Vascular Aging and COVID-19

Angiology
2023, Vol. 74(4) 308–316

DOI: 10.1177/00033197221121007
journals.sagepub.com/home/ang


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Abstract

Vascular age is determined by functional and structural changes in the arterial wall. When measured by its proxy, pulse wave velocity, it has been shown to predict cardiovascular and total mortality. Disconcordance between chronological and vascular age might represent better or worse vascular health. Cell senescence is caused by oxidative stress and sustained cell replication. Senescent cells acquire senescence-associated secretory phenotype. Oxidative stress, endothelial dysfunction, dysregulation of coagulation and leucocyte infiltration are observed in the aging endothelium. All of these mechanisms lead to increased vascular calcification and stiffness. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can involve the vascular endothelium. It enters cells using angiotensin-converting enzyme 2 (ACE-2) receptors, which are abundant in endothelial cells. The damage this virus does to the endothelium can be direct or indirect. Indirect damage is caused by hyperinflammation. Direct damage results from effects on ACE-2 receptors. The reduction of ACE-2 levels seen during coronavirus disease 2019 (COVID-19) infection might cause vasoconstriction and oxidative stress. COVID-19 and vascular aging share some pathways. Due to the novelty of the virus, there is an urgent need for studies that investigate its long-term effects on vascular health.

Keywords

vascular aging, coronavirus disease 2019, angiotensin-converting enzyme 2, pulse wave velocity, endothelial senescence

Introduction

The current pandemic of SARS-CoV-2 has been and still is a challenge for all healthcare systems. Symptoms of COVID-19 range from acute respiratory distress syndrome and multi-organ failure to very mild or even asymptomatic cases.[1] Recently, the concept that COVID-19 is an endothelial disease has emerged.[2] It has been known for some time that various viruses (eg Human immunodeficiency virus, Herpes simplex virus) can induce arterial stiffness and early vascular aging.[3,4] This has also been observed for COVID-19.[1]

In the first part of this narrative review, we present a short overview of the pathology of vascular aging. We then discuss why COVID-19 can be considered an endothelial disease.

Methods

The literature search for this narrative review was conducted using PubMed. Only papers available in English were selected. The literature search used these keywords and their combinations: 'vascular age', 'early vascular aging', 'supernormal vascular aging', 'endothelial senescence', 'oxidative stress', 'inflammaging', 'vascular calcification', 'ACE-2' and 'COVID-19'. Both authors reviewed each citation and abstract whenever available. Discrepancies in inclusion were revised and discussed. According to our keyword list, each article was accessed in terms of scientific content, methods used, and completeness in reporting factors.

Vascular Aging

Concepts of Early Vascular Aging and Supernormal Vascular Aging

Vascular age is determined by changes in functional and structural arterial wall properties. Chronological age defines the person's age. Several tools based on multivariate regression models are available for calculating vascular age.[5]

Vascular age, when measured by its proxy – carotid-femoral pulse wave velocity (cfPWV), is a better predictor of cardiovascular (CV) mortality as well as all-cause mortality when compared with chronological age.[6] Vascular age is a better predictor of CV risk than chronological age when studying patients with type 1 diabetes and for death when studying patients with chronic kidney disease.[7,8]

Disconcordance between vascular and chronological age might mean two things: either a person is in better vascular health than his peers of the same sex or the opposite – their

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vascular health is worse than their peers of the same sex. For example, vascular age much higher than chronological age implies early vascular aging (EVA).[9] This phenomenon has been observed in patients suffering from inflammatory bowel disease, chronic kidney disease, diabetes mellitus and obesity.[10-13] Indeed, EVA can be defined as an abnormally high cfPWV for a person's age and sex.[9] For example, patients with chronic bowel disease have increased cfPWV despite normal blood pressure.[11] Chronic low-grade inflammation in these patients mimics that seen in inflammaging.[10] Unfortunately, there are no exact cut-off values for EVA yet – neither for cfPWV or other vascular function and structure defining variables – the concept is new, and the studies are ongoing.[14]

The opposite to EVA, super normal vascular aging (SUPERNOVA), can be attributed to patients if their cfPWV is much lower compared with people of the same sex and age.[14] These individuals seem to be protected from vascular aging (measured by cfPWV) by genetic and epigenetic means. Moreover, SUPERNOVA means a reduced risk for CV events.[14] The exact mechanism of why these people are protected has not been established yet.

Interestingly, the Lancet Commission on Hypertension has named several avoidable thresholds leading to CV disease: elevated blood pressure, subclinical target organ damage and CV events.[15] Since preventive and destiffening strategies are still under development, the subjects with EVA phenotype move through these thresholds faster than those with SUPERNOVA.

The Pathology of Age-Related Vascular Remodelling

Senescence and Endothelial Dysfunction

Vascular aging starts from endothelial senescence; the hallmarks of endothelial cell senescence are dysregulation of vascular tone and stiffness, increased endothelium permeability, altered angiogenesis and mitochondrial biogenesis.[16] Cell senescence might be caused by oxidative stress and sustained replication.[17] Senescent cells lose their ability to divide and begin to produce pro-inflammatory and matrix-degrading molecules, and this is referred to as senescence-associated secretory phenotype (SASP).[16] This paracrine activity of SASP cells leads to inflammation, degradation of extracellular matrix and vascular remodelling.[16] Senescent endothelial cells also tend to produce less vasodilator nitric oxide (NO) but more vasoconstrictive endothelin-1.[18] Endothelial cell senescence is regulated by the p53 protein pathway, activated in response to telomere dysfunction and deoxyribonucleic acid (DNA) damage.[19]

Vascular endothelial dysfunction per se is characterized by vasoconstriction, pro-coagulation, pro-inflammation and proliferative effects regulated by endothelium through paracrine or autocrine means.[18] All these effects have been observed at a greater level in older people when compared with younger ones, even in the absence of CV disease.[18] Endothelial dysfunction is often measured by endothelium-dependent dilation (EDD).[20] The endothelium promotes vasodilation through NO synthesized by endothelial NO synthase (eNOS) and other, less critical vasodilators – prostaglandins and hyperpolarizing factors.[21] The decreasing EDD with age is attributed to a decrease in NO availability.[22] The reduction of NO synthesis could be explained by the decline in tetrahydrobiopterin (BH4), an essential cofactor for NO synthesis in eNOS.[23] Vasodilating effects can be modified by reduced eNOS expression, inactivation of NO, and conversion of NO to pro-oxidant molecules.[24] As a response to angiotensin II or oxidized low-density lipoproteins, the endothelium produces endothelin-1, which has a robust vasoconstrictive effect.[25] These regulatory mechanisms are essential in regulating peripheral blood pressure, but when a disease influences these pathways, it can restrict local blood flow.

Oxidative Stress

When the endothelial cell is not stressed, it has several antioxidant systems, like superoxide dismutase, glutathione peroxidase and heme oxygenase, all of which work against local oxidative stress.[26] When influenced by pro-inflammatory cytokines, endothelial cells can produce nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, and this process leads to the synthesis of superoxide anions and local oxidative stress.[27] Production of reactive oxygen species (ROS) is associated with hypertension, hyperlipidemia and diabetes.[28] Markers of oxidative stress are found in arteries of aging humans and animals.[29] Aging-related increase of ROS might be explained by more active NADPH oxidase, heightened mitochondrial production and a process caused by the decrease of BH4, leading eNOS to uncouple and shift from NO production to ROS production.[30] ROS can activate a cascade resulting in nuclear factor kappa-B entering the nucleus and activating genes responsible for synthesizing pro-inflammatory cytokines.[31] ROS produced during oxidative stress also interact with NO, leading to the accumulation of peroxynitrite – an extremely reactive and toxic metabolite.[31] The causal interaction between oxidative stress and reduction of NO levels and endothelial function is further illustrated by experimental data, which shows that the administration of antioxidants improves both NO availability and ED.[32] In aged endothelium, a vicious cycle of oxidative stress and inflammation exists, each fuelling another.[16]

Thrombogenesis

Endothelial cells play a crucial role in preventing the blood from clotting. It produces heparan sulfates, thrombomodulin, NO and prostacyclin to promote anticoagulation and anti-aggregation.[33] If these systems fail and a thrombus is formed, endothelium also produces plasminogen activators, which have a profibrinolytic effect.[34] Normally, the endothelium has

anticoagulant, antiplatelet and profibrinolytic qualities. However, the endothelium can act oppositely. Some triggers (eg, pro-inflammatory cytokines) can promote clot formation through von Willebrand factor and tissue factor expression and inhibit fibrinolysis through plasminogen activator inhibitor-1.[35] While the endothelium protects the body from blood clotting under normal circumstances, when exposed to inflammatory signals, it can act oppositely, leading to thrombosis.

Leucocyte Infiltration

Under normal circumstances, the endothelium does not interact with leucocytes for extended periods.[36] Various selectins expressed on the endothelial wall help slow leucocytes down and increase the time they spend in contact with the endothelium.[37] Pro-inflammatory cytokines (eg interleukin 1 alpha (IL-1α), tumour necrosis factor-alpha (TNF -α)) promote the synthesis of selectins.[36] Once slowed down, leucocytes bind to the endothelial wall through adhesion molecules like intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1).[37] Leucocytes, bound to the endothelium, can be affected by chemoattractants to enter tissues.[38]

Inflammation and Inflammaging

Inflammaging is a concept that defines constant chronic low-level inflammation in the absence of apparent infection and is usually attributed to the elderly population.[39] During the last decade, inflammaging has been related to cardiovascular pathology in populations with pulmonary diseases, chronic kidney disease, diabetes and obesity.[40,41]

Inflammatory mediators can worsen vascular endothelial and smooth muscle cell function.[42] Accumulation of inflammatory cells in vascular walls is related to hypertension in experimental animal studies.[43] Macrophages within the arterial wall produce ROS, which increases adhesion molecule expression, activation of matrix metalloproteinases and reduced amount of NO, leading to vascular remodelling and dysfunction.[44] Additionally, increased ROS production and inflammation promote telomere shortening, which is associated with atherosclerosis and major CV events.[45] Furthermore, increasing pro-inflammatory mediators and reducing anti-inflammatory mediators might lead to arterial stiffening and vascular calcification.[46,47]

Inflammaging is closely associated with perturbations in gut microbiota with aging. Based on previous evidence, it is well established that aging is associated with reduced microbiota diversity.[48,49] Studies agree that dysbiosis harms the host. On the other end of the spectrum of microbiota diversity stand supercentenarians, whose microbiota tends to be even more biodiverse.[50] The type of composition of microbiota determines levels of inflammatory markers, dysfunction of the blood-brain barrier and increased circulating bacterial DNA.[51] The well-functioning blood-brain barrier ensures brain health and prevents central nervous system damage.[52]

Lastly, the lessons about the association between inflammation and CV disease should be learned from pharmaceutical studies. Statins impact low-density lipoprotein cholesterol and inflammation, and statin-related CV event reduction results from both effects.[53] Colchicine, an anti-inflammatory drug, was associated with a lower incidence of myocardial infarction.[54]

Vascular Calcification

There is much evidence that vascular calcification increases with age.[55] It is an active process mainly defined by the phenotypic transformation of vascular smooth muscle cells (VSMCs). The osteogenic transformation of VSMCs is preceded by apoptosis, macrophage infiltration and inflammation.[56]

The phenomenon of arterial wall thickening due to precipitation of calcium phosphate that results in arterial stiffening is referred to as arteriosclerosis.[57] It is an essential part of vascular aging and a CV risk factor.[58] There are two significant types of arteriosclerosis – calcification of intima and calcification of media.[59] Medial sclerosis is prevalent in patients with type 2 diabetes and chronic renal disease and is also associated with aging.[60] Oxidative stress is shown to induce aging-associated vascular calcification.[61] When medial calcification affects arteries of the extremities, it is referred to as Mönckeberg medial sclerosis.[62] It is the most common type of medial sclerosis. Arteriosclerosis in microvasculature leads to an increase in cfPWV and pulse pressure, eventually leading to reduced perfusion.[63]

Atherosclerosis is mainly defined by intimal thickening that develops as early as the second decade of life and is associated with aging.[64] Higher than average amounts of senescent cells, reduced cell proliferation, DNA damage and telomere shortening are found in atherosclerotic lesions, indicating a close connection between aging and atherosclerosis.[65,66] As the result of intimal thickening, endothelial barrier becomes more permeable, allowing cholesterol and phospholipids to enter the subendothelium.[67] Increased permeability combined with mild hypercholesterolemia or hypertension is thought to be the driving force for early atherosclerotic lesions.[68] This has been demonstrated in animal studies, where older rabbits acquired more severe atherosclerotic plaques than younger rabbits when fed an atherogenic diet.[69] Coronary artery calcium can be used to measure coronary atherosclerosis. Calcium score measured from computed tomography is a good prognostic tool for adverse CV events though the radiation and economic burden should also be considered.[70]

How can COVID-19 Damage the Vasculature?

While COVID-19 is still a new entity, we already have some studies on the relationship between COVID-19 and arterial aging. Evidence suggests that the SARS-CoV-2 virus can

spread into the cardiovascular endothelium.[71] Further evidence shows that epitheliopathy is present in COVID-19 and is related to the severity of the disease and death.[72]

SARS-CoV-2 enters cells using ACE-2 receptors; increased ACE-2 production makes it easier for the virus to enter the cell.[73] The disease course is worse in individuals with comorbidities like endothelial dysfunction, diabetes, hypertension and CV disease – all of which are associated with elevated ACE-2 receptor expression.[74] Once infected, various pathways discussed later lead to a decrease of ACE-2 receptors in endothelial cells.[73]

ACE-2 receptors are found in various tissues and are targeted as a binding protein for different viruses.[75] ACE-2 messenger ribonucleic acid (mRNA) is found in most human cells, mainly in alveolar epithelial cells, enterocytes in the small intestine and vascular endothelial cells, and arterial smooth muscle cells.[76] ACE-2 plays a role in anti-inflammation by promoting vasodilation.[77] Under normal conditions, a physiologic equilibrium exists between oppositely acting angiotensin derivatives synthesized by ACE and ACE-2. If the concentration of ACE-2 receptors is reduced (as seen in COVID-19), the balance shifts towards vasoconstrictive, oxidative and pro-inflammatory responses.[74]

ACE-2 typically reduces vasoconstriction and promotes vasodilation, thus reducing hypertension.[78] Reduction in ACE-2 levels in peripheral vasculature disturbs the anti-hypertensive role of these enzymes in small vessels.[79] The decrease of surface levels of ACE-2 leads to an increase of angiotensin-II, which leads to vasoconstriction.[80] This has been a proven pathological response to the SARS virus.[80]

The damage SARS-CoV-2 does to the endothelial wall can be split into two parts: direct and indirect damage. Indirect damage is caused by hyper inflammation and an increase in circulating cytokine levels.[81] Previous studies have shown that acute infection can result in increased cfPWV, possibly by decreasing NO bioavailability.[82] In vivo studies have shown that C-reactive protein reduces eNOS expression and activity in endothelial cells, thus leading to functional stiffening of the arteries.[83] cfPWV increase during acute infection also strongly correlates with C-reactive protein, IL-6 and matrix metalloproteinase-9 (MMP-9) levels.[84] The increase in MMP-9 levels leads to reduced elastin synthesis and fragmentation.[85] Healthy elastin prevents vascular smooth muscle cells from changing their phenotype from normal contractile phenotype to pathologic secretory phenotype.[86] Increased arterial stiffness leads to increased damage to the arterial wall due to changes in pulse pressure.[87] This arterial damage itself leads to atherosclerosis and inflammation, and these effects both contribute to arterial stiffening.[88,89] Thus, a vicious cycle is formed.[1] Increased arterial stiffness has been proven to cause target organ damage, it is also used to predict CV events and mortality. Infection of the endothelial cell leads to endothelial dysfunction through impaired smooth muscle cell function and vascular extracellular matrix remodelling.[83]

Direct damage results from SARS-CoV-2 infecting vascular endothelial cells. The SARS-CoV-2 virus uses the ACE-2 receptor to infect its host.[90] These receptors are also present on endothelial cells.[91] It has also been shown that in vitro grown human capillary organoids can be infected by SARS-CoV-2.[90] Diffuse endotheliitis, infiltration of mononuclear cells into endothelium and evidence of endothelial cell death was found in post-mortem studies of patients who died from COVID-19.[92] All these findings prove that the virus can infect endothelial cells. Infected cells become a target for recruited immune cells, which may lead to apoptosis and endothelial dysfunction.[92]

A recent study shows that SARS-CoV-2 S protein alone can be sufficient to cause endothelial cell injury, even without genetic material typically found in the virus. The ways in which S protein damages endothelial cells are various. When affected by S protein, endothelial cells undergo mitochondrial fission, which might lead to apoptosis. Endothelial cells, when exposed to S protein, undergo reduced eNOS activity. This leads to decreased NO bioavailability and endothelial dysfunction. Authors also noticed increased glycolysis in endothelial cells, which led to increased ROS and inflammation. The increase in ROS leads to ACE-2 destabilization and a decrease in ACE-2.[93]

Systemic inflammation and direct viral damage could be responsible for the progression of atherosclerotic plaques or rupture of older plaques, as seen in Influenza infections.[94] SARS-CoV-2 infects endothelium.[92] Cells infected by SARS-CoV-2 have been shown to produce increased amounts of MMP-9.[95] MMP-9 is associated with increased cfPWV and elastin fragmentation.[84] Healthy elastin protects vascular smooth muscle cells from phenotype shift from the normal contractile phenotype to SASP.[86] MMP-9 has been shown to promote the formation of new atherosclerotic plaques and instability of plaques.[96,97] In vitro studies have demonstrated that SARS-CoV-2 spike protein is enough to induce the synthesis of adhesion molecules (VCAM-1 and ICAM-1) and pro-inflammatory cytokines (TNFα, IL-1β and IL-6) in human umbilical vein endothelial cells.[98] Pro-inflammatory cytokines contribute to the progression of atherosclerosis, and the expression of adhesion molecules is crucial for the development of atherosclerosis.[99,100] This shows a reasonable theoretical pathway of SARS-CoV-2-induced atherosclerosis.

During vascular aging, vascular smooth muscle cells transition from normal constrictive phenotype to pathological SASP. This transition is irreversible and is driven by the p53 protein pathway as a response to telomere shortening or DNA damage.[101] DNA can be damaged by oxidative stress resulting from increased ROS production, associated with SARS-CoV-2 infection, and telomere shortening was noticed in patients after COVID-19.[102]

SARS-CoV-2 endothelial infection and pathological changes present in aged vasculature share common pathways. Oxidative stress and pro-inflammatory cytokines activate nuclear factor kappa-B, leading to pro-inflammatory cytokine

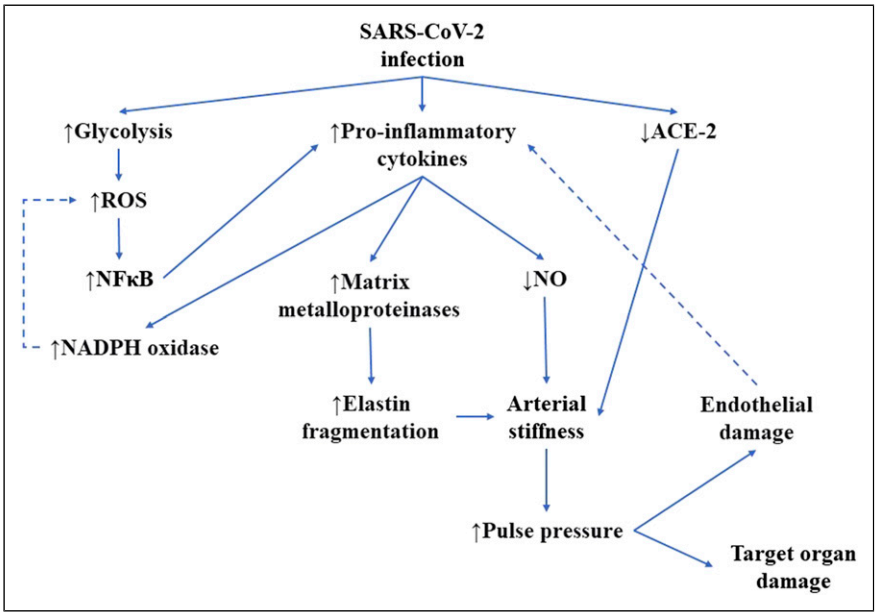

Figure 1. Common pathways for SARS-CoV-2 infection and arterial aging. Dotted lines represent the possible formation of the vicious cycle. ACE-2, angiotensin-converting enzyme 2; NADPH, nicotinamide adenine dinucleotide phosphate; NFκB, nuclear factor-kappa B; NO, nitric oxide; ROS, reactive oxygen species; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

gene transcription, and increased ROS production in NADPH oxidases.[16] Pro-inflammatory cytokines reduce the bioavailability of NO and increase the expression of matrix metalloproteinases, both of which lead to arterial stiffness.[83,85] Another common pathway leading to arterial stiffness is a decreased number of ACE-2 receptors, typical for both COVDI-19 and aging.[103] Arterial stiffness is associated with increased pulse pressure and endothelial damage.[104] The arterial damage triggers the production of pro-inflammatory cytokines.[16] The common pathways of arterial aging and SARS-CoV-2 infection have been summed up in Figure 1.

Conclusions

Aging vasculature undergoes a variety of biochemical changes and structural remodelling. Certain risk factors and genetic and epigenetic factors can influence the rate at which vascular aging occurs. Together these known and unknown factors are responsible for EVA and SUPERNOVA individuals. SARS-CoV-2 has been proven to, directly and indirectly, damage endothelium and promote changes like those seen in vascular aging. Vascular aging and COVID-19 share common pathways. COVID-19 could lead to early vascular aging. However, due to the novelty of the virus, there is still an urgent need for studies that investigate its long-term effects on vascular health. Also, there is a need to establish if certain medications could decrease any premature arterial aging. Fundamental studies are needed to identify possible therapeutic targets for the prevention and treatment of early vascular aging induced by COVID-19.

Author Contribution

All authors contributed to: (1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, and, (3) final approval of the version to be published.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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Abbreviations

ACE-2	angiotensin-converting enzyme 2
BH4	tetrahydrobiopterin
cfPWV	carotid-femoral pulse wave velocity
COVID-19	coronavirus disease 2019
CV	cardiovascular
DNA	deoxyribonucleic acid
EDD	endothelium-dependent dilatation
eNOS	endothelial nitric oxide synthase
EVA	early vascular aging
IL	interleukin

ICAM-1	intercellular adhesion molecule-1
MMP-9	matrix metalloproteinase-9
NADPH	nicotinamide adenine dinucleotide phosphate
NO	nitric oxide
ROS	reactive oxygen species
SARS	severe acute respiratory syndrome
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SASP	senescence-associated secretory phenotype
TNFα	tumour necrosis factor-alpha
SUPERNOVA	Super normal vascular aging
VCAM-1	vascular cell adhesion molecule-1
VSMCs	vascular smooth muscle cells