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An evidence-based debate on epigenetics and immunosenescence in COVID-19

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ABSTRACT

Immunosenescence contributes to the decline of immune function leading to a reduced ability to respond to severe coronavirus disease 2019 (COVID-19) in elderly patients. Clinical course of COVID-19 is widely heterogeneous and guided by the possible interplay between genetic background and epigenetic-sensitive mechanisms underlying the immunosenescence which could explain, at least in part, the higher percentage of disease severity in elderly individuals. The most convincing evidence regards the hypomethylation of the angiotensin-converting enzyme 2 (*ACE2*) promoter gene in lungs as well as the citrullination of histone H3 in neutrophils which have been associated with worsening of COVID-19 outcome in elderly patients. In contrast, centenarians who have showed milder symptoms have been associated to a younger "epigenetic age" based on DNA methylation profiles at specific genomic sites (epigenetic clock). Some large prospective studies showed that the acceleration of epigenetic aging as well as the shortening of telomeres were significantly associated with lymphopenia and poor outcome suggesting prognostic biomarkers in elderly COVID-19 patients. Furthermore, randomized clinical trials showed that statins, L-arginine, and resveratrol could mediate anti-inflammatory effects *via* indirect epigenetic interference and might improve COVID-19 outcome. Here, we discuss the epigenetic-sensitive events which might contribute to increase the risk of severity and mortality in older subjects and possible targeted therapies to counteract immunosenescence.

1. Introduction

The frailty elderly issue is well documented (Cacciatore et al., 1998, 2019, 2020; Napoli and Cacciatore, 2009; Napoli et al., 1999). Although severe coronavirus disease 2019 (COVID-19) occurred also in the healthy population irrespective of age, the highest morbidity and mortality rates were observed in older adults, with individuals aged >80 years having 20-fold increased fatality risk as compared to adults aged 50–59 years (Williamson et al., 2020). Older males showed a higher mortality rate than females (Mostaza et al., 2020), likely due to sex-specific differences in their immunological background (Klein and Flanagan, 2016). Frail elderly subjects having a general higher prevalence of traditional cardiovascular risk factors and comorbidities, mainly cancer, were more prone to enter in intensive care unit (ICU) during pandemics (Fiorentino et al., 2023; Damayanthi et al., 2021; Wang et al., 2020; Zhou et al., 2020; Napoli et al., 2020a, Suleyman et al., 2020; Henry and Vikse, 2020, Niu et al., 2020, Vietri et al., 2015, de Nigris et al., 2013). This is because SARS-CoV-2 virus may inflict a direct attack to the heart and vasculature impacting on cardiac aging and exacerbating the pro-inflammatory state, known as "cytokine storm" which, in turn, was associated to acute respiratory distress syndrome (ARDS) onset and high mortality (Wang et al., 2020; Zhou et al., 2020; Napoli et al., 2020a; Liu et al., 2020; Evans et al., 2020; Hojyo et al., 2020).

The host-related immune system efficiency in fighting SARS-CoV-2 virus may explain, at least in part, the discrepancy in disease severity

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between frail elderly and younger patients. The "immunosenescence" and "inflammaging" are physiologic related processes already present at different grade in frail aged individuals (Fulop T et al., 2017; Clegg et al., 2013).

Immunosenescence refers to the age-related dysregulation of both innate and immune systems and inflammaging describes a chronic low-grade inflammation (Fig. 1). These results from genetic and epigenetic-sensitive events (DNA methylation, histone modification, chromatin remodeling) accumulating across the lifespan (Ray and Yung, 2018) which may prepare the soil for the SARS-CoV-2-related immune escape (Crimi et al., 2019, 2020a, 2020b) and might account for the variability in vaccine responses (Tang et al., 2022, Grimaldi et al., 2022). Immunosenescence and inflammaging may be further aggravated upon Sars-CoV-2 infection leading to a more severe form of COVID-19 (Schmitt et al., 2022; Napoli et al., 2020b; Napoli et al., 2020b; Pietrobon et al., 2020). Although vaccination campaign significantly prevented hospitalization and death in elderly patients (Arregocés-Castillo et al., 2022; Haas et al., 2021), immunosenescence-related signatures were observed in older subjects (>66 years) who were classified as "non-responders" to vaccination as compared to younger individuals (Collier et al., 2021; Demaret et al., 2021; Moline et al., 2021). Understanding the epigenetic-sensitive molecular routes underlying immunosenescence may provide novel prognostic biomarkers and offer, owing to their reversible nature, additional targeted therapies able restore the immune function in frail older COVID-19 patients, as already showed in cardiovascular field (Benincasa et al., 2022, Napoli et al., 2021b, Napoli et al., 2020c, Mansueto et al., 2020) (Fig. 1).

2. Immunosenescence and clinical epigenetic-sensitive events in COVID-19

Classical hallmarks of immunosenescence include decreased lymphopoiesis owing to thymic involution (Liang et al., 2022), reduced maturation of B and T cells in both bone marrow and thymus, impaired function of mature lymphocytes in secondary lymphoid tissues (Goronzy and Weyand, 2019; Montecino-Rodriguez et al., 2013), accumulation of $CD4^+$ and $CD8^+$ T cells with short telomeres and without co-stimulatory surface protein CD28 making them unresponsive to antigenic stimulation (Dumitriu, 2015), hyper-reactive myeloid cells (monocytes and neutrophils) (Shaw et al., 2013), as well as exaggerate accumulation of pro-inflammatory cells and mediators in the lungs and extra-pulmonary organs (Bajaj et al., 2021) (Fig. 1). Inflammaging is a chronic low-grade pro-inflammatory state acting as a defence mechanism toward persistent antigenic stress, but it may become deleterious in older adults with unbalanced anti-inflammatory players (Fulop et al., 2017). COVID-19-related severity and mortality were significantly associated to marked lymphopenia as well as rising interleukin (IL)-6 levels (Zhou et al., 2020; Napoli et al., 2020a, 2020b, 2020c, 2021a) which triggered the cytokine storm mainly *via* STAT-3 signaling (Hojyo et al., 2020). Although the attempts to treat severe COVID-19 patients with tocilizumab (TCZ, a recombinant humanized monoclonal anti-IL-6R antibody) had a particular resonance in the early phase of pandemics, the Phase III COVACTA trial (NCT04320615) toned down the emphasis because TCZ failed to improve clinical status and mortality in hospitalized adults (https://www.clinicaltrialsarena.com/news/roche-actemra-covid-data/). Thus, identifying which epigenetic-sensitive events are associated to immunosenescence may provide additional targeted therapies to treat severe COVID-19.

2.1. DNA methylation, ACE-2 regulation, and "biological aging" in COVID-19

More convincing data is coming from associative studies between DNA methylation, *ACE-2* gene expression, and biological age in COVID-19. A pioneer study showed that SARS-CoV-2 could dramatically reshape DNA methylation profiles of peripheral blood mononuclear cells in COVID-19 patients receiving mechanical ventilation or supplemental oxygen than controls. Hypermethylation in regulatory regions of the interferon (*IFN*)-related genes as well as hypomethylation of *ACE-2* gene as well as inflammatory genes including *NLRP3* inflammasome and antiviral *MX1* associated significantly with severe COVID-19 (Corley et al., 2021). Independently from their immunosuppressive therapy, systemic lupus erythematosus (SLE) patients infected with SARS-CoV-2 showed a significant hypomethylation and overexpression of *ACE-2* gene as well as demethylation of *INF*-regulated genes, *NFκB*, and key cytokine genes in $CD4^+$ T cells than healthy controls, likely exacerbated

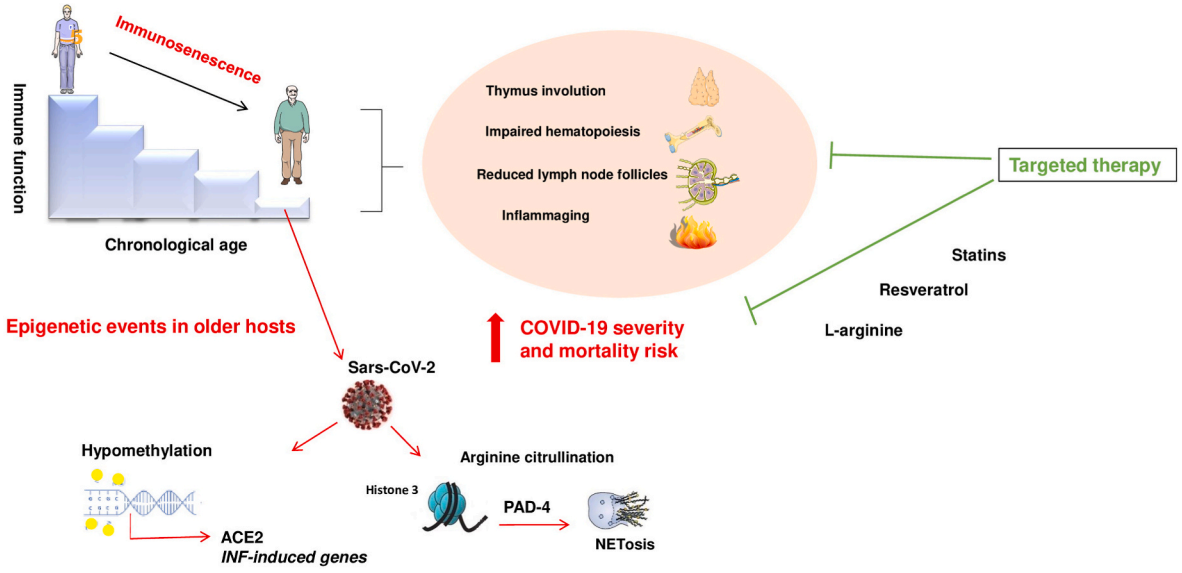

Fig. 1. Aging-related epigenetic events increasing the risk of COVID-19 severity and fatality.
The lack of efficiency of the immune system to rise a robust antiviral response in older individuals is mainly associated with immunosenescence, as a gradual reduction of functional competence of the immune cells. One of the key epigenetic events contributing to increase vulnerability to severe COVID-19 in older subjects is the DNA hypomethylation which may induce the expression of *ACE-2* receptor and *INF*-related genes. COVID-19 patients showed a marked upregulation of histone citrullination on immune response genes leading to NETosis, as a process involved in development of thrombotic events. Abbreviations: ACE-2: Angiotensin converting enzyme 2.

by oxidative stress (Sawalha et al., 2020). DNA methylation levels of the *ACE-2* gene significantly differed by sex and age in lung tissue isolated from COVID-19 patients. In addition, DNA methylation levels near the transcription start site of the *ACE-2* gene in airway epithelial cells strongly associated with "biological age" of subjects (Corley et al., 2020). Together, this evidence supports that *ACE-2* expression may be regulated by DNA methylation and that a methylation defect may increase predisposition to disease severity.

In gerontology and anti-aging medicine field, there is a much attention in distinguishing between biological and chronological age. While chronological age is simply the sum of years, biological age refers to the DNA methylation alterations at specific genomic sites which may be useful for a more accurate diagnosis or prediction of aging-related diseases at personalized level as well as serving as surrogate markers for evaluating therapeutic interventions including rejuvenation approaches (Ahadi et al., 2020). Chronological age increases at the same rate for general population whereas biological age is highly heterogeneous because as cells age DNA methylation alterations shifts and some of them may mark time (epigenetic clock). The first multi-tissue epigenetic estimator, known as Horvath's epigenetic clock, measured biological age based on DNA methylation levels at 353 CpG dinucleotides (Horvath, 2013) and showed the ability to predict all-cause mortality later in life (Chen et al., 2016; Marioni et al., 2015; Gibbs, 2014).

Despite chronological age is associates with immunosenescence, subjects with the same chronological age may show a highly heterogeneous decline of immune system ("immunological aging") (Alpert et al., 2019). The potential clinical utility of measuring biological age in combination with telomere length as non-invasive surrogates for predicting risk of COVID-19-related severity and mortality in adults was observed in some observational studies (Fig. 2) (Cao et al., 2022; Mongelli et al., 2021).

A genome-wide DNA methylation study using whole blood isolated from 232 healthy subjects and 194 non-severe and 213 severe COVID-19 patients found that a parallel accelerated biological age and reduced telomere length may be non-invasive surrogates to predict COVID-19 patients requiring hospitalization and increased mortality rates (Cao et al., 2022). A cohort of 117 adult patients with long-COVID-19 syndrome including fatigue, dyspnea, memory loss, sleep disorders, and difficulty concentrating showed a significant positive DeltaAge of 10.45 ± 7.29 years (epigenetic age acceleration) in parallel with telomere shortness and reduced *ACE-2* expression in peripheral blood biospecimens as compared to 144 non-infected volunteers (Mongelli et al., 2021).

Besides, a longitudinal study using DNA methylation data from peripheral blood isolated of 36 healthy participants showed that two-dose mRNA-based COVID-19 vaccination decreased the epigenetic age in older individuals suggesting the epigenetic clock as a biomarker of COVID-19 vaccine responses (Pang et al., 2022).

Surprisingly, in seminal Italian studies, nonagenarians/centenarians seem to have a more pronounced resistance to COVID-19 pandemics with a mortality rate lower than individuals aged between 50 and 80 years (Caruso et al., 2022; Marcon et al., 2020). Some studies showed that centenarians had a young epigenetic age compared with their chronological age (Daunay et al., 2022; Armstrong et al., 2017; Horvath et al., 2015). Although this phenomenon is still unclear, we hypothesized that a younger epigenetic age of the immune system than the predicted chronological age might overcome the inflammaging and immunosenescence associated with chronological aging (Fig. 2).

2.2. Histone modifications and NETosis in COVID-19

Some evidence supported that SARSCoV-2 virus may trigger an excessive tissue damage *via* neutrophil-extracellular trap (NETosis) which, in turns, is associated with onset of thrombotic events (Cicco et al., 2020; Leppkes et al., 2020; Veras et al., 2020). Although precise mechanisms of NETosis induction are still controversial, higher levels of cell-free citrullinated histone H3 (marker of NETosis) were significantly associated with increased levels of cytokine IL-8, platelets, leukocytes,

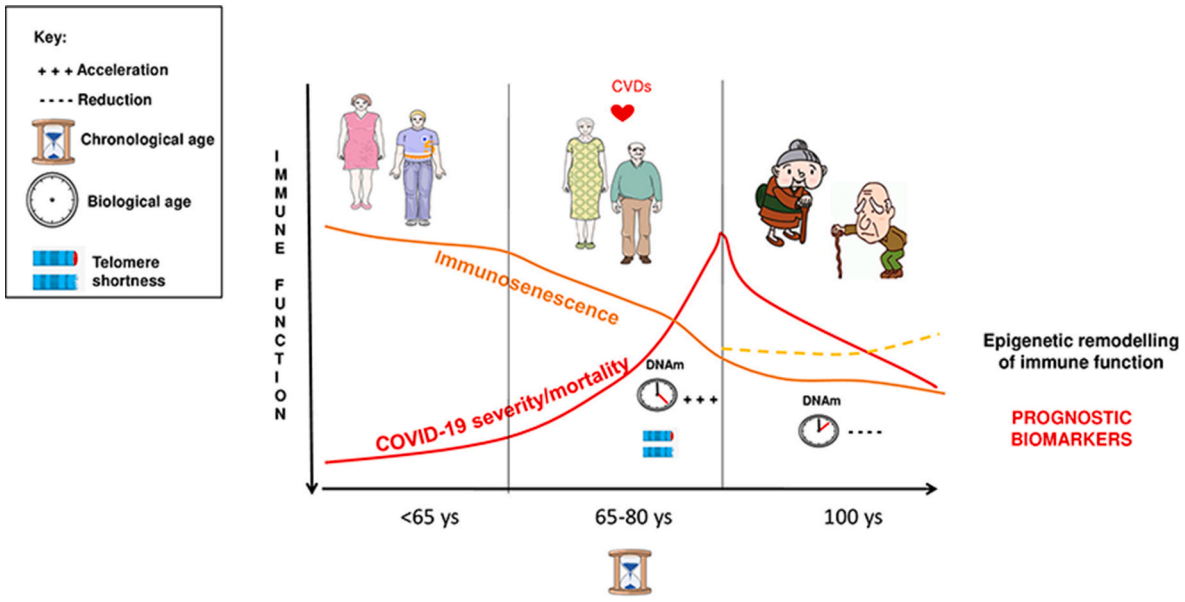

Fig. 2. Biological age affects severity and mortality in COVID-19.
Beginning with the sixth decade of life, the immune function starts its decline undergoing dramatic changes which continuously progress toward a state of immunosenescence (orange line) which manifests as increased susceptibility to infections. Immunosenescence aggravated COVID-19 clinical outcome in frail elderly subjects which present a higher prevalence of cardiovascular comorbidities (red line). Measuring accelerated biological age, based on levels of DNA methylation at targeted genomic regions (epigenetic clock) in combination with telomere shortness suggested possible non-invasive biomarkers for predicting risk of COVID-19-related severity and mortality in adults aged 65–80 years. As not expected, during pandemics centenarians showed a reduced risk for COVID-19 severity and fatality. Since biological age of centenarians could be reduced than predicted chronological age, we hypothesized that a possible epigenetic-sensitive remodeling of immune cells might counteract immunosenescence and help to fight Sars-CoV-2 infection (orange dashed line). Abbreviations: DNAm: DNA methylation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and granulocytes in severe COVID-19 patients (Leppkes et al., 2020). Citrullination of histone H3 is catalyzed by the arginine deiminase 4 (PAD) and promotes decondensation of chromatin and downstream increased transcription of pro-inflammatory genes. NETosis was significantly elevated in plasma, tracheal aspirate, and lung autopsies tissues from severe COVID-19 patients than controls, and the trigger of NETosis in SARS-CoV-2–infected neutrophils depended on the ACE2–TMPRSS2 axis. (Veras et al., 2020). In addition, treatment of neutrophils isolated from COVID-19 patients with Cl-Amidine, an inhibitor of PAD4, reduced the ability to trigger NETosis in vitro suggesting the requirement of PAD-4 in vivo (Veras et al., 2020). Taken together, this data suggests that histone citrullination plays a significant role in inducing NETosis during SARS-CoV-2 infection, thus increasing the susceptibility to severe COVID-19.

Thus, correlative studies suggested that epigenetic-sensitive events may occur upon Sars-CoV-2 infection and contribute to reshape host immune system modulating the severity of COVID-19.

3. Compounds useful to modulate immunosenescence

Although vaccines improved clinical outcome in older subjects, the is a great interest in discovering additional compounds useful to reinforce immune system. The existence of epigenetic-sensitive events which associated with severe COVID-19 opened the way to investigate novel targeted therapies. Controlled clinical trials are evaluating the effects of statins, L-arginine and resveratrol which might act also *via* indirect epigenetic interference in improving COVID-19 outcome, as already widely diffused for cardiovascular patients (Napoli et al., 2020c, 2021b; Sarno et al., 2021). In Table 1, we summarized the list of completed clinical trials with published results which were registered on https://clinicaltrials.gov/. Although established anti-inflammatory properties, no clinical trial is evaluating the possible beneficial effects of direct epigenetic drugs (small molecule acting as agonists or antagonist of targeted epigenetic mechanisms), such as vorinostat (SAHA), valproic acid (VPA), and apabetalone (BET inhibitor), in modulating immunosenescence in elderly COVID-19 patients (Bhat et al., 2022). Statins, acting as inhibitors of histone deacetylase enzymes (HDACi), may regulate NETosis through lipid-independent epigenetic mechanisms (Kow and Hasan, 2022). However, the preliminary results are mixed (Masana et al., 2022; Hunt et al., 2022; Kow and Hasan, 2022; Bikdeli et al., 2022). Observational studies showed that the use of statins in COVID-19 patients was associated with a significantly reduced risk of death (Masana et al., 2022; Hunt et al., 2022), likely due to the inhibition of NET formation by statins during the early stage of illness (Kow and Hasan, 2022). In contrast, a randomized controlled trial (INSPIRATION-S trial) which investigated the possible efficacy of atorvastatin found no significant difference in all-cause mortality between critically ill COVI-19 patients randomized to atorvastatin as compared to placebo (Bikdeli et al., 2022). L-arginine is a semi-essential amino acid representing the substrate used for nitric oxide production by endothelial nitric oxide syntase (eNOS) (Costa et al., 2019b, Napoli and Ignarro, 2003), and seems to act as an epigenetic regulator of T regulatory lymphocytes *via* inhibition of DNA methylation at promoter of *IL-10* promoter gene (Yu et al., 2017). A randomized, placebo-controlled trial adding oral L-arginine to standard therapy in patients with severe COVID-19 significantly decreases the length of hospitalization (Fiorentino et al., 2022). Besides, a combined supplementation of L-Arginine and Vitamin C has showed beneficial effects in attenuating the typical symptoms of long COVID-19 syndrome (Izzo et al., 2022). Resveratrol is a natural polyphenol compound which is present in grapes and other plants. Resveratrol can modulate the immunity system by activating sirtuin 1 which, in turn, is able to reduce levels of pro-inflammatory cytokines and maintain periphery T cell tolerance (Malaguarnera, 2019). A randomized placebo-controlled clinical trial has evaluated the safety and the efficacy of combining resveratrol and vitamin D3 in reducing hospitalization and morbidity in patients aged >45 years with early COVID-19 (McCreary et al., 2022). Patients treated with resveratrol had a lower incidence of hospitalization and pneumonia as compared to placebo group (McCreary et al., 2022). Thus, standard therapy in combination with compounds able to modulate immunosenescence and inflammaging, likely also *via* epigenetic interference, could be useful to protect older subjects from the severe form of COVID-19.

Table 1
Registered clinical trials of repurposed drugs and natural compounds with indirect epigenetic interference in COVID-19 patients.

Epigenetic effects	Current indication	Possible effects on immunosenescence	NCT
Statins			
HDACi	To decrease cholesterol levels in dyslipidemic patients	-Interference with Sars-Cov-2 cellular uptake,-Anti-inflammatory effects,-Modulation of NETosis	NCT04486508, NCT04407273
L-arginine			
DNMTi	Dietary supplement	IL-10 promoter DNA hypomethylation in Treg	NCT04637906
Resveratrol			
SIRT1a	Dietary supplement	Anti-oxidant and anti-inflammatory activity	NCT04400890

Abbreviations: **DNMTi**: DNA methyltransferase inhibitor; **HATi**: histone acetyltransferase inhibitor; **HDACi**: histone deacetylase inhibitor; **HMG-CoA**: 3-hydroxy-3-methylglutaryl-CoA; **IL:** interleukin; **NET**: neutrophil extracellular trap; **PUFA:** polyunsaturated fatty acids; **SIRT1a**: sirtuin 1 activator; **Treg**: regulatory T cells.

4. Conclusions

The COVID-19 pandemics has caused a higher percentage of deaths within the elderly patients. Epigenetic-sensitive changes occurring in ageing immune system are described, at least in part, as promoters for an increased severity and mortality for COVID-19 in the elderly. Two relevant examples are hypomethylation of *ACE-2* gene promoter and citrullination of H3 in NETosis which may exacerbate immunosenescence and, thus, have been proposed as possible drug targets. No less important is the effect of diet on epigenetic mechanisms that, in concert with the individual genetic architecture, can promote longevity and help prevent or treat diseases (Zhang and Kutateladze, 2018; Costa et al., 2019a). The interplay between diet and immunosenescence in elderly *via* epigenetics should be investigated in COVID-19. Preliminary controlled clinical trials showed mixed results regarding the efficacy of statins in reducing the inflammaging in COVID-19 patients whereas diet supplementation with L-arginine and resveratrol may mediate anti-inflammatory effects, at least in part *via* epigenetic-sensitive interferences. Taken together, this evidence warrants larger clinical prospective studies aimed at identifying epigenetic-sensitive biomarkers able to stratify the high-risk patients before entering in ICU and possible targeted therapies.

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Claudio Napoli: Conceptualization. **Enrico Coscioni:** Writing – review & editing. **Ugo Trama:** Writing – review & editing. **Maria Grazia Strozziero:** Writing – original draft. **Giuditta Benincasa:** Writing – original draft.

Declaration of competing interest

All the Authors declare no conflict of interests.

Data availability

No data was used for the research described in the article.

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