Review Article

Endotheliitis after COVID-19 Infection Requires Optimization of Chronic Disease Prevention

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Abstract

The outbreak of a new coronavirus–infection (2019-nCoV, so-called COVID-19) started in Wuhan, China[1] and shortly became a pandemic to create an enormous burden on the global health care and economic system.[2,3] In August 2021, the cumulative infected cases and the cumulative death, per world health organization (WHO) reporting, were over 266 million and almost 4.4 million worldwide, respectively.[2] In Thailand, the number of polymerase chain reaction (PCR) confirmed COVID -19 cases increased beyond one million cases in August 2021.[4] Although the majority of cases had only mild symptoms, critical cases developed severe pneumonia with respiratory failure, and many died.[5,6] Most of the deaths occurred in elderly patients who had chronic illness (non-communicable disease (NCD)) including diabetes mellitus (DM), hypertension (HT), chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD) and cardiovascular disease (CVD).[6-8] Studies revealed that the endothelial and epithelial cells that express angiotensin-converting enzyme 2 (ACE-2) receptor are the entry site for COVID -19 viral invasion. The subsequent inflammation, so-called endotheliitis, induces micro thrombi generation in capillary beds of lungs, kidneys, and myocardium, and contributes to cardiovascular morbidity and mortality.[8-11] This review summarizes the link between COVID -19 virus induced endotheliitis and poor prognosis in those with prior endothelial dysfunction. This relationship urges improvements in strategies to control and prevent these chronic conditions.

Keywords: endotheliitis, COVID -19 infection, chronic disease prevention

The global impact of COVID -19 viral pandemic

In December 2019, WHO reported the occurrence of a new coronavirus infection epidemic (2019-nCoV, SARS-CoV2, so-called COVID -19) in Wuhan, China.[1] With rather short incubation period, median of 4 days, COVID -19 rapidly spread over China within two months.[2] This outbreak became one of the fastest pandemics the world has ever seen, and the number of global infected cases rose from 750,890 in March 2020 to over 266 million in August 2021, with the death toll increasing from 36,405 to nearly 4.4 million.[3] Therefore, this novel virus created serious burden worldwide, not only for healthcare services but also the global economic system. It was estimated that COVID -19 virus reduced the annual global economic growth rate to -3.4% to -7.6% in 2020.[4] In June 2020, the World Bank reported that Thailand's economy had been shrinking by at least 5 % and it is expected to take more than two years for a full recovery to occur.[5] In Thailand, the number of PCR confirmed COVID-19 cumulative cases rose to over one million (1,120,869) cases, the cumulative death rate reaching 10,314 with peak mortality rates over 200 cases per day in August 2021.[6] Although the majority of cases had only mild symptoms, critical cases developed severe pneumonia, respiratory failure, and many people died.[7-10] Most of the death occurred in elderly patients, specifically those with chronic, non-communicable disease (NCD) such as DM, HT, CVD, HT, CKD, and chronic lung disease.[7-11] At the beginning, the pathogenic link between NCD and the COVID -19 infection was not entirely clear.

ACE-2 and clathrin-mediated endocytosis are both required for 2019-nCoV (COVID -19) cellular invasion

In 2000, the new human homologue of angiotensin converting enzyme, ACE-2, was discovered.[12,13] Its main function was to remove the single C-terminal Leu residue from angiotensin-2 (Ang-II) turning it into angiotensin 1-7, and changing angiotensin-I to angiotensin 1-9.[13,14] Ang1-7 had vascular protective effects, by counteracting the vasopressor, pro-inflammatory, pro-fibrotic and growth-promoting cellular actions of Ang-II, see **Figure 1**.[12] It had been shown that ACE-2 was localized in various human organs including the epithelium of gastrointestinal (oral mucosa, stomach, small intestine, colon), respiratory (nasal mucosa, nasopharynx, alveoli) system, lymph node, brain, vascular smooth muscle cells and particular in vascular endothelium of capillaries, venules, intra-myocardial vessels, medium-sized coronary arteries, arterioles, spleen and kidney.[13-15]

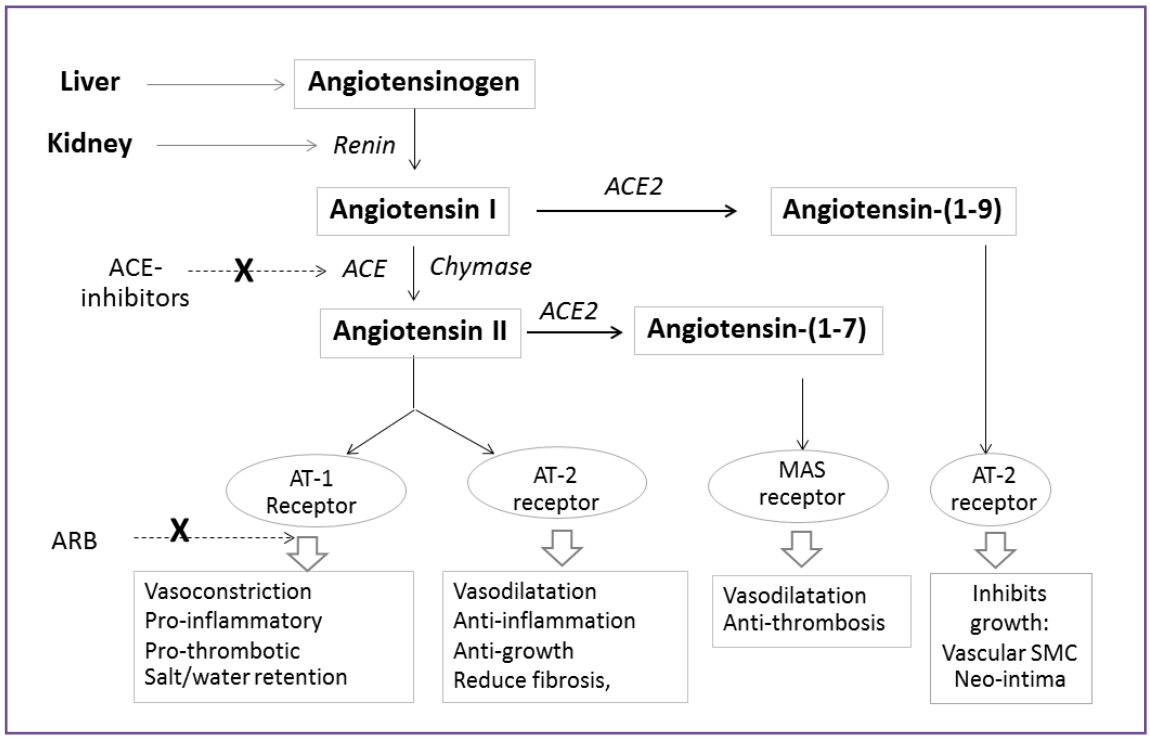

Figure 1: Renin-Angiotensin pathway and vasoactive effects: ACE=Angiotensin converting enzyme inhibitor, ACE-2: Angiotensin converting enzyme inhibitor-2, AT-1: Angiotensin II receptor type 1, AT-2: Angiotensin II receptor type 2, ARB: Angiotensin Receptor Blocker, MAS: the proto-oncogene that functions for Ang-(1-7) receptor. MAS came from an abbreviation of Massey, the surname of the person who donated the human tumor from which this proto-oncogene was identified.

In 2003 the coronavirus (SARS-CoV2) that caused the outbreak of SARS (severe acute respiratory syndrome) was identified.[16] Later, it was found that the spike (S) protein of this virus used the angiotensin-converting enzyme 2 (ACE-2) as a functional cellular receptor to attach to host cells.[17] Since the SAR-CoV and 2019-nCoV belong to the ϐ genus of the single-stranded RNA virus, they shared a similar sequence of the S-protein, in the range of 50-78%.[18] It was not surprising to find out that the ACE-2 receptor was also the entry site of 2019-nCoV.[19,20]

The single-stranded RNA, **2019-nCoV,** is surrounded by a lipid membrane, and includes the spike protein, composed of two subdomains. The S1 is the receptor-binding domain for ACE-2, whereas the S2 is the fusion site to the host cell membrane.[21] After binding to the ACE-2, the spike protein uses the host cell surface enzyme, the transmembrane serine protease 2 (TMPRSS2), to cleave the S1/S2 and the S2' sites to allow S2-membrane fusion.[22] In addition, in cell culture models it has been shown that the 2019-nCoV also requires **clathrin-mediated endocytosis to enter the host cell.**[23] This finding supported the therapeutic effect of some drugs that block (chloroquine) or disrupt (chlorpromazine) clathrin-mediated endocytosis.[23] After fusion of the viral membrane with the endosome, the viral RNA then passes into the cytosol for multiplication and viral replication, see Figure 2.[23,24]

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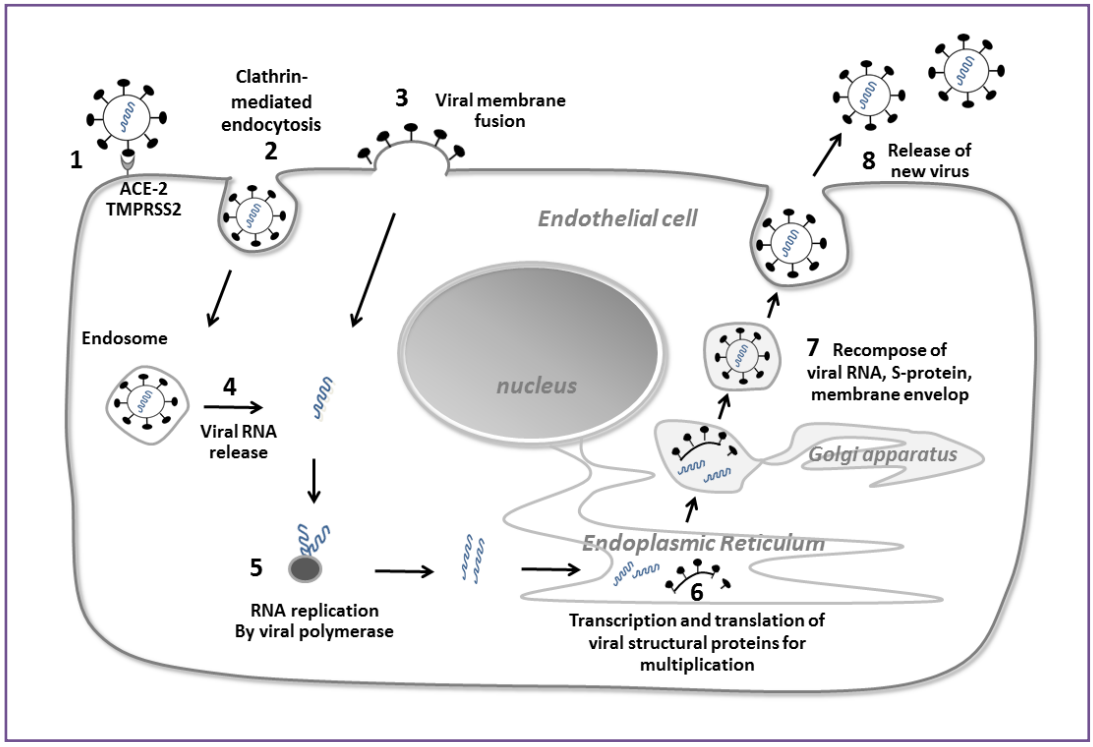

Figure 2: Simplified major steps of how COVID-19 virus entered and replicated inside the endothelial cells, modified from ref 23,24. The S-protein attached to ACE-2 receptor (1) at cellular surface and used the host cell surface enzyme, the transmembrane serine protease 2 (TMPRSS2), for cleavage the spike protein. Virus was taken into cell by clathrin-mediated endocytosis (2) into endosome or by membrane fusion (3). Viral RNA was then released to cytoplasm (4) and replicated by viral polymerase (5). Specific proteins, i.e. S-protein, membrane envelope, capsid, were resynthesized (6) and recomposed the viral RNA into the new envelope (7) which was later released from cell (8).

Endothelial cell, the viral target

Histologically, the endothelial cell (EC) forms a single cellular layer that separates the blood components from surrounding tissues.[25] The EC monolayer is in close proximity with **pericytes,** vascular supporting cells, which also attach to the basement membrane.[26,5] Functionally, the EC layer is metabolically active and protects vascular homeostasis by controlling vascular tone, permeability, inflammation, preventing thrombosis and maintaining vessel integrity by producing vasoactive substances including endothelium-derived relaxing factors (i.e. nitric oxide, prostacyclin), endothelium-dependent hyperpolarization factors and endothelium-derived contracting autacoids (i.e. endothelin-1, thromboxane A2, angiotensin II, superoxide anion), **Figure 3**[25,27]

The pathological evidence of direct EC invasion by COVID-19 virus was clearly documented by **Varga et al.** [28] in 2020. In three cases of COVID-19 infection, the presence of viral element within EC causing EC inflammation (so-called endotheliitis), and inflammatory cell death were illustrated in the **lung, heart, kidney, liver and small intestine.**[28] **Puelles VG et al.,**[29] studied 27 autopsies of COVID-19 cases and found viral particles in multiple organs, including the **lungs, pharynx, heart, liver, brain, and kidneys.** Regarding the heart, **Maccio et al.,**[30] studied the coronary histology of six COVID-19 patients and found that most of endotheliitis occurred in the small epicardial and intra-myocardial vessels, at the capillary level, where ACE2 and TMPRSS2 are strongly expressed, while the main coronary artery had only mild inflammation. Interestingly, they found evidence of neuritis, with lympho-monocytic inflammation of peri/epicardial nerves and strong ACE-2 expression, that might explain myocardial injury and arrhythmias in severe COVID-19 cases.[30] In brain, acute neutrophillic microvascular endotheliitis and acute type 3 hypersensitivity vasculitis were also reported in ten autopsied brain cases from COVID-19 infection, suggesting the role of autoimmune vascular inflammation.[31] Evidence of endotheliitis was also reported in limb amputation specimens of COVID-19 patients.[32] These findings strongly support the notion that EC was the target site and that endothelial dysfunction likely contributed to a pro-thrombotic state and excess cardiovascular mortality in COVID-19 infection cases.

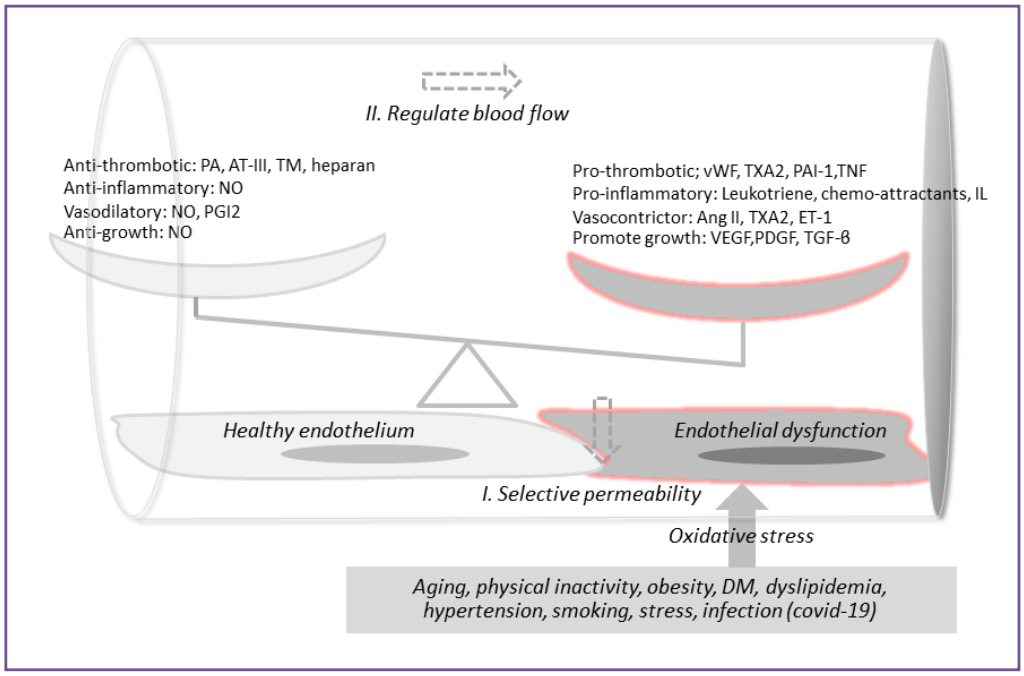

Figure 3: Major functions of endothelial cells are balancing vascular homeostasis by selective permeability and regulation of blood flow through several vasoactive mediators i.e. PA: plasminogen activator, AT-III: anti-thrombin III, TM: thrombomodulin, NO: nitric oxide, PGI2: Prostacyclin, vWF: von Willebrand factor, TXA2: thromboxane-A2, PAI-1: Plasminogen activator inhibitor, TNF: tumor necrotic factor, IL: interleukin, Ang-II: angiotensin II, ET-1: endothelin 1, VEGF: vascular endothelial growth factor, PDGF: platelet derived growth factor, TGF-ő: Transforming growth factor beta. Non-communicable diseases (NCD), unhealthy behavior and aging had created oxidative stress and caused endothelial dysfunction.

Pre-existing endothelial dysfunction in NCD, CVD cases and COVID-19 infection, a bad combination

Evidence of endothelial damage/dysfunction has been well documented in various NCD,[33] including in cigarette smokers[34,35] and patients with DM,[36-39] metabolic syndrome,[40] obesity,[41] HT,[42-45] dyslipidemia,[46-48] CAD,[49-51] CHF[52] and aging[53]. These conditions not only cause cellular toxicity but also induce apoptosis and endothelial loss.[54] Thus, **pre-existing endothelial dysfunction** in these conditions would make the COVID-19 infected cases at increased risk for CVD morbidity and mortality.

Recent studies demonstrate an association between NCD, CVD and high mortality from COVID-19 infection. In a prospective cohort study of 179 patients with COVID -19 pneumonia, four risk factors were identified to predict mortality, including age ≥ 65 years (OR:3.76,95%CI 1.14-17.39, p = 0.023), pre-existing concurrent cardiovascular or cerebrovascular disease (OR:2.46, 95%CI:0.75-8.04, p = 0.007), low CD3+CD8+ T-cell ≤ 75cells/µL (OR:3.98, 1.13-14.0, p < 0.001) and elevated cardiac troponin I, ≥ 0.05 ng/ml (OR:4.07,1.16-14.25, p <0.001).[7]

Further, at least three systematic reviews of COVID -19 studies confirmed a positive association between NCD and hospital mortality[8-10] **Qui and colleagues**[8] performed a systematic review and meta-analysis of 2,401 cases from 15 studies and found that common co-existing diseases in fatal COVID -19 cases were **HT** (38.6%:95% CI: 25.84-52.12%), **chronic cardiovascular disease** (17.54%, 95%CI: 13.38-21.69%), **DM** (22.2%,95% CI: 19.30-25.10%) and **chronic cerebrovascular disease** (15.58%,95%CI:10.05-21.12%). **Mehraeen et al.,**[9] reported the results of a systematic review, covering 114 studies involving 310,494 COVID -19 cases from various countries, which show that the most significant predictors for high mortality (>10%) were **older age, HT,** and **DM.** However, in this report, only DM was an independent predictor by multivariate analysis. **Nicoloski and colleagues**[10] reported the result of a systematic review of 45 COVID -19 studies and found that the co-existing chronic illness (NCD) including: DM, HT/CVD, COPD, CKD and liver diseases were highly prevalent among COVID -19 cases and much likely to predict worse outcomes such as severe respiratory failure and/or death.

To explain the strong link between those chronic comorbidities (NCD) and COVID-19 infection, several hypotheses have been proposed. **First**, over expression of ACE-2 receptors, which facilitate viral entry into the host cell, **second**, a hyper-inflammatory host response (AKA: cytokine storm),[10] and **lastly, a pre-existing EC dysfunction/activation from NCD,** which likely contributes to serious pulmonary/vascular complications and to high fatality. Meanwhile, with less ACE-2 activity, lingering Ang-II creates pro-inflammatory, pro-thrombotic conditions and vasoconstriction, see **Figure 4.**

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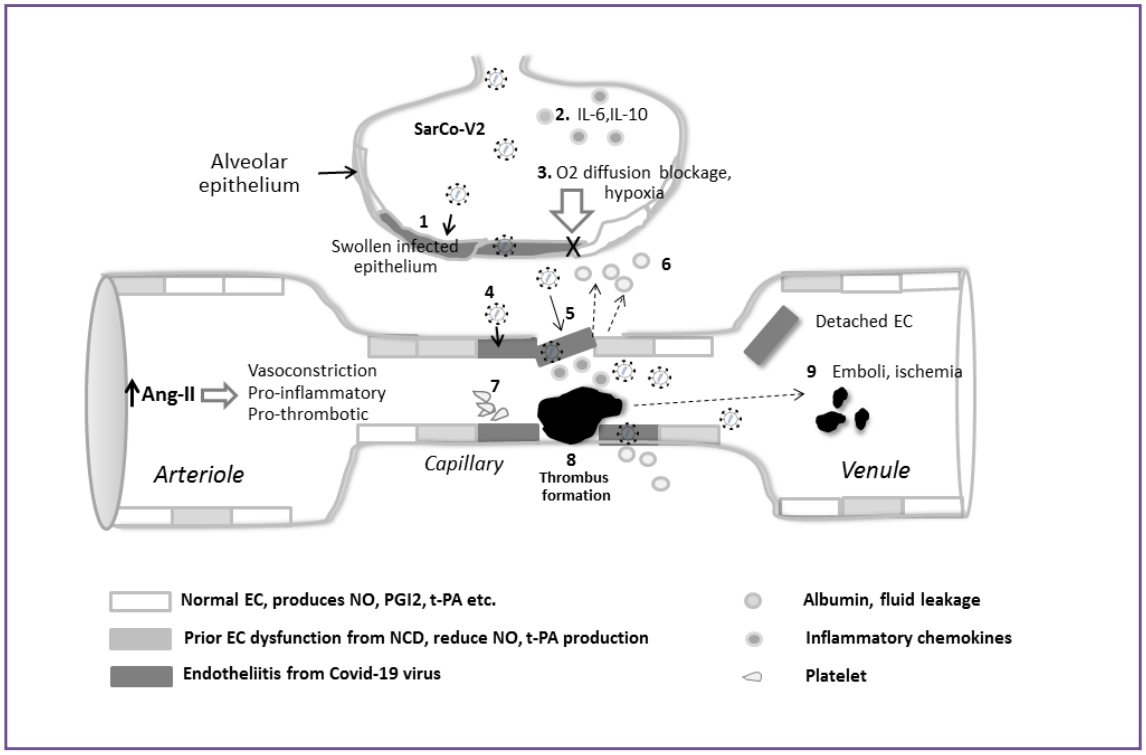

Figure 4: Simplified view of consequences after SARS-CoV2 (COVID 19) infected alveolar epithelium causing cellular edema (1), production of inflammatory cytokines.i.e. IL-6, IL-10 (2) and blocking oxygen supply (3). COVID -19 further entered endothelial cells (EC) via ACE-2 receptor, causing endotheliitis (4), disrupted EC (5) leading to albumin and fluid leakage (6), cytokines production, platelet aggregation (7), thrombus formation (8), embolism (9) and organ ischemia. Meanwhile, with less ACE-2 activity, lingering Ang-II creates pro-inflammatory, pro-thrombotic conditions and vasoconstriction.

Controlling CVD risk factors, the way to improve endothelial functions and reduce COVID-19 mortality

Following final differentiation, the mature EC has limited regenerative capacity,[54] so the repairing process requires proliferation from the nearby intact EC and from circulating Endothelial Progenitor Cell (EPC) from bone marrow, spleen and other tissues.[54-57] Thus, the severity of endothelial dysfunction depends not only on the extent of endothelial damage from NCD but also the capacity of reparative processes.[54]

Several therapeutic interventions improve EPC mobilization and restore endothelial function,[58] including exercise,[59-62] smoking cessation,[63] adoption of a Mediterranean diet,[64] glycemic control,[65,66] and medications such as nebivolol,[67] carvedilol,[68] angiotensin receptor blocker (ARB),[69] ACE inhibitor (ACEI)[70] and statins[71-75]. Among those mentioned, statins have been recently studied for their ability to reduce mortality in COVID-19 infections.[76,77] Although some conflicting data exist,[78] pooled data analysis and meta-analysis support mortality benefit of statin use,[79,80] even in the presence of comorbid conditions and risk factors such as aging, HT, DM and ischemic heart disease.[79] By pooling adjusted risk estimates in 14 observational studies, involving 19,988 COVID-19 patients, the use of statin was found to significantly reduce the risk of adverse outcomes (OR 0.51; 95% CI 0.41 to 0.63, $p < 0.0005$).[79] A recent meta-analysis confirmed that chronic statin use was independently associated with mortality reduction among COVID-19 cases, with aOR 0.67 (95%CI:0.52-0.86, from 11 studies) and aHR of 0.73(95%CI:0.58-0.91, from 10 studies).[80]

Some beneficial mechanisms of statin have been proposed including improved EC function, due to both LDL lowering and pleotropic effects. Beside blocking HMG-CoA reductase (rate-limiting enzyme for cholesterol synthesis), which reduces plasma cholesterol levels and sub-endothelial cholesterol deposits, and restores EC function, statins also have several pleiotropic functions including anti-thrombotic, anti-inflammatory, anti-oxidative and immune-modulatory effects.[81] In addition, in rabbits with dietary cholesterol induced atherosclerosis atorvastatin enhanced ACE-2 expression in the heart and kidneys, counter-acting renin–angiotensin system (RAS) by suppressing Ang II-induced contractile responses and enhancing AT2 receptor-mediated aortic responses.[82] All of these mechanisms could contribute to the reduced inflammation, thrombosis and mortality in COVID-19 infected cases under statin treatment.

Prognosis after COVID-19 infection

After 12 weeks of COVID infection, some survivors still

experience various symptoms, so-called "post- COVID-19 syndrome" or "long COVID".[83-85] These include cardiopulmonary (chest pain, palpitation, shortness of breath, cough), neuro-psychological (headache, concentration and cognitive deficit, dysgeusia, anosmia or parosmia, insomnia, anxiety, depression) and non-specific symptoms (fatigue or malaise, muscle and joint pain, vibratory sensation, fever).[83-85] The prognosis linked to these symptoms is not entirely clear and is often of difficult ascertainment due to the heterogeneous group of patients. For example, acute cardiovascular events caused from COVID-19 are quite varied, from acute myocardial infarction, myopericarditis, stress cardiomyopathy, arrhythmias, stroke, and arterial and venous thromboembolism.[86-89] Second, patients who had endothelial dysfunction from NCD or prior CVD[8-10] or had CVD complications trended to have higher fatality and morbidity.[86-89] Third, some treatment, i.e. chloroquine, hydroxychloroquine and azithromycin, could exacerbate arrhythmias by prolonging QTc.[90]

Thus, to better analyze long-term outcomes, patients should be re-classified, based on severity, in at least 3 groups:[88]

1. Patients who initially presented with asymptomatic, mild or moderate acute COVID-19 and continue to have symptoms ('long-hauler' COVID-19)

2. Patients who had mild or moderate-to-severe COVID-19 who had persistent structural changes i.e. myopericarditis (by MRI), interstitial lung disease (by CT), cardiovascular complications (heart failure, arrhythmias, vascular embolism), evidence of cardiac injury (i.e. elevated troponin levels) and/or reduced left ventricular ejection fraction.

3. Patients who had delayed recognition and treatment of acute coronary syndromes, stroke, pulmonary embolism and other cardiovascular disorders (so-called collateral damage).

A large retrospective registry study focusing on long-term cardiovascular outcomes was conducted in 2019, but results are still pending.[91] Hopefully, it will provide more data for the short and long-term cardiovascular mortality and cardiac events at one year of follow-up. Further large prospective studies are still needed to elucidate the epidemiology, pathophysiology, and long-term outcome of COVID-19 before this pandemic subsides.

Conclusions

The pandemic of COVID-19 virus is based on virus entry via targeted EC, causing endothelial dysfunction and a pan-vascular pro-thrombotic state which explains the high mortality in NCD and CVD cases with prior endothelial damage. With less ACE-2 activity, lingering Ang-II creates pro-inflammatory and pro-thrombotic conditions with vasoconstriction. Thus, the COVID-19 virus not only reminds of the importance of an intact EC barrier, but more importantly it emphasizes the need of better control and prevention of these chronic conditions to manage this and the next infectious epidemic.

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