response is activated through receptor activation, such as toll-like receptors (TLRs), in cells near those infected [3]. This triggers cytokines, leukocyte recruitment, interferons, and other antiviral

responses [49]. As the innate immune system uses more unspecific methods of viral clearance, the adaptive immune system gears up for T-cell and B-cell specific defense through an antibody and cytotoxic mediated offensive [49,50]. SARS-CoV-2 can upregulate or downregulate various aspects of the immune system that can predispose infected patients to severe outcomes involving innate immune system activation and adaptive immune system suppression [51,52].

Notable cytokines IL6, IL10, IL2, IL7, G-CSF, IP10, MCP1, MIP1A, and TNFα are produced as a result of severe COVID-19, with a suppression of type I interferon [53,54]. IL6 elevation is often associated with a hyperinflammatory state, which often correlates with MODS and ARDS, driving cytokine release/storm syndrome [55,56]. Similar cytokine storm syndromes have been studied within juvenile idiopathic arthritis and systemic lupus erythematosus [57]. The profile of cytokine production, end organ damage, and systemic immune activation has overlaps to additional immune pathologies including macrophage activation syndrome, hyperferritinemic sepsis, and hemophagocytic lymphohistiocytosis (HLH) [58–60].

2.4. Neutrophil extracellular traps

The overactive immune system of patients with COVID-19, and the cytokine profile, results in a system wide response known as Neutrophil Extracellular Traps (NETs). NETs are a secretion by neutrophils that contains histones, DNA, and multiple antimicrobial proteins that are used as a last resort to trap and kill bacteria and viruses [61,62]. NETs have been shown to be driven by platelets and genes such as *TLR4* [63], activating a controlled cell death process that begins with elevated intracellular reactive oxygen species followed by dissolving of the nuclei, granules, and cell membrane [64]. While beneficial as a last resort for the innate immune system, NETs present many chronic issues, primarily involving vasculature and capillaries in tissues, a process termed NETosis [65].

Sera from patients contains components of NETs, including citrullinated histones and myeloperoxidase-DNA, and is able to activate cultured neutrophil NET production [66,67]. The isolation of neutrophils from the peripheral blood of patients with COVID-19 has NET activation [68] suggesting the cytokine profile drives NET production in the blood and not just within lung tissue. The NETosis in COVID-19 has been connected to epithelial cell death in the lung, global thrombosis, elevation of vascular occlusion, and mortality [69–73]. NETs, along with platelet activation, have thus been suggested to be a critical intermediate bridging immune activation to coagulation complications of COVID-19.

2.5. Elevated coagulation, endotheliopathy, and vascular dysfunction

Hypercoagulability plays a large role in the prognosis, management, and outcome of SARS-CoV-2 infected patients. Documented presentation of thromboembolic COVID-19 infection includes stroke [74], myocardial infarction [75], pulmonary embolism [76], and deep vein thromboses [77]. Disseminated intravascular coagulation has also been noted in severe cases [78]. These thromboembolic events associated with SARS-CoV-2 infection have a predominance in severe cases where patients have underlying comorbidities. Severe cases result in sepsis and a cytokine storm [79], which places patients into a hypercoagulable state. Pro-inflammatory cytokines, such as IL1 and TNF-α, are involved with hyperactivation of platelets and downregulation of antithrombotic pathways [80]. These signals are upregulated during cytokine storm, shifting to a pro-coagulable state. Sepsis upregulates platelet adhesion molecules and increases circulation of platelet-leukocyte aggregates [81], which boosts coagulability.

COVID-19 contributes to vascular dysfunctions through endotheliopathy, the overactivation of endothelial markers of damage response [82,83]. Endotheliopathy is common to severe sepsis, a result of excessive stimulation of the sympatho-adrenal axis [84]. As thrombocytopathy and endotheliopathy have similar timing in COVID-19, it is hard to decipher the contributions they have on each other, but it is likely that the two work together to drive many of the coagulation pathologies of COVID-19 [85]. Thus, the combination of exaggerated cytokine signaling, NETosis, pyroptosis, thrombocytopathy, endotheliopathy, and platelet activation create a positive feedback loop of severe damage in patients with COVID-19. Interestingly, *in vitro* studies have shown that the preincubation of COVID-19 plasma added to the blood of healthy subjects blunts platelet activation, further studies are needed to tease apart the clinical relevance of this finding [86].

2.6. Neurologic complications

Severe patients with COVID-19 have shown multiple neurological phenotypes including confusion, headache, encephalopathy, and agitation [87–89]. Most of these neurological symptoms occur early in the clinical course and often in severe patients with COVID-19 [90]. The most distinguishing feature of COVID-19 in a subset of patients is the loss of smell (anosmia) and taste (dysgeusia) that can occur even in the absence of other symptoms [91]. The mechanisms of COVID-19 anosmia and dysgeusia are poorly understood, with evidence pointing to inflammation and sustentacular cells altering nerve cell signaling [92]. Consistent with vascular dysfunction, around 5.7% of COVID-19 cases have been reported to have large-vessel stroke or cerebrovascular disease [74,93].

2.7. Chronic symptoms

Two months after acute infections, 87.4% of the patients still report chronic symptoms including fatigue, labored breathing, joint pain, and chest pain [94]. The severe pneumonia and inflammation results in chronic lung pathologies including fibrotic tissue, bronchiectasis, and pulmonary vascular disease [95]. Many of the high risk and vulnerable patients suffer damage from an overactive immune response, which sustains cytokine production in the lung tissue, causing resident immune cells to infiltrate and do continuous damage [96]. The NETosis, pyroptosis, thrombocytopathy, endotheliopathy, and vascular dysfunction result in excessive tissue damage that will likely take months to recover. This will result in increased complications of heart,

kidneys, and liver while putting the blood-brain barrier at risk for cerebral complications [97–100].

2.8. Atypical manifestations

Patients can present with diverse phenotypes that are either connected to COVID-19 or to additional physiology that manifest as atypical manifestations. Case studies are a source of the broad atypical presentations including delirium, localized pain, hemoptysis, appendicitis, mesenteric adenitis, encephalomyelitis, balance issues, conjunctivitis, and ocular dysfunction to name a few [101–-101–110]. Elderly and those in long-term care facilities with many additional pathologies independent of COVID-19 can often present atypically, including hypothermia with fluctuating temperatures and falls [111]. Literature review of atypical manifestations is rather challenging as the early COVID-19 cases often annotate atypical presentations, but as cases expanded, these conditions have elevated in observance. Abdominal, GI, and testicular pain have been reported in several patients [112], which might be related to the high level of ACE2 in these tissues [113,114]. Some patients develop cutaneous changes including rash, acro-ischemia, chilblain-like edematous, small monomorphic vesicles, and androgenetic alopecia [115]. Guillain–Barré syndrome, a distinct acute autoimmune peripheral neuropathy [116], has been reported in around 4/1000 COVID-19 cases [117]. In young children and infants, MIS-C, an inflammatory overactivation that impacts multiple systems, has been seen for COVID-19 [118, p.,119]. In a small subset of patients, atypical Sweet syndrome can occur [120–122].

3 Clinical Risk Factors and Molecular Signatures

Both COVID-19 morbidity and mortality are connected to multiple risk factors. From hospitalization[20] to lethality [123], increase in age is a known risk factor. In a meta-analysis of thirteen studies, top-risk factors for SARS-CoV-2 infections include male sex, over 65 years old, and smoking, while cardiovascular disease (namely hypertension and coronary heart disease), type 2 diabetes, nonasthmatic chronic pulmonary disease, chronic kidney disease, liver disease and obesity contribute to severity and mortality risk [21,26]. Clinically, measurements of cytokines (IL2R, IL6, IL10, and TNF-α) and Sequential Organ Failure Assessment (SOFA) scores are associated with a higher mortality [123,124]. SOFA is a scoring system that quantitatively and qualitatively assesses failed organs, with proven utility in sepsis cases of adults and in-hospital mortality [125,126]. The integration of these risk factors into our molecular understanding can open the door for a more robust understanding of mechanisms and therapeutic options.

Secondary infections (viruses, bacteria, or fungi) have been identified in 6.9% of patients, with significant enrichment within critically ill patients, and has been shown as a risk factor for mortality in COVID-19 [127,128]. Common secondary infections include *Pseudomonas species, Escherichia coli, Klebsiella species, Staphylococcus aureus, Aspergillus fumigatus*, and *Candida albicans* [129]. This has led several clinical sites to consider the use of broad antibiotics when treating COVID-19 [130]. The elevation of IL6 has been suggested to correlate with the activation of several dormant viral infections including Epstein-Barr virus [131], but very little research has been done to suggest the additive role of other viruses on the pathology of COVID-19.

3.1. Human genetic risk factors

Human genetics has grown to allow for identifying the risks associated with disease within the genome, which can point to mechanisms of the virus to yield phenotypes/symptoms (Figure 2). Both the common variants (those found in >1% of the general population) and the rare variants (those not observed in the general population) contribute to COVID-19 severity. Sex, male vs. female, is determined by the genetics of the X and Y-chromosomes and is associated with multiple factors (cases, hospitalization, and death) of COVID-19 [132,133]. Genes on the Y-chromosome impact multiple biological processes of the immune and cardiovascular systems [134,135], including known roles of the *SRY* gene to regulate the expression of Renin Angiotensin genes including *ACE2* [136,137], the host receptor for the SARS-CoV-2 virus. *ACE2* expression in the lung alveolar epithelium is known to be impacted by both sex and age, with males having higher levels [138]. Testosterone, whose expression is regulated by the Y-chromosome, is thought to be the primary regulator of sex differences on *ACE2* expression levels [139]. Both testosterone [140] and the Y-chromosome [141] are known to modulate immune responses [142,143], which have been established to contribute to sex differences in the viral immune response [144–146] including COVID-19 [147,148].

Common variants linked to phenotypes are determined by using statistical strategies such as Genome Wide Association Studies (GWAS). A consortium of academic, clinical, and industry partners came together to form the COVID-19 Host Genetics Initiative (www.covid19hg.org/) to determine genomic regions contributing to COVID-19 outcomes [149]. The first GWAS report found two sites of the genome, 9q34.2 and 3p21.31, to associate with respiratory failure [150]. The 3p21.31 locus has been replicated and remains the strongest signal for severe COVID-19 [149]. This locus has multiple genes, falling near a block of cytokine receptors; however, further work needs to be done to narrow the association to causal genes and mechanisms. The 9q34.2 loci is that of the ABO blood alleles, gaining a large media following; however, the signal at this locus has been poorly replicated across independent GWAS, especially when normalizing for comorbidities, and does not reach significance in the newest meta-analyses. The Genetics Of Mortality In Critical Care (GenOMICC) consortium found genetic regions associated with immune process genes (*OAS1, OAS2, OAS3, TYK2, DPP9*, and *IFNAR2*), suggesting that many of the common genetic risk loci function through modulation of the host immune system [151].

The overactive immune responses, such as cytokine storm, are not unique to COVID-19, but are seen less frequently in other viral infections. Recently, our team discovered in a 16-year-old girl with Epstein-Barr virus (EBV) the process viral-induced genetics (VIG) [152], which modulated the immune system. VIG is where the virus suppresses host cell degradation of the virus by nonsense mediated decay, resulting in the activation of host

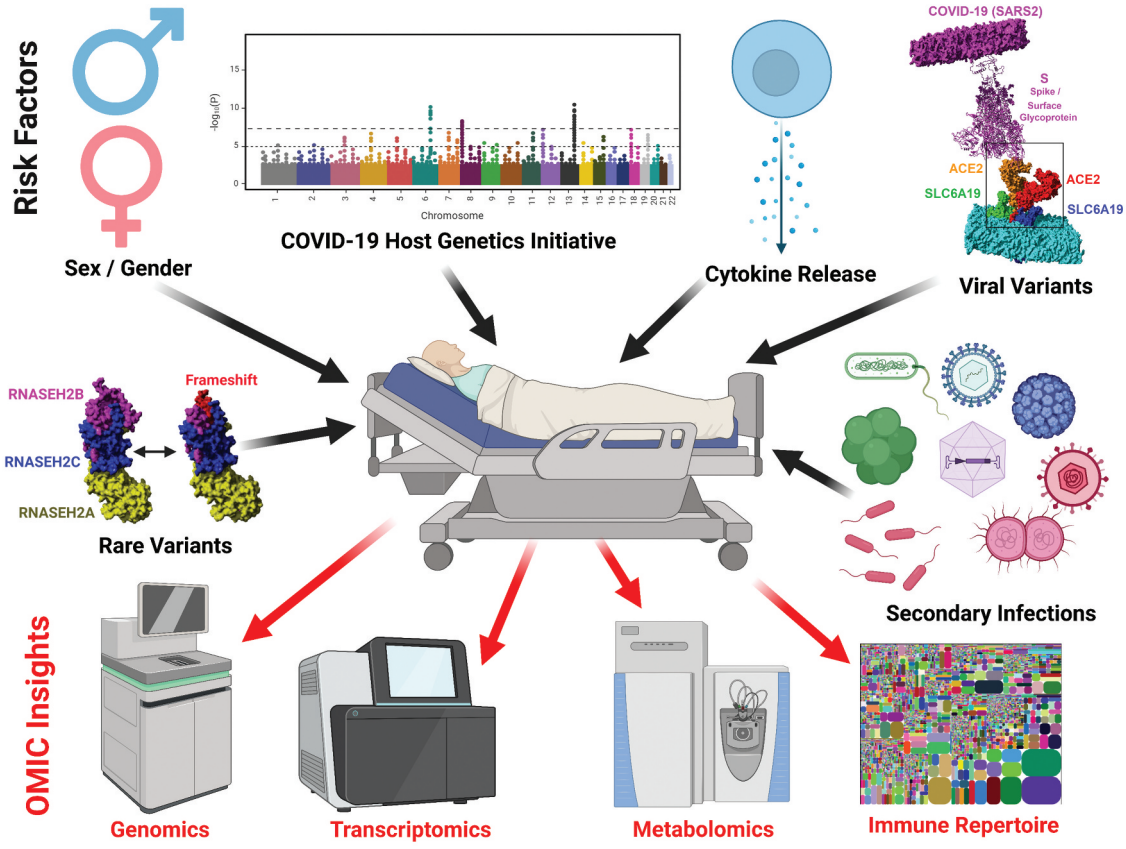

Figure 2. **Risk factors and omic insights into severe COVID-19**. Figure was generated in BioRender.

nonsense and frameshift variants that have dominant-negative function. In the case of our patient, we showed that EBV elevated a rare inherited variant in *RNASEH2B*, which resulted in hyperferritinemic sepsis similar to severe outcomes in COVID-19. Moreover, we have shown that the mechanisms for VIG are present in SARS-CoV-2 [153], but further work is needed to determine how rare variants interplay in COVID-19 VIG.

The most noted rare variants (<0.001 allele frequency) associated with severe COVID-19 are found within genes (*IRF7, IFNAR1, TLR3, UNC93B1, TICAM1, TBK1, IRF3, IRF7, IFNAR1, IFNAR2, TLR7*) connected to Type I interferon response and autoantibodies to the system [154–156]. In an assessment of variants connected to ACE2 and its co-regulating proteins (SLC6A19/TMPRSS2), 47 missense variants and two noncoding variants were identified that might impact biological processes of the proteins [157]. One of the variants in TMPRSS2, Val197Met, is decreased in prevalence in severe Patients with COVID-19 relative to controls [158]. Much work is still needed to connect rare variants to phenotypic outcomes in COVID-19.

3.2. Omics and COVID-19

Various strategies have been used to understand the cellular and systemic changes that occur because of infection with SARS-CoV-2. Clarifying essential factors necessary for infection and understanding the immune system response may be key to controlling and mitigating long-term effects of infection. Building on genomics, additional omics from RNA sequencing (RNAseq), Single-cell RNAseq, immune repertoire, proteomics, and metabolomics have been used to determined mechanisms and severity risks. Advancing knowledge in this area will undoubtedly have an overall benefit to human health surrounding infectious disease and the immune system complexity, discovering biomarkers and mechanisms of severity [159].

One important technique used to examine cellular activity and changes is RNAseq. RNAseq involved isolating RNA, converting it into copy DNA (cDNA), preparing the cDNA into libraries, sequencing the pieces of cDNA, assessing sequenced reads to human genes, and comparing mapped reads across samples. RNAseq of both the infected epithelial cells, peripheral tissues, and blood reveals a modified Type 1 and Type III interferon (IFN-I and IFN-III) response resulting in inappropriate immune activation [160]. Epithelial cells highly expressing *ACE2* and *TMPRSS2* have a tendency to be entry points of infection and be more adversely affected by SARS-CoV-2 [160,161]. Upon entering the cells, RNAseq shows that unlike other viruses, the IFN-I and IFN-III are not activated by SARS-CoV-2 but instead have a proinflammatory cytokine activation [160], suggesting potential mechanisms of intracellular immune modulation to non-viral proinflammatory response [162].

RNAseq can be advanced with additional methods including separating cells and performing RNAseq analysis on each individual cell (Single-cell RNAseq) or by amplifying and focusing RNAseq on the B and T cell recombination sites (immune repertoire). Profiles of single-cell RNAseq from patient samples can provide essential information to aid in decisions pertaining to future interventions. These Single-cell RNAseq were used from control datasets (non COVID-19) to determine the expression of

SARS-CoV-2 receptor (ACE2) and associated factors, to show details of cells infected by the virus [163]. Single-cell RNAseq of the blood of patients with COVID-19 has shown a suppression of B- and T-cells (lymphopenia), peripheral activation of monocytes, and T-cell exhaustion with a TNF/IL1β–driven inflammation signature in the peripheral blood [164–167]. Immune repertoire sequencing suggests the T-cell repertoire to decrease in early infection followed by an expansion in convalescent production phase, while the B-cell repertoire has isotype switching and clonal expansion [168]. These responses have been shown to last for 6–8 months [169], with further need for longitudinal follow up to determine the length of adaptive resistance to the virus. This immune repertoire sequencing combined with single-cell RNAseq has been pivotal in the rapid determination of neutralizing antibodies to the SARS-CoV-2 virus [170], opening the door for the rapid generation of viral neutralizing drugs.

Additional omic technologies used for COVID-19 have included metabolomics and proteomics, which commonly rely on mass spectrometry strategies. Metabolomics of severe patients with COVID-19 revealed marked changes in mitochondrial biology (including NAD and ATP), amino acid and fatty acid metabolism, and tryptophan-nicotinamide pathway [171–173]. Proteomics of patients with COVID-19 show an alteration of complement, coagulation, platelet degranulation, cytokine, metabolism, angiogenesis, and immune processes [174–177]. Integrating these diverse techniques over the course of SARS-CoV-2 infection shows a heterogenous biological response to COVID-19 marked by immune and blood issues, giving rise to the clinical pathologies of COVID-19 [178,179]. As our biomarkers strengthen to accurately predict those individuals with adverse outcomes in combination with early detection of SARS-CoV-2, it is possible to treat those individuals at highest risk with modulators (anti-viral, immune suppression, or risk normalizing treatments) earlier to prevent hospitalizations. Patients at outpatient clinics and testing sites may one day be screened for additional biomarkers of adverse immune responses, genetic risk, and clinical risk indicators to suggest precision treatment when sent home.

4. Therapeutics of severe COVID-19

When SARS-CoV-2 infections are caught early through contact tracing or routine testing, additional monitoring of risk factors and biomarkers can be supported with antiviral compounds to slow viral replication and immune modulators to prevent over reaction [22]. Remdesivir, a hepatitis C RNA-dependent RNA polymerase inhibitor, has been shown in a double-bind randomized trial to outperform placebo control in hospital recovery [180]. Use of anti-inflammatory drugs in non-hospitalized patients has conflicting data and suggests the need for further trials to determine the balance of risk and reward [181]. A balanced assessment of antiviral and immune suppression agents relative to the development of acquired future resistance and immune response needs more studies [182].

COVID-19 therapy has been primarily supportive and based essentially on supporting failing lungs, treating infections, and ameliorating the proinflammatory cascade using anti-inflammatory agents such as steroids and biologics. The adverse activation of the immune system has led to a promising avenue for treating severe COVID-19 to normalize the altered immune system signals (Table 1). Dexamethasone, a corticosteroid that suppresses the immune activation, has shown promise, reducing mortality within severe patients [183].

While small retrospective cohorts have shown potential benefit to convalescent plasma administration in other viral illnesses, prospective studies have failed to demonstrate this same benefit in COVID-19 [184]. Early reports were underpowered, but showed improvement in clinical status in five critically ill patients who received therapeutic convalescent plasma [185]. These early results led to the US food and drug administration (FDA) emergency use authorization (EUA) for hospitalized patients. Initial findings reported that in the first 5,000 patients infused, no increased adverse events were detected as compared to what would be expected from fresh frozen plasma [186]. However, this was a retrospective cohort, with no randomized control group, no central studying monitoring or systematic reporting of side effects, making it unclear how one can interpret these results. The theoretical risk exists of transfusing blood products to critically ill patients remain [187] with no prospective randomized controlled trials and a recent meta-analysis that have shown mortality benefit to administration of convalescent plasma [188–191]. In addition to a lack of proven benefit in hospitalized patients, the NIH has recently halted a trial of convalescent plasma therapy in emergency room patients with mild symptoms after an interim analysis showed no benefit.

Most recently, a randomized placebo-controlled trial has shown no benefit in patients receiving 'high titer' convalescent plasma [189]. Emerging data has shown up to 10.2% of patients admitted with severe COVID-19 may have anti-type 1 interferon autoantibodies that contribute to severity of disease [155], as discussed in the genetics risk section, leading to theoretical concern that convalescent plasma containing these autoantibodies could contribute to worsening of disease. The phenomenon of potentially worsening disease needs to be investigated in prospective trials.

Multiple monoclonal antibodies have been authorized for further study by the FDA. One tested in non-hospitalized patients with COVID-19, showed two neutralizing monoclonal antibodies against SARS-CoV-2 spike protein, used in a combined cocktail (REGN-COV2) were able to decrease viral multiplication [192]. However, SARS-CoV-2 antibodies have been shown to be needed to be administered early in

Table 1. Therapeutics of severe COVID-19.

Drug	Type	Use
Dexamethasone	Steroid	Suppress immune activation
Convalescent plasma	Antibody	Neutralize virus
Monoclonal antibody	Antibody	Neutralize virus
Tocilizumab	Anti-IL6 monoclonal antibody	Suppress immune activation
Anakinra	IL1 inhibitor	Suppress immune activation
Baricitinib	JAK inhibitor	Suppress immune activation
Remdesivir	Polymerase inhibitor	Suppress viral replication

exposure, often before patients come into clinical care for severe COVID-19. Another monoclonal antibody, an antibody blocker of CCR5 developed to treat HIV-1 infection, was administered as an open label compassionate use therapeutic for COVID-19 and showed some benefit in moderately ill patients with COVID-19 [193].

Early reports from China suggested an association between elevated IL6 with more severe disease course. Tocilizumab, an anti-IL6 monoclonal antibody, is currently used on label for cytokine release syndrome for chimeric antigen receptor T-cell therapy. First reports from China, in a retrospective cohort of 20 patients showed improved oxygenation, CT scan findings and laboratory normalization [194]. Further evidence has been evolving on the overactive cytokine response leading to severe COVID-19 [195]. These reports and early experience led to widespread off-label treatment of severe COVID-19 with anti-cytokine options. Since the initial reports of tocilizumab in severe COVID-19, multiple retrospective cohorts have been published showing various benefits of tocilizumab administration. To date, there are limited prospective randomized controlled trials that have shown benefit. In moderately ill patients with COVID-19 admitted to the hospital, tocilizumab was not effective in preventing intubation or death [196]. Another prospective randomized controlled trial showed potential benefit in decreasing risk for noninvasive ventilation, mechanical ventilation, and death at day 14, but no difference in mortality was seen at day 28 [197]. A study targeting tocilizumab administration in patients with COVID-19 admitted to the hospital with a Pao2/Fio2 ratio between 200 and 300 mm Hg did not show any benefit when compared with standard of care. In hospitalized patients not mechanically ventilated, tocilizumab administration decreased the likelihood to progress to composite outcome of progression to mechanical ventilation or death [198]. To further complicate the picture, showed benefit to all outcomes, including 90-day mortality in patients in the intensive care unit who received tocilizumab within 24 hours [199]. In the largest study of tocilizumab in patients admitted with COVID-19, with 2021 patients receiving intervention, tocilizumab improved 28-day survival regardless of baseline respiratory support. Inclusion criteria for this arm of the RECOVERY trial was hypoxia and evidence of systemic inflammation (CRP>75 mg/L) [200]. While current data appears to be conflicting, and if tocilizumab does benefit a subgroup of patients with severe COVID-19, it appears that hospitalized patients with evidence of systemic inflammation may be the subgroup most likely to benefit if given early in hospital course. Because of this most recent data, both the NIH and the Infectious Disease Society of America have updated their treatment guidelines to recommend tocilizumab 8 mg/kg (max of 800 mg) one time dose in addition to standard of care in subgroup of patients who are hypoxic and have significantly elevated inflammatory markers [201,202]. A small prospective, non-blinded trial of sarilumab, an IL6 receptor inhibitor, showed decreased progression to respiratory failure. Phase III trials in sarilumab are ongoing [203].

IL1β is an exocrine cytokine that is released after NLRP3 inflammasome activation [195]. RNA sequencing of patients in the early recovery stage of COVID-19 infection has shown an abundance of CD14++IL1β+ monocytes and further analysis predicted IL1β as a potential target for therapeutic intervention [164]. Anakinra is an IL1 inhibitor and has many clinical uses, including treatment of secondary hemophagocytic lymphohistiocytosis [204]. A retrospective trial from Italy has shown improved survival with high-dose anakinra and additional retrospective cohort demonstrated improved survival with anakinra in addition to dexamethasone [205,206]. A prospective cohort trial showed improvement of inflammatory markers, but was not powered to show difference in clinical outcomes [207]. While these and other cohort studies have shown potential for benefit, prospective randomized controlled trials of anakinra and canakinumab, an IL1β with an extended half-life, in treatment of severe COVID-19 are ongoing and selection of patient populations most likely to benefit prior to randomization is likely key to the success of these trials.

Because of molecular signaling of cytokines through Janus associated kinase (JAK) and signal transducer and activator of transcription (STAT), JAK inhibitors are being actively investigated as a treatment. Baricitinib recently received an EUA from the FDA for use in hospitalized patients in conjunction with remdesivir, due to a study showing reduced time to recovery, but no effect on mortality [208]. It is not clear if baricitinib is superior to dexamethasone and if safe to use in conjunction with dexamethasone. Adaptive COVID-19 Treatment Trial-4 (ACCT-4) designed to compare remdesivir/dexamethasone against remdesivir/baricitinib is currently recruiting participants (NCT04640168). Ruxolitinib, tofacitinib, and fedratinib are all in clinical trials for treatment of severe COVID-19 that have not yet been completed or published.

With the immunologic pathways in mind, multiple clinical trials of various immunomodulators are underway based on clinical observations, preliminary data, and mechanistic data. This includes CTLA-4 fusion protein administration and inhibition of BTK, C5, IFN-γ, TNF-α, IL17-A, and CCR2/5 among others. None of these randomized, prospective trials yet have published data.

5. Reinfection, vaccination, and SARS-CoV-2 variants

Lymphocytes are critical for developing immunity to coronaviruses, where B-cell driven antibodies provide short-term resistance (a few years) and the T-cell acquired response lasting much longer [209]. While most patients and animal models point to acquired response to SARS-CoV-2 after asymptomatic to mild infection [210], the risk in severe patients is not fully explored. As lymphopenia is a major alteration of severe COVID-19, it remains to be determined if Patients with COVID-19 will have acquired response upon second exposure. Some evidence points to individuals that have been reinfected [211], where reinfection was seen to lack the expression of neutralizing antibody to SARS-CoV-2 [212]. It is possible that many of the patients with genetic mutations, elevated Type I interferon autoantibodies, or cytokine storm are at risk of reinfection and severe disease. Further

work is needed to determine if these individuals would benefit from vaccination even if they already had COVID-19.

The most rapidly growing area of SARS-CoV-2 research is the emergence of variants in the virus that impact human biology and treatment options. As the virus has infected such a large portion of the population, and with each infection there is a risk of viral variants to arise, the genetic heterogeneity of SARS-CoV-2 has greatly expanded [213]. Variants in the Spike protein (K417N/T, E484K, and N501Y) have been shown to impact or evade the acquired response of antibodies [214–216]. In areas of the United States these variants have expanded to volumes that can have broad impact on the outcomes of the current vaccine strategies [217]. Any variant that can evade vaccine targeting can be spread throughout the world and create secondary pandemics if not controlled [218]. As the variant load is high throughout the world, it is needed to continue decreasing infection rates to minimize novel variant expansions that evade current or future vaccine development [219].

6. Expert opinion

Understanding the molecular pathways of severe COVID-19 opens the door for novel therapeutic design. Several immune modulator therapies are undergoing trials. However, as we have laid out in the current paper, a growing level of support suggests clinical risk factors, genetics, and secondary infections contributing to precision medicine outcomes that may require unique treatment strategies of severe COVID-19. Researchers and clinicians need to begin thinking about how to understand each individual patient through novel tools. Our group has begun implementing blood RNAseq of sepsis patients, where we reveal the complex nature of each patient's pathology [152]. After studying close to 200 patient samples to date, we have noted that no two patients ever present identical for both the blood RNAseq and for clinical course. Patients have infections on top of divergent genomes, different lifetime exposures, and complex biology occurring at the time, including variability of homeostasis and foreign organisms (whether normal flora or secondary infections). Blood RNAseq opens the door for the visualization of unique patient biology, which can allow for the full view of pathology and move toward treating each patient instead of treating an overly simplified disease annotation.

Most kids and adults think of futuristic medicine as something like that of Dr. Leonard McCoy within Star Trek, waving a wand over an individual to identify patient-level pathology and unique treatment options based on the survey. The idea of precision medicine is not new, yet the tools to make it a reality are nearly here. With tools like blood RNAseq and proteomics, we are moving into the realm of individualized medicine, giving a robust understanding of how an individual is divergent from normal, or the sum of population level insights. The statistical divergence can be linked to clinical insights that allow patient level correlations that can be detailed through more advanced biological experiments, where each patient is an n = 1 with mechanistic insights. Yet, we must continue to advance our understanding of patient-level statistics to decipher signal in all the noise in data. We must learn to integrate diverse tools and clinical information with these advanced assays to further find signals that truly correlate and cause pathology.

The physiologic understanding of disease has progressed slowly over time, particularly in situations of severe and critical illness. Many treatments are based on physiologic factors, but medical management is often based on aggregated best evidence and not necessarily tailored to the individual undergoing treatment. In addition, most clinical trials have not made an attempt to individually phenotype patients prior to randomization in a particular therapeutic clinical trial. The onset of the COVID-19 global pandemic showcased the challenges encountered when standard treatments provide differing responses from patient to patient, reinforcing the importance an individualized approach to specific disease processes will have in the near future. With such a large number of affected individuals, the diverse phenotypes of patients with COVID-19 provide large amounts of data which may allow for a better understanding of infectious responses in individuals and how these responses can differ from patient to patient on a genetic level. This will hopefully improve the use of tailored treatments for infectious etiologies of similar nature in the future. For COVID-19, the lack of a single, unifying explanation for a condition that has affected millions of individuals worldwide shows the need for a more individualized understanding of disease processes from a genetic level into the phenotypic expression to guide precision treatment and management.COVID-19 has highlighted the weaknesses in precision medicine, where we continue to advance tools and resources, but we struggle to apply these to individual patients. The field of biomarkers has been central to these challenges. Our data and others for immune responses in patients with COVID-19 highlights that end results (hospitalization or lethality) can occur by more than one mechanism, thus rarely does a single biomarker have the statistical power to identify 100% of a patient group. Patients continue to show phenotypes for months after infection, but we are unable to currently predict when these will occur. If we are wise, we will continue to develop high-level insights for patients with COVID-19 (and others) through tools like blood biomarkers and RNAseq, with focus not only on traditional statistics but reevaluating statistics more focused on convergent phenotypes by diverse mechanisms. If we reach this level, in the next decade it is possible to imagine a future where a few milliliters of blood could lay out acute and chronic risk for each individual, giving a better guide to how each patient should be treated.

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