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Why does COVID-19 disproportionately affect the elderly?

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Abstract

The severity and outcome of coronavirus disease 2019 (COVID-19) largely depends on a patient's age. Over 80% of hospitalizations are those over 65 years of age with a greater than 23-fold greater risk of death. In the clinic, COVID-19 patients most commonly present with fever, cough and dyspnea. Particularly in those over 65, it can progress to pneumonia, lung consolidation, cytokine release syndrome, endotheliitis, coagulopathy, multiple organ failure and death. Comorbidities such as cardiovascular disease, diabetes, obesity and hypertension increase the chances of fatal disease, but they alone do not explain the variability in COVID-19 symptoms. Here, we present the molecular differences between the young, middle-aged and elderly that may determine whether COVID-19 is a mild or life-threatening illness. We also discuss several biological age clocks that could be used in conjunction with genetic tests to identify both the mechanisms of the disease and individuals most at risk. Finally, based on these mechanisms, we discuss treatments that could increase survival in the elderly, not simply by inhibiting the virus, but by restoring patients' ability to clear the infection.

Introduction

Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2), which is responsible for the worldwide pandemic of coronavirus disease (COVID-19), originated in Wuhan, China, in late 2019, [1]. COVID-19 has so far killed more than 210,000 people [2], with the majority of deaths in people over the age of 65. The reasons the disease is particularly dangerous in older people is not yet known, and rarely discussed at the molecular level [3]. Even prior to SARS-CoV-2, human coronaviruses have been known to impact elderly people disproportionately [4], yet therapeutic strategies to protect this fraction of the population have largely failed. The severity of COVID-19 is, of course, strongly associated with comorbidities such as hypertension, diabetes, obesity, cardiovascular disease, and respiratory system diseases [3]. But simple explanations based on co-morbidities and a lack of resilience in the aged fail to explain why viral loads are not well controlled and why the immune system often reacts uncontrollably.

SARS-CoV-2 is spread by respiratory droplets or by direct contact. Entering the nose, mouth or eyes, the virus spreads from the back of the nasal passages, where it binds to and enters via the dimerized angiotensin-converting enzyme 2 (ACE2) [5] on the surface of airway epithelial cells [6]. From there it spreads to the mucous membranes of the throat and bronchial tubes, eventually entering the lungs where it infects type 2 alveolar epithelial cells called pneumocytes. This can lead to pneumonia, characterized by a loss of lung surfactant and an increase in oxidative stress and inflammation [7] (**Figure 1**).

Particularly in the elderly, severe cases of the disease are characterized by an acute respiratory distress syndrome (ARDS) that requires positive airway pressure with oxygen and pronation or invasive ventilation. This stage is characterized by neutrophilia, lymphocytopenia, bilateral nodular and peripheral ground glass opacities on chest X-rays, and lung fibrosis. The ACE2 protein is widely expressed on the surface of both epithelial and endothelial cells, which traverse multiple organs and can both be infected by the virus [8]. The recruitment of immune cells, either by direct viral infection of the endothelium or immune-mediated, can result in widespread endothelial dysfunction in the

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lung, heart, kidney, and liver and brain, with prominent endotheliitis of the submucosal vessels and apoptotic bodies [8].

Even if viral loads decline, a type of cytokine release syndrome can rapidly develop, characterized by disseminated intravascular coagulation (DIC), causing liver damage, renal dysfunction, cardiovascular inflammation, coagulopathy and death [9, 10]. In this perspective, we offer mechanistic explanations as to why COVID-19 advances in some people and not others, and especially in the elderly, including differences in the immune system, glycation, the epigenome, inflammasome activity, and biological age.

The aging immune system

The ability to control viral load is one of the best prognostics of whether a patient will have mild symptoms or advance to severe COVID-19 [11]. For the immune system to effectively suppress then eliminate SARS-CoV-2, it must perform four main tasks: (1) recognize, (2) alert, (3) destroy and (4) clear. Each of these mechanisms are known to be dysfunctional and increasingly heterogeneous in the elderly [12], but which aspect is most relevant to COVID-19 progression is not known [13].

During aging, the immune system changes in two major ways. One is a gradual decline in immune function called immunosenescence, which hampers pathogen recognition, alert signaling and clearance. This is not to be confused with cellular senescence, an aging-related phenomenon whereby any type of cell can arrest their cell cycle or otherwise be epigenetically locked into an inflammatory state. The other classic immune system change is a chronic increase in systemic inflammation called inflammaging, which arises from an overactive yet ineffective alert system [14].

An abundance of recent data describing the pathology and molecular changes in COVID-19 patients points to both immunosenescence and inflammaging as major drivers of the high mortality rates in older patients. Within immunosenescence, there are defects in both the innate and adaptive immune systems. Innate immunosenescence is characterized by ineffective pathogen recognition and macrophage activation, and a reduction in natural

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killer (NK) cell cytotoxicity, whereas adaptive immunosenescence is characterized by thymic atrophy and accumulation of anergic memory lymphocytes. In both cases, these age-related changes are thought to be due to pathogenic, genetic, and lifestyle factors that affect the cells' epigenetic status and the diversity of immune cells.

The aging innate immune system

The innate immune system is the body's first line of defense against coronaviruses. Sentinel cells, such as macrophages and dendritic cells, recognize structurally conserved viral proteins via single-pass membrane-spanning receptors called Toll-like receptors (TLRs) expressed on cell surfaces. Defects in TLR function of innate immune cells are well known to increase the severity of pneumonia in mice, especially in the context of aging and chronic inflammaging [15]. Alveolar macrophages (AMs) are mononuclear phagocytes that surveil the lungs for dust, allergens and the remnants of pathogens. When their TLRs detect an invader AMs respond by producing type I interferons, which attract immune cells to the site of infection and present antigens to lymphocytes [16, 17]. Although AMs increase in number during aging, their plasticity to convert between pro- and anti-inflammatory states is greatly reduced [18], exemplified by a weak cytokine response after TLR activation [19] (**Figure 1**).

The inability of AMs in older individuals to recognize viral particles and convert to a pro-inflammatory state likely accelerates COVID-19 in its early stages, whereas in its advanced stages, AMs are likely to be responsible for the excessive lung damage. Prolonged macrophage activation is a well-known cause of severe lung injury in rhesus monkeys [20] and in the cases of SARS (caused by SARS-CoV-1) higher numbers of pulmonary neutrophils and macrophages correlated with the development of ARDS and greater lung damage [21]. A decline in neutrophil activity might also be partly responsible because, during aging, these cells progressively lose their ability to migrate to sites of infection and kill infected cells [22, 23]. NK cells, a major component in innate immunity with potent cytotoxic activity, are an unlikely cause of COVID-19 severity. Their numbers are relatively stable during aging [24] and in a mouse model of SARS,

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they were not necessary for normal viral clearance [25]. To discern which of these cell types play the most destructive roles, more detailed analyses of COVID-19 patient autopsy tissue will be needed.

The aging adaptive immune system

Immunosenescence of the adaptive immune system is also a likely factor that determines whether a patient progresses to severe COVID-19 (**Figure 2**). Situated just above the heart, the thymus – a primary lymphoid organ and the site of T cell development and maturation of early thymic progenitors from the bone marrow – is one of the first tissues to experience aging. By age 60, the thymus is on average ~43% its original size [26], coincident with activation of the inflammasome component NLRP3 and Caspase-1, a pro-apoptotic protease [27]. A build-up of intrathymic adipocytes further reduces thymic cellularity and deteriorates the thymic microenvironment. The thymic atrophy with age also contributes to a reduction of naïve T cells and an accumulation of memory lymphocytes, resulting in defective in immunosurveillance and an exhaustion of B cells, cytotoxic T cells, and helper T cells [28]. Other common effects of aging on the adaptive immune system include a decline in the production of fresh naïve T cells, a less expansive T cell receptor (TCR) repertoire, T cell metabolic dysfunction, and weaker activation of T cells [29, 30]. Clonal populations of $CD8^+$ T cells expand during aging, limiting their diversity, whereas $CD4^+$ T cells remain fairly diverse TCRs [31] and, instead, suffer activation deficits [30].

Interestingly, one study found that supercentenarians – defined as those over 110 years old – tend to have an unusual population of cytotoxic $CD4^+$ T cells whose activation doesn't decline with age and can take on the cytotoxic functions usually performed by $CD8^+$ T cells [32]. This T cell behavior may explain why some elderly people, even some people over 100, are able to survive COVID-19. Measuring the repertoire and frequency of TCRs in patients from a spectrum of ages and disease severity should be performed to determine if a loss of T cell diversity is a reason why SARS-CoV-2 viral loads tend to spike in the elderly but not the young.

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Not only does the repertoire of T cells decline in aging, so do their numbers. Those over 60 years old increasingly have the low T cell numbers, a condition known as lymphopenia [33]. Because T cells express very low levels of ACE2, lymphopenia is unlikely to be caused by direct viral infection, as in the case of HIV [34]. One proposed cause of the T cell paucity is an exhaustion of the immune system driven by repeated exposures to viruses over one's lifetime [33, 35, 36]. This hypothesis is based on several studies that tracked the morbidity and mortality of people over 60 who had been chronically infected with human cytomegalovirus (CMV) [37, 38]. Cycles of CMV reemergence were associated with vast immune system remodeling, including a pronounced exhaustion of $CD8^+$ T cells that was more predictive of all-cause mortality than chronological age. Other studies indicate that T cell depletion is due to the cumulative exposure to many different pathogens [37, 39], in which case geographic regions and individuals with the high rates of pathogen exposure should have higher risk of COVID-19 fatality. A major cause of immune exhaustion is telomere shortening in viral-specific memory $CD8^+$ T cells, which induces cellular senescence, a state of cell cycle arrest and hyper-inflammation that prevents expansion upon re-infection [40]. The fact that in the most severe COVID-19 cases T cells express high levels of the immune-exhaustion marker PD-1 [33, 41] make this theory plausible.

Increased inflammation and cytokine storms in the aged

During the course of COVID-19, patients can bring down their viral titers, only to rapidly descend into a state of shock involving hyper-activation of the immune system and hypercoagulation in small blood vessels [33, 42]. This rapid and uncontrolled inflammatory signaling cascade, known as a "cytokine storm," exacerbates the dyspnea and hypoxemia and triggers inflammation in major tissues such as the lungs, kidneys, heart, liver and brain. The resulting vascular inflammation is emerging as a main cause of complement-associated microvascular injury and thrombosis in severe COVID-19 cases [43]. The initial trigger for cytokine storms is not yet known but it likely involves the immune system's detection of a large quantity of viral antigens released by dying cells.

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The cytokine profiles of late-stage COVID-19 patients are similar to secondary haemophagocytic lymphohistocytosis, a type of cytokine storm that can be triggered by systemic viral infection, including increased levels of interleukin (IL)-2, IL-6, IL-7, C-reactive protein (CRP), granulocyte-colony stimulating factor, interferon-γ inducible protein 10, monocyte chemoattractant protein 1, macrophage inflammatory protein 1-α and tumor necrosis factor-α [36, 44, 45]. Even more predictive of death than serum cytokine profile is an increase in the fibrin degradation product D-dimer that is a prognostic for DIC [7], a condition of abnormal, excessive generation of thrombin and fibrin in the bloodstream.

Why the elderly are particularly prone to cytokine storms is not known, but there are some likely causes. Levels of D-dimer, the main prognostic of coagulopathy, increase naturally with age, hence the D-dimer test has a high false positive rate in elderly patients. Why D-dimer increases with age is not known but it likely reflects a higher level of vascular inflammation [46]. In cytokine storms, high levels of IL-6 cause vascular endothelial cells to secrete fibrin, which causes small clots to form in the microvasculature of the body. In the lung, this may underlie the hypoxemia seen in patients with seemingly functional lungs. If left untreated, clots leach additional clotting factors from the bloodstream, increasing the risk of bleeding and multi-organ failure. Drugs such as tocilizumab (Actemra), which block IL-6 receptor activity, are currently being used in patients in advanced stages [47].

One in two fatal cases of COVID-19 experience a cytokine storm, 82% of whom are over the age of 60 [48]. Though there may be many simultaneous triggers of the storm, abundant evidence indicates that inflammaging is a major driver, exacerbated by obesity, poor diets and oral health, and sedentary lifestyles. For example, in rodents, inflammaging increases the risk of cytokine storm syndrome [49] and, in humans, age correlates with higher basal circulating levels of pro-inflammatory cytokines including IL-6, TNF-α, IL-1α and CRP [50, 51].

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A central player that could help explain a predisposition to cytokine storms is NLRP3, the major protein component of the inflammasome. During aging, there is a steady increase in the abundance and activity of NLRP3 in immune cells, including AMs of the lung which, upon stimulation, contribute to pulmonary fibrosis, a histological feature of COVID-19 [52]. NLRP3 inflammasome activation requires two steps, the first of which is the priming step, induced by TLRs or tumor necrosis factor receptor activation. This leads to the activation of NF-κB and promotes the expression of NLRP3, pro-IL-1β, and pro-IL-18. The second step, also called the activation step, is triggered by a range of stimuli that emerge during infections, such as tissue damage, nucleic acids, and invading pathogen proteins [53].

In older individuals, NLRP3 may be poised for hyperactivation by SARS-CoV-2 components. The control of NLRP3 activity is under the direct control of SIRT2, a member of the NAD^+-dependent sirtuin family of deacetylases [54]. Old mice, especially those deficient in SIRT2, have accelerated inflammaging, along with decreased glucose tolerance and increased insulin resistance. During aging, NAD^+ levels decline, reducing the activity of the sirtuin family of deaceylates (SIRT1-7) [55]. This decline, exacerbated by COVID-19, might therefore promote hyperactivation of NLRP3 and the trigger cytokine storms in COVID-19 patients [10]. Maintaining NAD^+ levels may therefore alleviate COVID-19 symptoms, a possibility supported by recent data showing that SARS-CoV-2 proteins hyperactivate poly-ADP-ribose polymerases PARP9, -10, -12, and -14 and deplete cellular NAD^+ [56] and the ability of NAD precursors to lower inflammation in human subjects [57, 58].

Mechanisms of infection in other coronaviruses support that hypothesis that NLRP3 activation is a second trigger of cytokine storms in the aged. The SARS-CoV-1 ORF3a protein, for example, is a potent activator of pro-IL-1β gene transcription and protein maturation, the two signals required for activation of the NLRP3 inflammasome [59]. In macrophages, SARS-CoV-1 ORF8b robustly activates the NLRP3 inflammasome by interacting directly with the Leucine Rich Repeat domain of NLRP3 in cytosolic dot-like structures [60]. Thus, we envisage a 2-step model in which inflammaging and the NLRP3

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basal overactivation is the first step and SARS-CoV-2 antigen-mediated hyperactivation is the second step, triggering a cytokine storm.

In chronic diseases, hyperactivity of the inflammasome plays a dominant role in the development of type 2 diabetes and other age-related diseases [61]. Indeed, in older adults, the upregulation of two inflammasome-related gene sets correlate with increased risk of hypertension, metabolic dysfunction, oxidative stress and was predictive of mortality [62]. Individuals over the age of 85 that expressed lower levels of these inflammasome modules are less likely to die within seven years [62]. Taking together the known effects of coronavirus proteins on NAD^+, NLRP3, and the two stages of inflammasome activation, these data provide an explanation as to why co-morbidities positively correlate with cytokine storms and fatality in COVID-19 patients.

Obesity is another major risk factor in COVID-19 fatality [63]. The increase in NLRP3 expression and activity caused by obesity may also explain a number of observations, including: (i) why obese mice produce higher levels of serum chemokines, and lower neutralizing antibodies and effector memory T cells during a viral infection [64]; (ii) why obesity is associated with lower survival in COVID-19, SARS-CoV-1 and MERS-CoV infections; and (iii) why obesity-related human diseases such as cardiovascular disease, chronic kidney disease, and diabetes, predispose patients to cytokine storms (**Table 1**) [65-67].

Epigenetic changes with age

The dysregulation of the epigenome and resulting changes in gene expression during aging are strongly implicated as biomarkers, and potentially underlying causes, of chronic disease states and aging itself. The “relocalization of chromatin modifiers” theory of aging postulates that symptoms of aging and the loss of resilience are a result of a lifetime accumulation of epigenetic changes [68, 69], driven in part by the redistribution of chromatin factors, such as the nuclear proteins SIRT1/6/7, HDAC1 and PARP1 to sites of dsDNA break repair causing epigenetic “noise” that obscures cellular identity [68-72].

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This process manifests as DNA methylation changes that set the pace of the biological clock in hematopoietic cells [73, 74].

Age-related changes to the host's epigenome compromise immune cell composition and function [75], negatively impacting viral defenses [76, 77], including adaptive immune memory during infection [78, 79]. Coronaviruses are also known to mediate epigenetic change, perhaps accelerating the rate the immune system ages. MERS-CoV, for example, antagonizes host antigen presentation by altering DNA methylation, which silences genes encoding major histocompatibility complexes [80], and SARS-CoV-1 infection can delay the activation of interferon response genes, accompanied by changes to histone methylation and long non-coding RNAs [81]. Testing DNA methylation age of immune cells and other tissues before, during and after infection will help elucidate both how the aged epigenome impacts disease severity, and how the virus alters the aged epigenome.

SARS-CoV-2 entry into cells may be epigenetically determined and contribute to the vulnerability of the aged. The virus enters cells via interactions between the viral spike glycoprotein receptor to ACE2 on the human cell surface [82]. While genetic differences in ACE2 are being pursued as a cause of COVID-19 severity [83], there is little attention being paid to epigenetic differences. In humans, ACE2 is ubiquitously expressed in epithelial tissues of the body, most highly in alveolar epithelial cells and enterocytes of the small intestine [84]. ACE2 is regulated in the body transcriptionally, post-transcriptionally, and post-translationally [85].

In both mice and rats, ACE2 expression decreases with age and is associated with an increase in aortic fibrosis and inflammation [86, 87]. In humans, ACE2 promoter hypomethylation in lymphocytes correlates with transcriptional activation in patients with lupus [88], implying that transcription of ACE2 is controlled by methylation. Age-related changes in ACE2 expression have not yet been systematically investigated in human airway epithelial cells [89, 90]. It is known, however, that methylation at one of seven CpGs in the ACE2 promoter decreases with age and these CpGs are bordered by long-range promoter-enhancer contacts that may change over time [90]. Bisulfite sequencing

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of the ACE2 gene paired with transcriptomic and four-dimensional chromatin analyses will be necessary to understand if there is a causal relationship between promoter methylation, ACE2 expression, and disease outcome.

The elucidation of SARS pathogenesis is complicated by the fact that ACE2 is also part of the renin-angiotensin system (RAS) that regulates immunity, fibrosis, blood pressure, and metabolism. By cleaving the product of ACE, angiotensin II, it counteracts vasoconstriction caused by angiotensin converting enzyme (ACE). Most likely due to its role in vasodilation and reducing inflammation, ACE2 partially protects against sepsis-induced- and SARS-induced severe acute lung injury in mice [91, 92] and asthma-induced airway inflammation in rats [93]. Changes in DNA methylation during aging are known to affect the RAS [10, 94, 95]. Analysis of ACE2 gene expression in the lungs of COVID-19 patients with pulmonary arterial hypertension, chronic obstructive pulmonary disease, and a history of smoking found a correlation between ACE2 expression and COVID-19 severity [96]. Thus, age-related dysregulation of ACE2 could explain why age is such a risk factor for COVID-19 complications and why cardiovascular disease and hypertension likewise predispose patients to develop a more aggressive form of COVID-19.

The effects of ACE inhibitors, used commonly beyond middle age to control blood pressure, are generally believed to be neutral in COVID-19 [97, 98]. Due to their opposing roles in the RAS, when ACE is inhibited, ACE2 expression appears to increase, likely providing a yet unknown protective function [99]. Inhibiting ACE2 expression or blocking ACE2 accessibility could prevent viral entry but may lead to vasoconstriction and hypertension. Instead, the most promising ACE2-targeted therapeutic strategy is to infuse human recombinant soluble ACE2 into the airway or blood stream to bind the SARS-CoV-2 spike glycoprotein receptor, preventing it from binding ACE2 on host cell surfaces [100] and slowing cell infection rates.

Sirtuins and NAD^+

The sirtuins (SIRT1-7) are a family of NAD^+-dependent lysine deacylases that control numerous aspects of stress resistance and pathogen defenses. SIRT1 is a nuclear histone deacetylase that suppresses viral replication and chronic inflammation [101]. By binding to the promoter region of ACE2, SIRT1 upregulates transcription under conditions of cell stress [102]. During aging and particularly during the course of COVID-19, levels of NAD^+ decline, likely due to increased NAD^+ consumption by the $CD38^+$ glycohydrolase [103] and increased transcription of the poly-ADP-ribosyl transferases, PARP9, PARP10, PARP 12 and PARP14 in mice and humans infected with SARS-CoV-2 [56]. Coronaviruses also possess an ADP-ribosylhydrolase that further depletes NAD^+, apparently to disrupt cell signaling, DNA repair, gene regulation and apoptosis [10, 104, 105].

As a co-substrate of the sirtuins, changes to the levels of NAD^+ affect immunity and coagulation. One of the most likely changes with age that would predispose the elderly to cytokine storms during COVID-19 is a decline in sirtuin activity. By negatively regulating activity of NLRP3, the main component of the inflammasome, SIRT1 and the related protein SIRT2, play key roles in suppressing acute lung inflammation during sepsis [54]. Mice lacking SIRT1, for example, display aggravated inflammasome activation, with increased production of lung proinflammatory mediators, including intercellular adhesion molecule-1 and high-mobility group box-1, and a dramatic reduction of lung claudin-1 and vascular endothelial-cadherin expression [106]. SIRT1 also attenuates the acute inflammatory response through deacetylation of H4K16 in the TNF-α promoter [107]. Another nuclear sirtuin, SIRT6 attenuates NF-kB signaling by deacetylating H3K9 [108]. Thus, during aging, a decline in NAD^+ and the known mis-localization of SIRT1 and SIRT6 across the genome during aging [68, 109], could be major contributors to the age-dependency of COVID-19 symptoms. Given the increasing evidence that lower NAD^+ levels in the lung and vascular endothelium contribute to poor COVID-19 outcomes, NAD boosters, such as the NAD^+ precursors NMN and NR [110], have been suggested as first-line treatments against COVID-19, especially aged patients [56].

Biological clocks

Over the past decade, a variety of biological clocks have been shown to predict human health and longevity more accurately that chronological age, including clocks based on DNA methylation patterns [111-114], inflammaging [115], gene expression patterns [116], frailty [117, 118], serum proteins [119], and IgG glycosylation [120-122]. Given that these clocks provide a quantitative measure of the rate of aging of an individual and their overall resilience, biological clocks may be useful for predicting who will likely progress to severe COVID-19.

Epigenetic Clocks

Estimates based on twin studies place the contribution of non-genetic factors on predicted COVID-19 phenotype at 50% [123] and on total disease burden in old age at about 80% [124]. Indeed, lifestyle factors that affect the epigenome such as calorie intake and smoking increase the susceptibility to COVID-19. Epigenetic age is greater than chronological age in various disease contexts and lower in long-lived humans, providing strong evidence that epigenetic age reflects biological aging [111, 125]. Age-associated changes to the epigenome have profound effects on the immune system, including T cell function, cytokine production and macrophage pattern recognition. DNA methylation is believed to set the pace of the aging clock in several mammalian tissues, including hematopoietic cells of the immune system [73, 74]. Epigenetic clocks that measure DNA methylation at specific CpG sites are the most widely used measure of biological age and disease susceptibility [111, 125]. Restoration of the thymus using a drug cocktail of metformin, growth hormone and dehydroepiandrosterone led to the reversal of features of immunosenescence, specifically increasing naïve T cells and a decreasing senescent PD-1^+ T cells, along with the reversal of the epigenetic clock by about 1.5 years [74]. Epigenetic age may be a better biomarker than chronological age in predicting how variation in lifestyle factors and age-associated comorbidities increase susceptibility to COVID-19, a possibility we hope to test by measuring the DNA methylation ages of

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DNA samples from thousands of COVID-19 patients and correlating them to medical records.

Glycosylation Clocks

Changes in glycosylation during aging may also predispose older individuals to severe COVID-19 [126]. Glycosylation is the enzymatic process by which carbohydrates called glycans, such as sialic acid, mannose and fucose, are covalently attached to proteins or lipids, typically on the cell surface or in the bloodstream. An individual's repertoire of glycans – a notable example being the type of N-glycans attached to immunoglobulins [127] – changes with age and environmental factors, such as smoking and poor diet [126]. They type of glycans attached to IgGs affects their pro- and anti-inflammatory properties [128]. Decreased galactosylation of IgGs is associated with central adiposity [129] and inflammaging in the context of diabetes [130]. Biological clocks based on IgG glycosylation are able to predict chronological age within 10 years, and can be improved by inclusion of clinical parameters [122]. Thus, changes to the glycome with age could serve both as an indicator of biological age and predict COVID-19 severity.

Aging also changes the glycome via non-enzymatic glycation, by which reducing sugars circulating in extracellular compartments covalently bind to proteins and lipids to form advanced glycation end products (AGEs). AGEs are present in large quantities in the Western diet, and greater consumption of dietary AGEs increases serum TNF-alpha [131]. AGEs tend to accumulate under hyperglycemic conditions and contribute to the pathology of many age-related disease such as type 2 diabetes and obesity [132]. AGEs may increase COVID-19 severity in the aged by inhibiting the NLRP3 inflammasome during the early stages of viral infection [133] when the inflammatory program is activated by the SARS-CoV-1 3a protein [134]. AGEs also play a role in activating pro-coagulation pathways [132], potentially contributing to the DIC observed in COVID-19 patients.

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Glycosylation patterns specific to the elderly may also impact viral entry. The spike protein is heavily modified by N-acetyl-glucosamine [135], modifications that are highly conserved between coronaviruses. SARS-CoV-2 shares 20 out of 22 of glycosylated N-linkages with SARS-CoV-1 [135]. In the case of the human influenza virus, variation in sialic acid structures on the surface of cells lining the upper and lower respiratory tracts dictates tropism and age-dependent binding efficiency of the virus [136] but how changes in the coronavirus spike protein during aging might affect viral transmission and pathogenesis is not yet known. If we are to use glycation as a prognostic marker for COVID-19, it will be necessary to map the glycome in hundreds of patient samples with varying degrees of COVID-19 severity, including asymptomatic individuals.

Immune Clocks

Immune system heterogeneity between individuals increases during aging [13] and may predict susceptibility to infectious diseases. A biological clock based on the immune system, IMM-AGE, was recently developed that predicts all-cause mortality in older adults more accurately than even DNA methylation clocks [115]. IMM-AGE overcomes the limitation of inter-human immune heterogeneity by tracking immune cell frequencies and gene expression changes longitudinally within individuals and then computationally predicting how an individual's homeostatic immune state changes over time. Though individuals exhibit variation in immune cell-type composition, these changes fall into three stages that converge on a common "attractor point" that correlates with age and is indicative of overall physiological resilience [115]. In this way, IMM-AGE measures the entropic relationship between age and immune system remodeling, the rate of which can predict survival. Because IMM-AGE is even able to capture and predict the effect of inflammaging on cardiovascular system, and because COVID-19 fatality is so closely tied to cardiovascular disease and inflammaging, this clock may prove to be the most accurate at identifying COVID-susceptible individuals. More studies are still needed to determine if and how viral infections alter these and other biological clocks, and whether variation in biological aging can truly explain COVID-19 severity.

Where do we go from here?

Why SARS-CoV-2 infections are more severe and fatal in the aged is not known but viable hypotheses are emerging that include changes to the immune cell repertoire, the epigenome, NAD^+ levels, inflammasome activity, biological clocks, and covalent modifications of human and viral proteins (**Figure 3**). But much remains to be elucidated. Besides understanding the basis of the cytokine storms and coagulopathy, it is not known why SARS-CoV-2 so easily infects such a broad array of tissues in the elderly but rarely in the young. Nor is it clear whether the elderly develop stronger or weaker functional immunity during seroconversion, or how long their protection will last compared to those with milder cases of COVID-19. In the aged, immune responses to vaccination are often weak or defective [13, 137, 138], while autoimmunity increases [139]. Therefore, in designing vaccines against SARS-CoV-2, it will be important to consider that older people may not respond as well to vaccines as young people. Studies that follow the long-term consequences of SARS-CoV-2 infection in older people will also be critical to understand the long-term health consequences of COVID-19 pathology, such as fibrosis and scaring of the lungs, cardiopulmonary dysfunction, and neuropsychological disability [140]. These could significantly reduce viral resistance and lifespan in the elderly and middle-aged people who recover from severe cases of COVID-19. The most exciting and potentially impactful technologies to treat COVID-19 are those that activate the body's defenses against aging [141]. It may even be possible to reset the age of cells and tissues [142-144] so currently high-risk individuals can respond to viral infections as though they were young.

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diseases by raising NAD levels. Other affiliations are listed here https://genetics.med.harvard.edu/sinclair-test/people/sinclair-other.php.

Abbreviations

SARS-CoV-1, severe acute respiratory syndrome coronavirus identified in 2003; SARS-CoV-2, severe acute respiratory syndrome coronavirus identified in 2019; MERS-CoV, middle east respiratory syndrome coronavirus; COVID-19, coronavirus disease 2019; ARDS, acute respiratory distress syndrome; TLR, Toll-like receptor; TCR, T cell receptor; DIC, disseminated intervascular coagulation; IL, interleukin; ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; RAS, renin-angiotensin system; SIRT1-6, sirtuin 1-6; AGE, advanced glycan end product; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; IMM-AGE, immune age; PARP, poly (ADP-ribose) polymerase; NAD, nicotinamide adenine dinucleotide; NMN, nicotinamide mononucleotide; NR, SARS-CoV-1 ORF3a, SARS-CoV-1 open reading frame 3a; NK cell, natural killer cell; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PD-1, programmed cell death protein 1; IgG, immunoglobulin G; IgE, immunoglobulin E; AM, alveolar macrophages; CRP, C-reactive protein.

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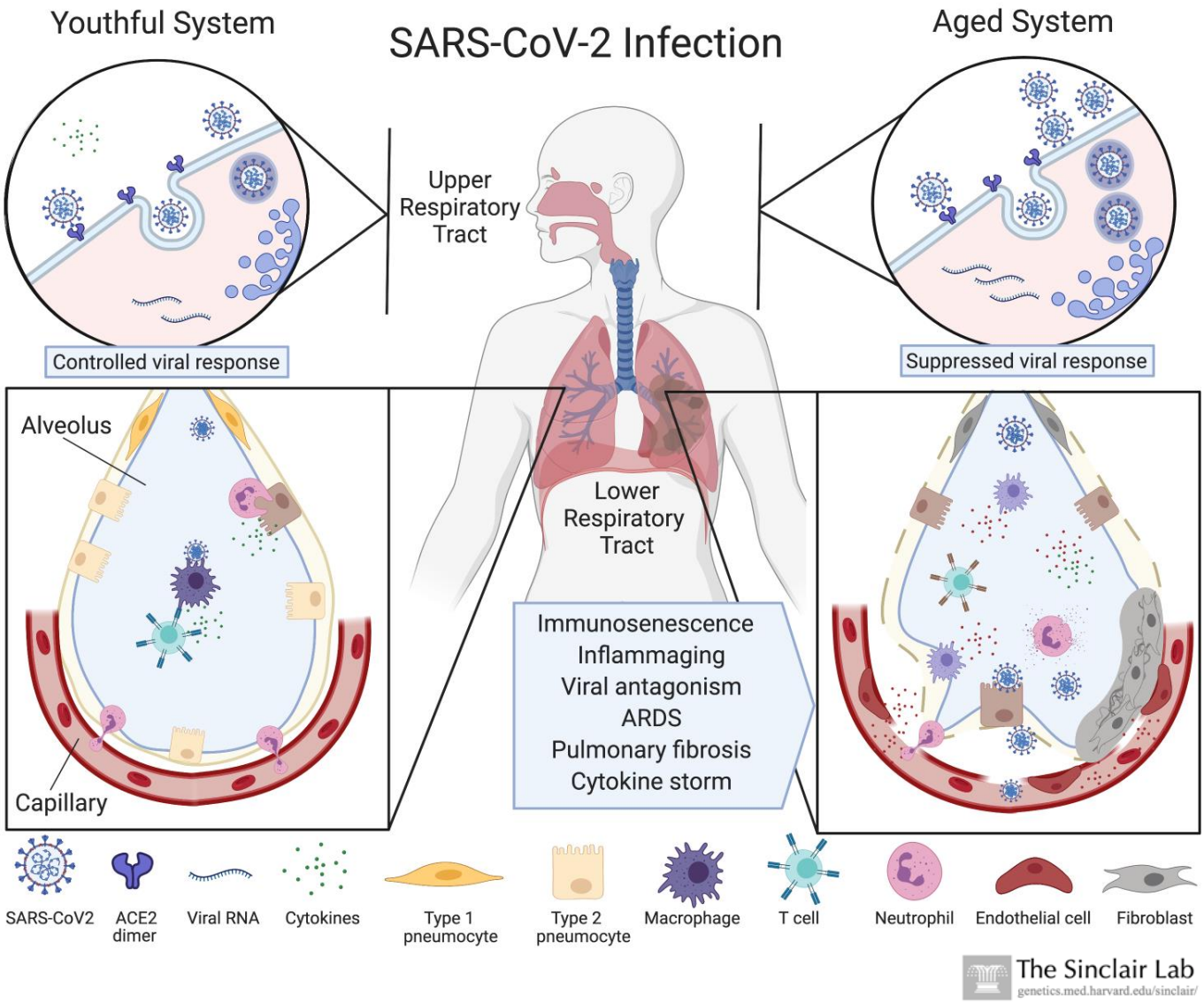

Figure 1. Ineffective clearance of SARS-CoV-2 infection in the aged respiratory system.

The SARS-CoV-2 virus binds to ACE2 enzymes on airway epithelial cells in the upper respiratory tract where they are endocytosed and replicated. Viruses then travel to the alveoli and infect type 2 pneumocytes which, in the youthful system (left), are recognized by alveolar macrophages (AMs) or dendritic cells (not pictured) that release cytokines and present antigens to T cells and other adaptive immune cells. T cells with the appropriate receptors activate other lymphocytes or directly kill infected cells, preventing the spread of the virus. Neutrophils migrate to the sites of infection to clear infected cell debris. In the aged system (top right), viral alert signals are slow, resulting in greater viral

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replication. Defective macrophages and T cells with a limited repertoire of receptors are less effective. More cells are infected, inducing high levels of inflammatory cytokine signaling. The endothelial cell lining of the capillary becomes inflamed, fibroblasts are activated and SARS-CoV-2, viral components, and cytokines enter the bloodstream. Fluid fills the alveolus, reducing lung capacity and the virus infects endothelial cells in other organs. A cytokine storm initiates microvasculature clotting ensues causing severe hypoxia, coagulopathy and organ failure. Created with BioRender.

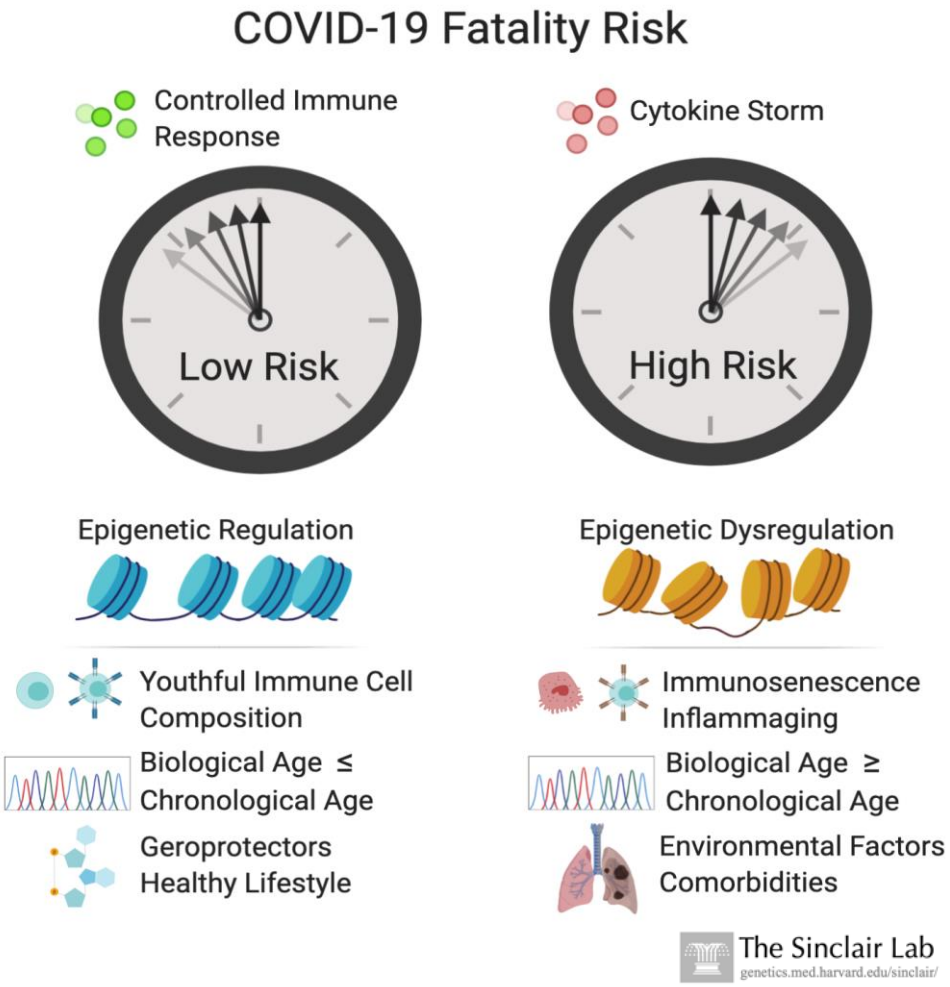

Figure 2. Factors that increase the fatality risk of COVID-19.

Epigenetic dysregulation, immune defects, advanced biological age, and other factors increase the risk of cytokine storm and COVID-19 fatality. Tightly controlled activation of the innate immune system is essential for viral recognition and clearance. Cytokine storm is the result of sustained activation of the inflammatory signaling cascade and can result in hypercoagulation in small blood vessels, which leads to tissue damage, DIC and

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multi-organ failure. Inflammaging and immunosenescence contribute to the development of cytokine storm. D-dimer, a fibrin degradation product and prognostic of DIC, and elevated levels of the cytokine, IL-6, are associated in the clinic with increased fatality. Epigenetic dysregulation of the immune system and of the RAS may increase fatality risk. A variety of biological clocks have been shown to predict human health and longevity more accurately that chronological age. An individual with a biological age greater than their chronological age is thought to be undergoing accelerated aging, which may increase the risk of COVID-19 fatality. Individuals with comorbidities such as diabetes, obesity, COPD, are at greater risk for COVID-19 fatality, as are chronic smokers. Conversely, individuals who live healthy lifestyles and consume geroprotectors agents such as metformin, resveratrol and NAD boosters may have a decreased risk of fatality. Created with BioRender.

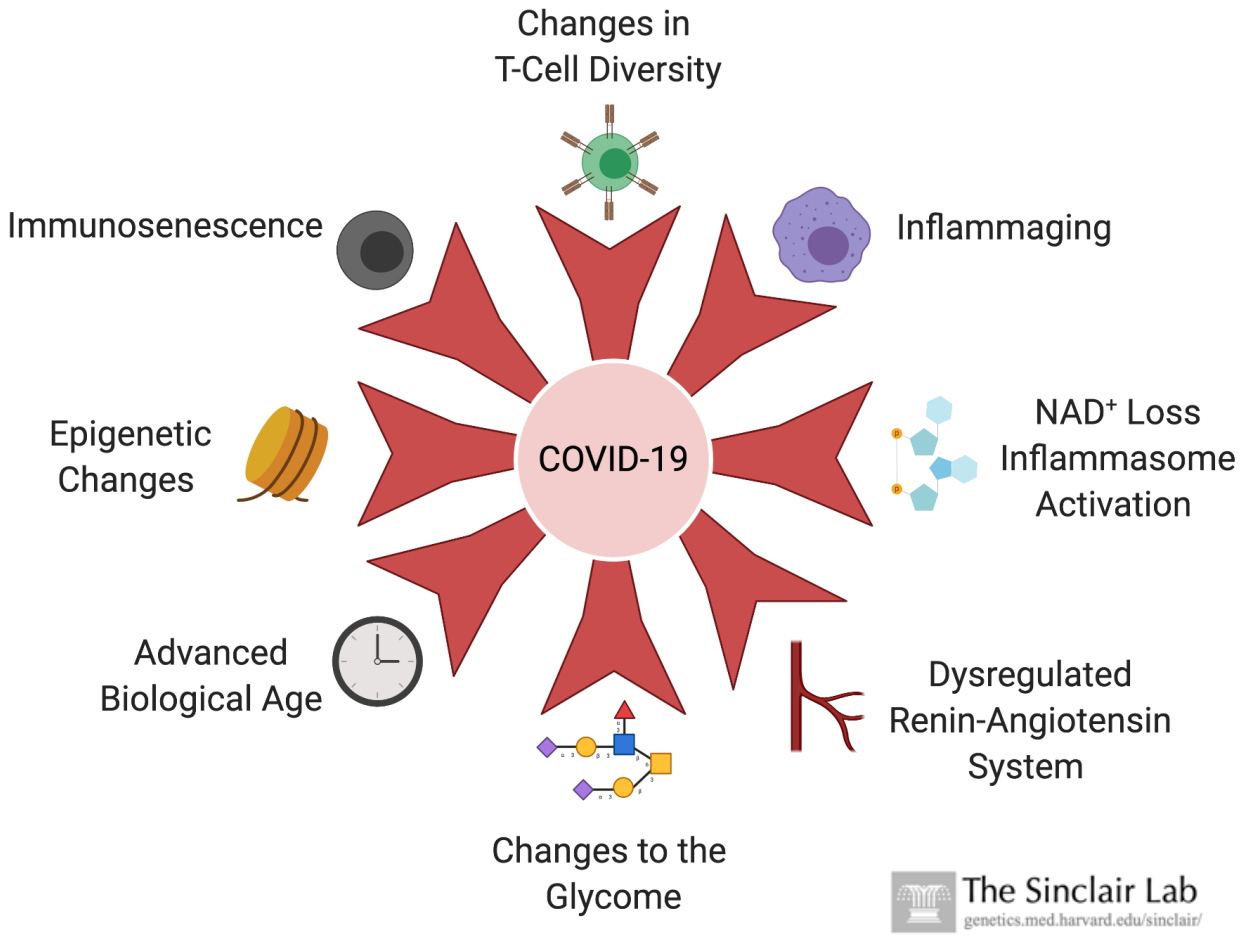

Figure 3. Age-related changes that increase COVID-19 susceptibility. The aging immune system undergoes immunosenescence, experiences changes in T-cell diversity

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and endures a chronic activation of the innate immune system, called inflammaging. These hallmarks of the aging immune system (1) cripple the body's ability to clear the SARS-CoV-2 virus and (2) initiate and sustain cytokine storms, which result in acute organ injury, DIC and multi-organ failure. An age-associated decline in NAD^+ results in derepression of NLRP3 and inflammasome in the elderly, further exacerbating the cytokine storm. Coronaviruses also possess an ADP-ribosylhydrolase that further deplete already-low NAD^+ levels in the elderly. Smoothening of the epigenetic landscape during aging results in changes in immune cell composition and function that decrease the immune system's ability to mount a response to infection. Epigenetic dysregulation of ACE2 may also impact increased viral loads in the elderly. Dysregulation of the RAS during aging and in the context of age-associated disease, such as cardiovascular disease, hypertension, COPD and chronic smoking, contributes to severity of COVID-19 infection. The glycome which controls a variety of immune signaling pathways changes during aging and in the context of metabolic disease, in part due to environmental factors such as smoking and diet. For example, decreases in IgG galactosylation contribute to chronic inflammation. Biological clocks that measure different biomarkers of biological age, may explain increased COVID-19 susceptibility more accurately than advanced chronological age. Created with BioRender.

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Table 1. Risk Factors for Adverse Outcomes to Respiratory Viral Infections

Virus/Pathogen	Risk Factors	Reference
Trivalent H1N1 vaccination	Chronic medication use	Agarwal et al., 2018
Respiratory Viral Infection	High CMV-reactive $CD4^+$ T-cells	Johnstone et al., 2014
MERS-CoV-1	Type 1 and 2 diabetes; obesity; cardiovascular diseases, hypertension, and cardio-artillery diseases	Badawi and Ryoo, 2016
MERS-CoV-1	Old age, male sex and underlying medical conditions, including diabetes mellitus, renal disease, respiratory disease, heart disease and hypertension	Matsuyama et al., 2016; Rivers et al., 2016; Yang et al., 2017
SARS-CoV-1 associated Pneumonia	Obesity	Frasca and McElhaney, 2019
SARS-CoV-1	Diabetes mellitus, end-stage renal disease, immunological, neurological, metabolic and dermatological diseases	Yang et al., 2017
SARS-CoV-1	Shared transcriptional networks with chronic heart failure, breast cancer, bone diseases, aging	Moni and Lio, 2004
SARS-CoV-1	Diabetes mellitus and plasma glucose levels	Yang et al., 2006
SARS-CoV-1 associated Pneumonia	Elevated levels of lactate dehydrogenase, C-reactive protein	Chiang et al., 2004
SARS-CoV-1	Hospital-setting exposure; diabetes	Booth et al., 2003
SARS-CoV-2	Diabetes mellitus and chronic kidney disease	Bhatraju et al., 2020
SARS-CoV-2	Older age, high SOFA score, and greater D-dimer	Zhou et al., 2020
SARS-CoV-2	History of smoking and elevated ACE2 expression in the lung	Pinto et al., 2020 (preprint)