The risk of SARS-CoV-2 infection does not appear to vary by age but the risk of symptomatic, severe, or fatal disease caused by this virus varies enormously with age. Children are low risk; data from New York City suggest about one death per 90,000 children and teenagers, including those with underlying conditions. The infection fatality rate for persons over 75 years was about one in 26 persons. Studies various countries have identified hypertension, type 2 diabetes, obesity, coronary artery disease and older age as risk factors for severe and critical COVID-19. At any age, have one or more chronic health problem increases the risk of mortality from COVID-19 by about 20 times. Thus, if we can identify and protect those at high risk, we can direct resources to save the lives of the millions of high risk people.

Hypertension, type 2 diabetes, obesity, coronary artery disease and aging all have endothelial dysfunction as a common feature. It is likely endothelial dysfunction that accelerates COVID-19 pathology.

Endothelial cells, the cells that make up the lining of the blood vessels and capillaries, bear the ACE2 protein that SARS-CoV-2 viral particles bind to, to enter the cell. Viral binding to ACE2 proteins on the endothelial cell surface inhibits ACE2 activity and allows for the cell to become infected. In autopsies of SARS patients (SARS-CoV-1) the viral infection of the heart was associated with decreased ACE2 expression[1], thus the virus depletes ACE2 activity.

ACE2 is an enzyme who's role is to converts angiotensin II (AT II), a vasoconstrictor and proinflammatory agent into angiotensin 1-7 (AT 1-7) in endothelial and other cells. AT 1-7 has anti-inflammatory, antioxidant and anti-fibrotic activity. Thus, COVID-19 is suspected to have proinflammatory effect on the vascular and pulmonary endothelium as a result of it shifting the endothelial balance towards vasoconstriction, inflammation and oxidative stress. This shift is most dangerous among those with preexisting endothelial compromise, including those with metabolic syndrome, diabetes, hypertension, obesity and heart disease.

The vascular endothelium regulates blood flow, and the adhesion of leukocytes (white blood cells – WBCs) and platelets to the endothelium. The endothelium controls vasodilation vs. vasoconstriction and helps mediate inflammation. The key endothelium-derived relaxing factor is nitric oxide (NO). NO promotes vasodilatation and reduces platelet aggregation and adhesion, and is therefore is anti-thrombotic. NO additionally inhibits adhesion of leukocytes and expression of pro-inflammatory cytokines, as well as prevents smooth muscle proliferation in the vasculature. NO is an endothelial-derived relaxing factor that negates the effects of endothelium-derived vasoconstrictors angiotensin II and endothelin-1.

The protective effects of eNOS[2]

- Promotes vasodilatation of vascular smooth muscle.
- Decreases platelet adhesiveness.
- Decreases adhesiveness of the endothelial layer to WBCs.

- Anti-inflammatory.
- Anti-oxidant. It scavenges reactive oxygen species.
- Anti-fibrotic.
- Anti-atherosclerotic actions on the arterial vessel wall.
- Counteracts smooth muscle cell proliferation.

The vasodilatory action of AT 1-7 is mediated by NO. In SARS-CoV-2 infection, with a loss of ACE2 activity and a shift away AT II towards AT 1-7, there is a shift in the balance of NO/angiotensin towards oxidation, inflammation, coagulation and vasoconstriction. This alteration underlies much of the injury experienced in COVID-19.

NO is a gasotransmitter (a neurotransmitter that is a gas) produced by endothelial nitric oxide synthase (eNOS). Tetrahydrobiopterin (BH4) and NADPH are required cofactors for the production of the NO. BH4 is also a cofactor for the production of several other neurotransmitters, including serotonin, dopamine, norepinephrine.

In normal conditions, the enzyme eNOS functions as a dimer, with two molecules of eNOS coupled together. The two molecules of eNOS are held together by a single atom of zinc in coordination with two molecules of BH4.[3] Normally, the enzymatic reaction occurs in pairs, with two molecules of l-arginine and O2 are converted into two molecules of l-citrulline and two NO are produced. In this reaction, two BH4 molecules are converted to two molecules of BH2 (7,8-dihydrobiopterin) and water is produced. The reaction is fueled by two molecules of NADPH, resulting in two NADP + 2 H^+.

After NO is produced, BH2 then needs to be recycled back into BH4. When it is not, bad things happen. When there is insufficient BH4 (or excess BH2), the eNOS dimer uncouples; the formation of NO continues, but rather than water being made as a byproduct, superoxide ($O_2^{\bullet}$), an extremely potent reactive oxygen species, is created. Some of the NO produced is then attacked by the $O_2^{\bullet}$, producing the nitrogenous free radical, peroxynitrite ($ONOO^-$).[4] $O_2^{\bullet}$ causes widespread intracellular damage to proteins and DNA, and peroxynitrite activates caspace 1 and promotes apoptosis.

The eNOS uncoupling mechanism is the product of evolution, and serves to promote inflammation and the “walling off” of areas of injury and infection. So in these circumstances, it is a good thing.

In order to survive bruises, bumps and bites, nicks and cuts, lacerations and lances, our bodies have mechanisms to curtail the loss of blood, fight infection at the point of injury, and repair the damage.

Platelets play a central role in coagulation. When there is injury of the blood vessels, including the tiny capillaries, platelets become activated as a result of exposure to collagen and Von Willebrand factor (VWF). VWF is normally stored in Weibel-Palade bodies, which are unique storage granules present only in endothelial cells. These granules also contain P-selectin,

endothelin-1, IL-8 and other cytokines. The granules open into the blood stream in response to inflammation and injury. VWF and P-selection are stored coiled but when activated by thrombin become threadlike and "sticky" to their ligands. They likely remain tethered to the Wiebel–Palade body, at least for a while; later the threads are cleaved by the antithrombotic enzyme ADAMTS13. The VWF "threads" also stick to each other forming larger sticky fibers. With thrombin activation VWF becomes sticky and binds to collagen, platelets, and to the coagulation protein, factor VIII. After activation by thrombin, P-selection also becomes sticky but specifically binds white blood cells, thus promoting an inflammatory response in the area. Thus, WVF and P-selectin act like spider webs or fishing lines in the blood stream hooking their target cells. VWF then binds the platelets to exposed collagen and forms plaques. The WBCs that get anchored by P-selectin can then migrate into intima of the blood vessel. VWF was been found to be five time higher than normal in a COVID-19 patient.[5]

ADAMTS13 is an enzyme that helps disintegrate VWF. There are several conditions in which ADAMTS13 is deficient; it is deficient in thrombocytopenic thrombotic purpura (TTP), which can be the result of a rare congenital deficiency, or more commonly form the development of anti-ADAMTS13 antibodies, and autoimmune disorder. A deficiency on ADAMTS13 can also occur as the result of sepsis or other severe infections such as malaria.[6] Plasmin also helps inactivate VWF[7] and degrade VWF-platelet complexes.[8]

Collagen becomes exposed when there is injury to the thin layer of endothelial cells that line the inside of blood vessels. Resting platelets maintain a low calcium level by constant calcium efflux. When there is traumatic injury and exposure of collagen, Factor VII (F VII) becomes activated (F VIIa). This activates factor X, and Factor Xa converts prothrombin to thrombin. Thrombin converts fibrinogen to fibrin. Thrombin also activates platelets that become adherent to VWF or fibrinogen at the site of injury.

When platelets become activated there is an increase in intra-platelet calcium that activates numerous enzymes in the platelet, and this alters the activity, shape, movement and other behavior of these cells. The activated platelets then amplify the coagulatory response to form clots and stop bleeding. This is great in injury, but intravascular coagulation form SARS-CoV-2 infection causes microemboli to the lung and thus hypoxia.

In addition to injury, thrombin and platelets can also become activated by other stimuli, including endothelial injury, or when metabolic factors inhibit the normal homeostatic balance that maintains anticoagulation. For example, endothelial prostacyclin binds to receptors on the platelets, and this prevents their activation. PAF (platelet-activating factor) is a phospholipid that can activate platelets and cause degranulation of white blood cells. PAF can be generated enzymatically as a result of environmental stressors, such as cigarette smoking and air pollution, but also by free radicals.[9] Additionally oxidized phospholipids, such as those produced by virally infected cells, may be recognized by PAF receptors, triggering inflammation and thrombotic events. There is evidence that dysregulated PAF signaling can contributes to DIC in sepsis and severe trauma.[10]

During the enzymatic reactions in which BH4 is used, for example, in which arginine is converted to citrulline or in which tryptophan is converted to the precursor of serotonin, BH4 is converted to dihydrobiopterin (BH2) and thus, BH4 needs to be replenished. This is not done primarily by creation of new BH4, but rather by BH2 being recycled back into BH4. This can be done by the enzyme QDPR (6, 7-Dihydropteridine Reductase: EC 1.5.1.34).

In vascular endothelial cells however, a different enzyme appears to be key for recyling BH4. In these cells, dihydrofolate reductase (DHFR), an enzyme which is part of folate metabolism, recycles BH2 into BH4. Knockdown of this enzyme in mice was found to increase the production of ROS, while knockdown of GTPCH, the rate-limiting enzyme for BH4 synthesis, did not; indicating that the recycling is of BH4 in the endothelial cell is paramount. Additionally, supplementing cells with BH2 increased ROS production. The accumulation of BH2 appears to inhibit NO production and signalling.[11] BH2 or an increase in BH2:BH4 ratio appears to cause eNOS uncoupling which shifts eNOS away from the production of NO and H_2O to the production of superoxide and ONOO- radicals.[12]

BH4 is thus essential to the formation of NO by endothelial cells, and plays a pivotal role in maintaining vascular health. NO is anti-inflammatory, antithrombotic, and prevents proliferation of smooth muscle that would increase vascular resistance. This is a cause of hypertension and heart disease. NO attenuates angiotensin and endothelin-1 activity. In contrast BH2 causes uncoupling of eNOS from BH2, causing shift from of NO production to the production of oxygen radicals ($O2^{\bullet}$) and nitrogen radicals (peroxynitrite: $ONOO^-$), causing endothelial dysfunction and promoting cell injury and thrombosis.

Aging is associated with a decline in eNOS coupling, and thus a tendency toward lower NO production, vascular dysfunction, and ROS production. Animal studies have shown greatly reduced life spans in animals with eNOS knockouts. eNOS activity is extended by eNOS protein binding with HSP90. HSP expression and binding with eNOS is reduced in aged endothelial cells. Glycosylation of eNOS also appear to decreases its activity, and thus hyperglycemia may inhibit eNOS function.[†] [13] Uncoupling of eNOS occurs more readily among those with cardiovascular disease and metabolic syndrome.[14] Oxidative stress also promotes uncoupling, and thus COVID-19 infection with its loss of ACE2 is thought to also promote the uncoupling of eNOS.

Abdominal obesity is closely associated with metabolic syndrome and endothelial dysfunction. Abdominal obesity is associated with reduced NO availability. INFγ promotes the activation of inducible nitric oxide synthase (iNOS) which has a long-lasting pro-inflammatory effect and which inhibits eNOS activity. iNOS behaves much like uncoupled eNOS. iNOS helps fight infections by way of it cytotoxic activity against pathogens, but also kills nearby host cells. Reduced eNOS function is correlated with elevated serum triglyceride levels and lower folate levels. High fat diets are thought to inhibit eNOS function.[15]

[†] Glycosylation of ACE2 also increases its binding to SARS-COV spike protein. Chloroquine is thought to inhibit the glycosylation of ACE2. Area for future investigation.

If BH2 is not recycled to BH4, BH2 gets metabolized into neopterin. Neopterin can be measured and used as an index of BH2:BH4 ratio. Neopterin levels are associated with increased lipid peroxidation and the formation of proinflammatory arachidonic acid products including prostaglandins and leukotrienes. Neopterin levels increase with age and are lower in healthier individuals. Neopterin levels have been found to be higher in person with a greater waste circumferences and insulin resistance, and found to be predictive of mortality risk.[16]

These data support the notion that COVID-19 risk may be associated with inadequate BH4 and NO. The inflammatory reaction caused by COVID, would worsen the loss of BH4 mediated NO production, and push towards vasoconstriction, ROS production, and intravascular coagulation.

The B vitamin, 5-methyltetrahydrofolate (5-MTHF), has been found to increase the binding affinity of BH4 to eNOS and to enhance the regeneration of BH4 from BH2. In elderly patients with coronary artery disease, 5-MTHF supplementation reversed eNOS uncoupling, reduced ROS production, and restored NO-mediated vasodilatation.[17] 5-MTHF can rapidly improve endothelial function. 5-MTHF has been determined to be a strong peroxynitrite scavenger and found to increased vascular BH4 and the BH4/total biopterin ratio. 5-MTHF helps reverseeNOS uncoupling and enhances eNOS activity.[18]

Resveratrol may help restore some eNOS activity, likely as a result of lowering NF-κB activity, but perhaps also by promoting eNOS coupling with BH4 and inducing eNOS transcription.

Multiple factors affect eNOS function: [19]

- Dimethylarginase (DDAH) activity. Asymmetrical dimethylarginine is an endogenous inhibitor of eNOS. DDAH metabolises asymmetrical dimethylarginine in the blood stream, thus DDAH prevent the impairment of eNOS.

- ROS reactive oxygen species, specifically $O_2^{\bullet}$ and ONOO- reduce DDAH activity. ROS can also convert BH4 into BH3, a non-functional form. Anti-oxidants such as vitamin C may be helpful, but anti-oxidant enzymes such as SOD and catalase are likely more important for quenching ROS and H_2O_2.

- Loss of a naturally occurring antioxidants diminished ROS scavenging capability. Oxidative stress also drives the balance towards BH2 and eNOS uncoupling in a feed-forward cycle.

- ACE, Angiotensin II, endothelin-1, hypertension, insulin resistance, type 2 diabetes, and hyperhomocysteinemia are associated with higher levels of asymmetrical dimethylarginine. Elevated LDL cholesterol and/or triglycerides decrease or impair DDAH. Homocysteine, elevated blood sugar, nicotine, and TNFα, inhibit DDAH enzyme and increases asymmetrical dimethylarginine.[20]

- Zinc is a cofactor for dimethylargininase. Activating compounds for DDAH include EGCG, taurine, vitamin E, aspirin, fenofibrate, estradiol, pioglitazone, pravistatin, probucol, losartin, and telmisartan. These agents may be helpful.
- Diabetes, with chronically elevated blood glucose may cause glycation of eNOS, which impairs its function. Diabetes promotes uncoupling of eNOS.
- Insufficient BH4 causes uncoupling of eNOS. Although generation of BH4 is important, the more common deficit is in the recycling of BH2 to BH4. 5-methyl tetrahydrofolate the active form of vitamin B9, improves BH2 recycling and eNOS coupling.
- C-Reactive Protein (CRP) down regulates eNOS.

C-Reactive Protein

C-reactive protein (CRP) is an inflammatory marker of endothelial dysfunction that is predictive of coronary artery disease, myocardial infarction, stroke and atherosclerosis. CRP is not just a marker; it causes a marked down-regulation of eNOS mRNA and eNOS expression. CRP also promotes the expression of the endothlin-1, a potent endothelium-derived vasoconstrictor. Additionally, CRP promotes the release of plasminogen activator inhibitor-1 (PAI-1) from the endothelial cells. PAI-1 inhibits the breakdown of fibrin clots by inhibiting activation of plasmin. CRP upregulates NF-κB and adhesion molecules such as ICAM and VCAM and monocyte chemoattractant protein (MCP). Thus, CPR serves to promote inflammation and adhesion of white blood cells to an area of injury and maintain fibrin clots. These activities help in survival and serve protective functions when there is an injury limited to time and place; however, CRP causes disease if chronically elevated throughout the vascular system.[21] The principal function of C-reactive protein is as a pattern recognition receptor that binds to lysophosphatidylcholine expressed on the surface of dying or dead cells, and then participates in the activation of the classical complement cascade as part of the C1 complex.[22]

An elevated CRP during COVID-19 infection is a potent predictor of disease severity. In a study from Wuhan, China, elevated CRP was associated with the number of lung areas affected and highly predictive of poor outcome.[23] In an American study of COVID-19 patients reporting to the emergency department of a hospital, 100% of patients had elevated CRP levels. Half of all patients had low sodium levels.[24] In another study, CRP, D-dimers (an indicator of intravascular coagulation, ferritin (another inflammatory marker), the inflammatory cytokine IL-6, and low lymphocyte count were found to predict risk of mortality in COVID-19 patients. COVID-19 survivors had median CRP levels around 40 mg/L while non-survivors had median levels around 125 mg/L.[25] The normal upper limit of CRP levels in health is 3.0 mg/L.

Inflammatory activation with TNFα, IL-6, and IFNγ promote the diversion of tryptophan into the kynurenine pathway. This depletes the formation of serotonin and melatonin, an antioxidant, and also depletes BH4, which causes uncoupling of eNOS and the production of ROS and ONOO-. Vitamin B6 is a cofactor for several enzymes in the Kynurenine pathway.

When there is inadequate vitamin B6 availability, it shifts kynurenine pathway towards more toxic and more inflammatory products. In a study of people living in Boston, vitamin B6 levels, as measures as plasma pyridoxal-5'-phosphate (PLP), were found to be inversely correlated with CRP levels.[26]

Oxidized Fatty Acid Metabolites

13-HODE(S) is another marker/mediator of endothelial dysfunction. 13-HODE(S) is formed from arachidonic acid and linoleic acid by the enzyme ALOX15. 13-HODE(S) is also from linoleic acid (LA) by the enzyme prostaglandin-endoperoxide synthase 2 (aka COX-2). 13-HODE can also form as result of oxidative action on linoleic or arachidonic acid by free radicals. IL1-β induces 13-HODE formation, apparently by increasing the expression of COX-2.[27]

Dietary linoleic acid is the major source of oxidized LA metabolites (OXLAMs) such as 13-HODE. A 12-week diet limiting linoleic acid was found to lower HODE and other OXLAMS.[28] The common dietary vegetable oils that are highest in linoleic acid are sunflower, corn, cotton seed, hemp, soybean, and peanut oils. Under risk of the COVID pandemic, I advise lowering the consumption of oils and fried foods. If oil is needed for example for baking, I recommend coconut oil.

Alcohol, other than as small amounts as red wine should be avoided, as alcohol when combined with linoleic acid increases the production of both 9-HODE and 13-HODE. ALOX15 contributes to liver injury from alcohol/linoleic acid as demonstrated by attenuated injury in ALOX15 deficient mice exposed to alcohol and linoleic acid. Exposure to linoleic acid and alcohol increased mRNA for TNFα, MIP-2α, MCP-1 and iNOS in M1 Macrophages.[29]

Potent ALOX15 inhibitors include: Apigenin, Baicalein, nordihydroguaiaretic acid, Fisetin, and quercetin, in approximate descending order.[30] Nordihydroguaiaretic acid (NDGA) is a compound found in the creosote bush.

Apigenin also reduces the TNFα induced expression of Plasminogen activator inhibitor-1 (PAI-1) (gene: SERPINE1) by 77%.[31] PAI-1 inhibits the activation of tPA and uPA, (plasminogen activators) and thus inhibits the conversion of plasminogen to plasmin, which breaks down fibrin clots.

A20 (TNFAIP3) is a protein that is required for vascular homeostasis and vascular endothelial cadherin (veCAD) expression. A20 limits endotoxins induced inflammation mediated by NF-κB activation and inhibits TNFα-induced apoptosis.

VeCAD is needed at the adherens junctions of endothelial cells, including those of the capillaries of the alveoli of the lung; veCAD helps maintains endothelial barrier integrity. LPS from gram negative bacterial induce loss of veCAD at the adherens junctions of endothelial cells and promotes vascular permeability, and accumulation of protein-rich edema, extravisation of inflammatory cells, and can trigger acute lung injury. [32] A20's enzymatic activity also inhibits NLRP3 inflammasome activation, and necroptosis by inhibiting RIPK3, CASP1, CASP8, and IL-

1β activation.[33] Thus, A20 appears to important for resolution and healing after infection. A2 may inhibit virally induced NF-κB signalling by restricting TRAF6 ubiquitination.[34]

Astragaloside IV, a compound found in the Chinese medicinal herb astragalus, increases A20 expression, and was found to inhibit virally induced NF-κB signalling.[35] Other natural agent that appear to upregulate A20 include zinc,[36] berberine,[37] vitamin D3 (as calcitriol),[38] and vitamin E.[39] MiR-128-3p upregulates the expression of A20,[40] but also promotes LPS induced myocardial inflammation.[41] miR-221 interferes with A20 signalling,[42] while miR-19b-3p directly targets A20 (TNFAIP3) mRNA.[43] [44] Induction of A20 may help resolve inflammation from viral infection; however, excessive down-regulation of immunity, as may be caused by corticosteroids, may need to be avoided early in disease.

Oxidative Stress

When the cell is stressed, it has several response mechanisms. A very simplified explanation would be to say that with mild to moderate cytoplasmic oxidative stress, the cell responds with Nrf2 and ARE, the Antioxidant Stress Element. This is a transcription factor for at least ten antioxidant and detoxification enzymes. With severe oxidative stress, the cell's responds with various forms of NF-κB, which act as transcription factors for inflammation. With extreme oxidxative stress and injury, the cell responds with NF-κB and AP-1 which can promote a strong inflammatory response with programmed cell death and the proliferation of inflammatory cells.

Antioxidant Response Element (ARE) Proteins[45]

ARE Genes	Functions
GCL and GCLM	Glutamate Cysteine Ligase and the Glutamate-cysteine ligase modulatory subunit are enzymes that work together on the rate-limiting step in the synthesis of glutathione, the body's most important antioxidant system.
GSR	Glutathione reductase reduces (recycles) glutathione disulfide (GSSH) into two molecules of glutathione (GSH), a critical cellular antioxidant.
SOD	Superoxide dismutase converts oxygen radicals into normal O2 and hydrogen peroxide that can then be reduced by glutathione.
CAT	Catalase decomposes the ROS hydrogen peroxide into O2 and H_2O.
HMOX1	Heme oxygenase breaks down heme from hemoglobin and produces a molecule of carbon monoxide that acts as an anti-inflammatory signal. HMOX1 is thought to protect the organs from damage from oxidative stress during injury and sepsis.
TXNRD1 and SRXN1	Thioredoxin and sulfiredoxin reductase repair oxidized disulfide bonds
NQO1	NAD(P)H dehydrogenase (quinone 1) detoxifies reactive quinones that cause oxidative stress and redox cycling.
GST	Glutathione S-transferase creates antioxidant conjugates with toxins so that they can be removed from the body, generally in the urine
UGT	UDP-glucuronosyltransferase catalyzes glucuronic acid conjugates, to help transport toxins into the bile or urine for elimination from the body
ABCC2	This ABC protein binds and transports negatively charged metabolic waste across cell membranes into the renal tubule or bile canaliculi for disposal.
G6PD	Glucose-6-phosphate dehydrogenase helps maintain energy to reduced NADP to NADPH and thus for the reduction of GSSH to GSH.

With aging there is a decline in the cellular presence of several ARE antioxidant proteins. In young animals these enzymes appear to be constitutively expressed in adequate amounts to protect the cell, however, basal levels of the ARE proteins fall with aging. This may result from decreased translation/transcription or from increased consumption due to greater oxidative stress. With aging the cell becomes increasing dependent on Nrf2-medidated transcription of the ARE to protect the cell from oxidative stress.[46] Thus especially for older persons, activation of Nrf2 and ARE transcription is protective from oxidative stress.

One of the ARE enzymes of special interest is heme oxygenase as it protects the organs from damage from oxidative stress during injury and sepsis. This protection occurs as the result of the production of the gasotransmitters CO (yes, carbon monoxide). CO release inhibits the ATP-induced NLRP3 inflammasome activation and attenuates myocardial dysfunction in mice with sepsis.[47] CO inhibits the generation of ROS from mitochondria in macrophages exposed to LPS and ATP, and inhibits the translocation of mitochondrial DNA into the cytosol.[48] CO treatment

has been demonstrated to inhibit ischemia-induced HMGB1 nucleocytoplasmic shuttling and release, and blunts the upregulation of TLR4, RAGE, TNF-α, IL-1β, IL-6, and MCP1 mRNA.[49] Another study also found that macrophages from CO treated mice had increased IL-6 and IL-10 in response to LPS, but decreases TNF.[50] Thus, heme oxygenase induction may down-regulate many of the cytokines that promote oxidative stress and sepsis in COVID-19.

Strategies for Improving Endothelial Function

Endothelial health is important throughout life, but may be critical to survival during the COVID-19 pandemic. Diseases that constitute metabolic syndrome; obesity, diabetes, hypertension, and heart disease, involve the endothelium and are associated with high risk of severe COVID disease. Thus, improving endothelial function should decrease risk from SARS-CoV-2 infection. It is probable that most Americans and Europeans will be exposed to this virus within a year of this writing and that millions will die from this disease. The time to act is now.

A risk score should be calculated for every individual. The score can be based on age, gender, BMI or waste circumference, menopausal status, and medical history (HTN, DM, CAD, etc.) and other know COVID-19 risk factors. Some risk calculators are available online, but an official state or CDC recognized calculator should be made so that there is consistent scoring, and so that updates can be made at a central site as information and estimates are refined.

Testing: I encourage C-reactive protein (CRP) as a marker of endothelial dysfunction for adults over the age of 45 and as well for younger patients with metabolic syndrome or other chronic disease who are thus at high risk for severe COVID-19. The goal of testing is to help judge individual risk and help target preventive treatments to lower risk. Neopterin may be another marker of COVID-19 severity risk. These tests may be used to refine risk assessment and help motivate behavioral adaptation.

The purpose of this scoring has several purposes. The first to identify risk. Those at highest risk need to be afforded the highest protection. This may mean workplace and other accommodations to lower risk of exposure as much as possible. When a vaccine becomes available, these should individuals should have priority.

A second use of risk evaluation is to identify those who can benefit from risk-lowering modalities, and to help them accommodate to adopt those strategies. Also this can help direct resources to insure these individuals have easy access to personal protective equipment, sufficient understanding to health behaviors to protect themselves and have access to nutritional supplements and dietary changes that can lower their risk.

A third use of risk evaluation is to help in decisions making as to which patients be treated with anti-viral medications, especially if those medications are in short supply.

If a person over the age of 45 tests positive for *early* SARS-CoV-2 infection, (in the first days of onset) I encourage CRP and ferritin testing. High levels that are associated with high risk of severe COVID-19 indicate likelihood of benefit from anti-viral therapy. Anti-viral therapy with

chloroquine and hydroxychloroquine appears to be most, and perhaps only effective if given early in the course of the disease. I consider that there is sufficient evidence to treat most high risk individuals (other than those with contraindication to these medications) with antiviral therapy. CRP can identify those who may be at high risk that do not have other known risk factors.

Same-day SARS-CoV-2 testing is needed so that high-risk individuals can be treated at the onset of symptoms. Pharmacists should be authorized to prescribe anti-COVID-19 antiviral treatment to high risk individuals under a prescribed CDC protocol as an emergency measure during the pandemic.

Improving Endothelial Function

The following list contains research generated ideas for improving endothelial function. Some are included as possible areas of research rather than as treatment recommendations.

1. N-acetyl cysteine (NAC) is a nutrition supplement that has been used as a medication for the treatment of acetaminophen toxicity and as a mucolytic agent. It is a precursor of glutathione, the body's most important antioxidant. NAC has been demonstrated to reduce oxidative stress and inflammatory response in patients with community acquired pneumonia using 1200 mg/day.[51] In hemodialysis patients, 600 mg of NAC twice daily (BID) lowered oxidative stress and improved endothelial dysfunction.[52] In another study of chronic kidney disease patients, 600 mg NAC BID use for 8 weeks was associated with highly significant declines in hsCRP, TNFα, IL-1, IL-6, C3 (complement factor 3) and other inflammatory markers.[53] In a study of recurrent unexplained pregnancy loss, NAC 600 mg/day with folic acid doubled the live birth rate compared to folic acid alone.[54] In a six- month, flu season, randomized double blinded clinical trial using 600 mg of NAC BID, both groups has similar seroconversion of A/H1N1 influenza, however 79% of control patients developed symptomatic disease while only 25% of those using NAC did. Thus, it did not prevent the infection, but greatly increased the rate of asymptomatic infection.[55] In a review of NAC trial for the prevention of exacerbations of chronic bronchitis, using 1800 mg of NAC per week to 1200 mg per day, found a 23% decrease in the number of events.[56] In another study, NAC 600 mg BID was found to delay the onset of ventilator associated pneumonia and decrese hospital stay for ICU patients.[57] Thus, NAC should be expected to decrease oxidative stress and lung inflammation.

   More specific to COVID-19 pathology, NAC has anti-thrombotic properties. In mice, NAC inhibited VWB-dependent platelet aggregation and NAC treatment led to the rapid resolution of induced thrombi.[58] NAC has found to be effective in preventing thrombotic thrombocytopenic purpura (TTP) in both mouse and baboon animal models of this disease, nevertheless, it failed to help resolve preexisting thrombus formation and organ damage. Thus, NAC appears to prevent TTP injury, but not resolve it.[59] In animal models of ferric chloride induced thrombotic stroke, intravenous NAC had thrombolytic activity that helped disintegrate VWF cross-linking, and reduced the injury size and neurologic outcome of the stroke.[60]

Thus, NAC is a precursor for the production of glutathione, an essential antioxidant and detoxifying agent, has anti-inflammatory effects, decreases hsCRP, inflammatory cytokine, and C3 levels, lowers the rate of symptomatic influenza for at least some strains of the virus, and perhaps most importantly for COVID-19, may decrease the development of microthrombi and intravascular coagulation. None of the trials cited above used more than 1200 mg of NAC per day and none of these trials did dose-effect testing.

A trial in which NAC was used in the treatment of retinitis pigmentosa, a hereditary degenerative eye diseases, doses of 600 mg, 1200 mg and 1800 mg twice a day for 12 weeks and then three times a day (TID) for another 12 weeks. At the highest doses there were gastrointestinal complaints that resolved with lowering of the dose. The best gain in visual function occurred with the 1200 mg (BID – TID) dose, with less benefit observed at the 1800 mg TID dose than the 600 mg TID dose.[61] In a study of NAC 1800 mg BID in patients with COPD for 8 weeks they was no improvement as compared to controls, but only one dose was tried,[62] while other studies suggest that 1200 mg of NAC per day prevents exacerbations of chronic bronchitis, and that among with chronic bronchitis but without COPD, 600 mg per day is sufficient.[63]

N-acetyl cysteine is generally available as 600 mg capsules. For the prevention of COVID-19 in high risk individuals, I recommend NAC 600 mg BID or TID. For the treatment of high risk COVID-19 patients, I recommend 2400 to 3000 mg of NAC, divided into two or three doses.

NAC may be helpful for children with pediatric multisystem inflammatory syndrome (PMIS), a novel COVID-19 associated syndrome similar to Kawasaki syndrome. Doses of NAC that has been used safely in children are about 35 to 60 mg/kg body mass.[64] A dose of 40 mg/ kg would be a reasonable trial dose. NOTE: This should not considered a recommendation of NAC as a treatment for PMIS, but rather as a point of data for beginning the planning of a clinical trial.

2. Consume a diet high in natural folate or take a 5-MTHF supplement. 5-MTHF supplements may act quickly to improve endothelial function. I recommend 800 mcg of 5-MTHF per day for improving endothelial health for those at risk.

3. Zinc supplement or a diet with sufficient zinc. The rate-limiting enzyme for BH4 production, GTP cyclohydrolase I (GTPCH), requires zinc as a cofactor.[65] eNOS coupling also requires zinc and zinc is a cofactor for dimethylargininase. Zinc may upregulate A20. Low dose zinc supplementation, sufficient to avoid zinc deficit is suggested. I recommend about 10 mg of elemental zinc/day in the form of zinc acetate or zinc citrate.

4. Vitamin D3. Low vitamin D levels appear to be correlated with elevated risk of severe COVID-19 and poor outcome. [66] [67] I recommend 400 to 600 IU of vitamin D3 daily.

5. Nrf2 activation of SOD, catalase and other antioxidant enzymes of the Antioxidant Response Element can be promoted by sulforaphane (broccoli and cauliflower) and allicin (garlic).[68] A few ounce serving of broccoli or cauliflower twice a week is likely

sufficient to provide this benefit. The vegetable will only provide sulforaphane if properly prepared. Boiling these vegetables destroys the sulforaphane. Thus they should be eaten raw or lightly steamed just before consumption.

Garlic can also be used to induce ARE transcription. It too is only effective if consumed in a way that delivers allicin. An appropriate amount is a medium sized clove, crushed or chewed, a few times a week. Garlic powder can be used; it should be sprinkled on food so that it is moistened before being eaten to form allicin. Cooking temperatures break allicin down, so cooked garlic has little or no effect.

6. Avoid high linoleic fatty acid diet, high fat diet, and maintain a low n3:n6 fatty acid balance. This would avoid corn-fed beef, in favor of range fed animals and the consumption of cold water fish. Eicosapentaenoic acid, found in fish oil appears to down-regulated miR-221 expression, and thus may increase A20 expression. A high fat diet lowers eNOS levels. It takes months however, to replace or reduce fatty acids stored in the body. Today is a good day to begin. Fired foods and vegetable oils should be avoided.

7. Avoid Metabolic Syndrome: Avoid high fructose and/or high fat diet. Table sugar is 50% fructose; its intake should be limited. High-fructose corn syrup and beverages sweetened with it should be avoided.

8. Low vitamin B6 levels are associated with elevated CRP. Most individuals get sufficient vitamin B6 in the diets, but it can become "sequestered" and unavailable as a result of inflammation. Thus, those with chronic inflammation or metabolic syndrome may benefit from the use of an active vitamin B6 supplement. Only a low dose should be required, but the natural, active from, pyridoxal-5-phosphate (P5P), should be used.[69] Persons with inflammatory conditions may benefit from low dose P5P supplements (<50 mg day).

9. Homocysteine inhibits DDAH and increases asymmetrical dimethylarginine that inhibits NO production. Elevated homocysteine levels are associated with cardiovascular and cerebrovascular disease. Homocysteine levels can often be normalized with adequate 5-MTHF, vitamin B6 (P5P), and vitamin B12 (as methylcobalamin).

   The need for methylcobalamin depends on the nutritional status and health of the individual, and the need for supplementation increases with age. Supplementation dose is best determined individually from blood tests. A blanket recommendation to cover a wide population requires a high enough dose for those with poor absorption, but low enough to be safe for everyone. I recommend 500 mcg per day for moderate-risk, middle aged individuals, and 1000 mcg per day for elderly individuals, preferably as a lozenge to help increase absorption. Elderly with who have poor B12 absorption may require vitamin B12 shots.

10. Exercise that increases blood flow velocity helps promote vascular health. "Acute exercise stress" causes a short term stress which stimulates the activation of Nrf2, and transcription of the ARE.[70] Thus, vigorous exercise in which the person's heart rate

accelerates, the individual gets short of breath and sweats, should increase the production of the ARE enzymes.

11. Individuals should work towards normalizing body mass and waist circumference. Keep in mind that sedentary behavior is a greater risk factor for heart disease than is obesity, and that an increase in activity may be easier and quicker than weight loss.

12. Avoid alcohol consumption and especially binge drinking. Alcohol should be limited to one ounce of red wine per 30 pounds of ideal body weight per day if used to improve vascular health.

13. EGCG from green tea, taurine, vitamin E, or low dose aspirin may increase DDAH activity and may improve NO production. Low dose aspirin can inhibit platelet function, but higher doses inhibit endothelial prostacyclin production and thus may promote endothelial dysfunction. If low dose aspirin is used, I recommend one-half of a baby aspirin (half of an 81 mg tablet, thus 40.5 mg) per day.

14. Apigenin inhibits ALOX15 and inhibits many TNFα induced inflammatory proteins. Apigenin is present in parsley, and it can be used as a tea. A therapeutic dose for a person with COVID-19 is likely around 200 mg of apigenin per day in divided doses. A therapeutic dose for a person with severe endothelial dysfunction is likely about 100 mg per day of apigenin.

15. Low dose statin medications (i.e. 5 mg of simvastatin) may improve endothelial function.

16. A20 (TNFAIP3) promotes endothelial integrity and inhibits NF-κB signalling. Garlic, zinc, vitamin D3, eicosapentaenoic acid (found in cold water fish), genistein (found in beans), vitamin E, astragalus, and berberine, have been found to induce A20 in cell cultures. I recommend use of those known to improve endothelial function by additional pathways (underlined).

17. Avoid nicotine and smoking. It only takes a few days for smoking cessation to begin lowering mortality risk.

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