Atherosclerosis as pathogenetic substrate for Sars-Cov2 ‘‘cytokine storm’’

Mattia Vinciguerra MD[1], Silvia Romiti MD[1], Ernesto Greco MD PhD[1].

1. Department of Clinical, Internal Medicine, Anesthesiology and Cardiovascular Sciences, Sapienza University of Rome, Rome, Italy.

Corresponding Author: Dr. Mattia Vinciguerra, Department of Clinical, Internal Medicine, Anesthesiology and Cardiovascular Sciences, Sapienza University of Rome, Rome, Italy.

Email: mattia_vinciguerra@libero.it

Word count: 3,369 words

Abstract

Sars-CoV-2 outbreak represents a public health emergency, affecting different regions around the world. Lung is the organ more damaged due to the high presence of Sars-CoV-2 binding receptor ACE2 on epithelial alveolar cells. Severity of infection varies from absence of symptomatology to be more aggressive, characterized by sudden acute respiratory distress syndrome (ARDS), multiorgan failure and sepsis requiring treatment in Intensive Care Unit (ICU).

It is not still clear why in a small percentage of patients, immune system is not able to efficiently suppress viral replication.

It has been documented as affections of cardiovascular system such as heart failure (HF), coronary heart disease (CHD) and risk factors for atherosclerotic progression, hypertension and diabetes among others could result predictive factors for severity and susceptibility during Sars-CoV-2

Atherosclerotic progression, as chronic inflammation process, is characterized by immune system dysregulation leading to pro-inflammatory pattern, including Interleukin 6 (IL-6), Tumor Necrosis Factor α (TNF-α) and IL-1β raise.

By reviewing immune system and inflammation profiles in atherosclerosis and laboratory results reported in severe Sars-CoV-2 infection we have supposed a pathogenetic correlation. Atherosclerosis may be a pathogenetic ideal substrate to high viral replication ability leading to adverse outcomes, as reported in patients with cardiovascular factors. Moreover, level of atherosclerotic progression may affect a different degree of severe infection; in a vicious circle feeding itself, Sars-CoV-2 may exacerbate atherosclerotic evolution due to excessive and aberrant plasmatic concentration of cytokines.

Key-words: atherosclerosis; Sars-CoV-2; COVID-19; pathogenesis of Sars-CoV-2;

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak is affecting different regions of the world since the end of 2019, representing a public health emergency.

Up to the date (i.e. 16 May, 2020) more than 4,677,129 cumulative cases have been confirmed by the Center for System Science and Engineering (CSSE) at Johns Hopkins University (JHU) and the number of deaths is 308,038 until now (https://coronavirus.jhu.edu/map.html).

The incubation period of Sars-CoV-2 to develop symptomatology is of 4-5 days, extending up to 14 days [1]. Severity of infection vary from absence of symptomatology, to having fever, cough, shortness of breath, anorexia and fatigue up to most severe cases characterized by severe pneumonia, acute respiratory distress syndrome (ARDS) and sepsis [2-4]. Respiratory failure, shock and multiorgan system dysfunction describe a critical illness, representing approximately 5% of cases and requiring mechanical ventilations in Intensive Care Unit (ICU) [5].

Pathogenesis of COVID-19 severity is not well known [6,7].

Angiotensin Converting Enzyme 2 (ACE2) is the functional receptor of Sars-Cov-2 representing the entry site to the human cells and may be ubiquitous although more expressed by epithelial cells of lungs, myocytes and vascular endothelial cells [8]. Interestingly referring to Sars-CoV similarities of shared ACE2 structure, it may be present on macrophages, monocytes and lymphocytes triggering the immunological activation [9]. Immunological response to the viral infection results the main cause for acute organ injury due to excessive activation. Moreover, in patients who need ICU and those with severe/critical manifestation of Sars-CoV-2, the immunological pattern is more dysregulated, being characterized by pro-inflammatory figure [9], leading to an abnormal and disproportionate activation of cytokine host cascade labeled as ‘‘cytokine storm’’ [7].

The causes of immune response exacerbation are largely unknown and is not still clear why only this small percentage of patients develop a persistent and dangerous infection, potentially leading to deteriorating of his condition and eventually to death [9].

A meta-analysis conducted on 53,000 infected patients in Wuhan has established as risk factors for severity of COVID-19: older age, sex male, smoking and any comorbidity. In fact Hypertension (HTN), diabetes mellitus, cardiovascular disease (CVD), cerebrovascular diseases, chronic obstructive pulmonary disease

(COPD) and chronic kidney disease (CKD) have shown a higher significative incidence in severe cases (54,9%) than in less aggressive ones (27,6%) [10].

Except for CKD and COPD, the other risk factors documented for severity are involved or are a direct consequence of atherosclerotic process, which is characterized by a well-known progression strongly related with immune system dysregulation.

Our aim is to review the pathogenetic mechanism of severe COVID-19, focusing on atherosclerotic process and describing it for profiles related to immune system and inflammation.

Pathogenesis of COVID-19

Sars-CoV-2, as causative agent of the novel respiratory infectious disease COVID-19, is a single-stranded RiboNucleic Acid (RNA) virus characterized by a high transmission human to human mainly through respiratory droplets [11,12].

The virus colonizes primarily lungs after an invasion of mucous membranes and causes the 'viremia phase' as defined by Lin et al, entering in the systemic blood system. Typically fever and cough represent the early most common symptoms detectable in this phase [6].

The high documented transmissibility of COVID-19 may be explained by the high viral load during ''viremia phase'' and by the molecular efficient mechanism to recognize binding protein ACE2 allowing the invasion of alveolar epithelial human cells [8,12].

The immune function of patients affected is involved to control the virus during the 'acute phase'; it seems in fact that acute organ damage which follows the first 'viremia phase' , occurs approximately after 7 days the onset of symptoms, when Sars-Cov-2 is not efficiently suppressed by immune system.

In the ''acute phase'', exploiting ACE2 binding receptor, COVID-19 leads to worsening respiratory functionality causing pneumonia [6].

The severity of respiratory disease and impairment varies from a picture of mild symptoms such as cough and shortness of breath up to Acute Respiratory Distress Syndrome (ARDS), as expression of critical illness [13].

The ubiquitous presence of ACE2 and patient's susceptibility may be associated besides to multiorgan failure, including acute myocardial causing directly myocarditis, kidney and liver injury leading to a systemic impairment [14].

The immune system, triggered by viral replication, plays a crucial role to damage organ during the ''acute phase'' due to the excessive activation [9].

An abnormal inflammatory response leads to exuberant amounts of cytokines and chemokines among others. The postulated pathogenetic mechanisms involved, due to the high affinity for ACE2, are associated to the massive viral replication in targeting cells such as alveolar epithelial, endothelial, macrophages and lymphocytes causing respectively apoptosis and pyroptosis in the cells of the immune system.

Moreover, basing on the pathogenesis of COVID-19, the role of viral induction on ACE2 may be involved due to downregulation and shedding of receptor leading to renin-angiotensin system dysfunction and increasing vascular permeability. Deficiency of ACE2, being the enzyme involved in the modulation of Renin Angiotensin Aldosterone System (RAAS), is linked with Angiotensin II (Ang II) raise, leading to the largely known deleterious effects such as Hypertension, vascular leakage, hypertrophy, fibrosis and consequently to severity of infection [9,15]. These findings may be supported by documented deficiency of ACE2 in African descendent populations such as African American, explaining the high incidence of severe manifestations and rate of mortality in this community. The biological variability in the different expression of ACE2, associated in black population with environmental selection, overexposes them to early HTN, atherosclerotic progression and CVD and may play in two different ways towards Sars-CoV-2 infection. Although deficiency of ACE2 may limit the adhesion to human cells and act potential as a form of ''immunity'' against infection, once they acquire COVID-19 are more susceptible to develop ARDS, sepsis and multiorgan failure, as summarized in the ***Figure 1*** [16]. Similarly, in the cited ***Figure*** is also described the possible pathogenetic mechanism for higher incidence of severe COVID-19 in males, highlighting furthermore gender difference of infectability due to the higher presence of a substrate to viral replication.

Moreover, ACE2 is implicated in the inactivation of des-Arg9 bradykinin, as component of kallikrein-kinin molecular system, causing angioedema in the lung due to the local vascular leakage effect [17].

Instead is controversial the role of anti-S-protein-neutralizing antibodies that it seems to facilitate acute lung injury [9].

Immune system results dysregulated showing a pro-inflammatory pattern affecting mainly the lung tissue for a large amounts of cells infiltration.

COVID-19 increases the plasmatic secretion of IL-1β, Interferon- γ (IFN-γ), interferon γ-induced protein 10 kDa (IP-10), Monocyte Chemoattractant Protein-1 (MCP-1), IL-4, and IL-10. The activation of complement system is found involved in the pathophysiology of ARDS, with increased plasmatic levels of C5a and directly in the autoptic evaluation with the high presence of C3a and C3-fragments, playing a primary role potentially useful for an effectiveness therapy [18].

After severe infection, pathway of T Helper (Th) 1 lymphocytes is hyperactivated causing an excessive production of CD14++ CD16+ inflammatory monocytes, responsible of inflammatory exacerbation and increased plasmatic concentration of IL-6 [19].

IL-6 is the key-cytokine triggering increased liver production of acute-phase proteins (APPs) such as C-reactive protein (CRP) and fibrinogen causing a hypercoagulable disease, characterized by thrombotic and embolic events, and may predict severity of infection [20,21]. Clinical pictures as deep acute venous thrombosis and pulmonary embolism are probably related to such disorders. Moreover, IL-6 is the target for therapy with Tocilizumab, a monoclonal antibody that has demonstrated efficacy in severe infection and actually in trial to be approved against Sars-CoV-2.

In addition to IL-6 a high plasma levels of IL-2, IL-7, IL-10, granulocyte colony stimulating factor (GCSF), IP-10, MCP-1, macrophage inflammatory protein 1-alpha (MIP-1α), and TNF-α have been documented in the host representing the well described ‘‘cytokine storm’’. In ICU patients decreases the plasmatic concentration of lymphocytes due to pulmonary aggregation and sequestration [7,9].

The pathogenetic mechanism to explain the different degree of immune system hyperactivation able to give a severe picture is not still clear [10].

However, analyzing statistic data from Italy and China of severe infection or related death are manifest some predisposing patterns able to provoke a bigger susceptibility to COVID-19.

In the meta-analysis conducted in Wuhan on 53,000 infected patients, severe illness and death occurred in the 20, 2 % and 3, 2% of cases. Old age (60 yrs.), sex male and the association of any comorbidity such as hypertension, diabetes and CVD among others [10] represented risk factors for severity, as reported in the introduction.

Data about COVID-19 related-death in Italy (https://www.epicentro.iss.it/coronavirus/sars-cov-2-decessi-italia), until today (i.e. 16 May, 2020), report 31,763 died patients with an average age of 80 years, 60,2% were men and only 3,9% of cases without any comorbidity. Moreover, focusing on the prevalence of pre-existing comorbidities, the most common is hypertension with approximately 70% of cases, diabetes more than 25% and coronary heart disease (CHD) reaching to 20%, followed by CKD, atrial fibrillation (AF), heart failure (HF) and COPD.

The role of atherosclerosis

Atherosclerosis is the leading origin of CVD producing worldwide the first causes of deaths, including stroke and CHD.

It is a chronic inflammatory disease characterized by infiltration, deposit and lipid oxidation which activates and promote a self-maintenance inflammatory state [22-25].

The most common risks factors are smoking, Hypertension, dyslipidemia, metabolic dysregulation such as obesity and diabetes mellitus and non-modifiable factors as advanced age, familiarity and gender. All these conditions represent also the main risk factors and pre-existing comorbidities in patients COVID-19 with worst clinical manifestation and evolution [3,4,10,13] suggesting a link between atherosclerosis process and severity of COVID-19.

In Atherosclerosis, mechanical stress and endothelium damage enable the accumulation of several plasma lipoproteins, in particular LDL, in the sub-endothelial space where they are modified into oxidized-LDL and trigger inflammation of arterial wall. Both innate and adaptive immune system play a crucial role in lesion formation and plaque characterization maintaining and promoting a pro-atherogenic state.

Oxidized-phospholipids and cholesterol crystals acquiring properties of damage-associated molecular patterns (DAMPs) are recognized by toll-like receptors (TLRs) and nod-like receptors (NLRs) and activate NLRP3 inflammasome pathway which results in proteolytic cleavage of proIL-1β and pro-IL18 to mature IL-1β and IL18 [26]. In the atherosclerosis disease, inflammatory signaling pathways activated are TLR4/NF-κβ and the JAK/STAT which contributed to boost inflammatory state raising cytokines expression and consequent activation of innate and adaptive immunity cells [24].

Therefore, immune system is dysregulated, due to chronic inflammation related to high plasmatic concentration of cholesterol, leading to pro-inflammatory pattern.

IL-1β has pro-inflammatory effects inducing expression of cytokines such as IL-6, TNF-α, IL-8, chemokines, improving the susceptibility of macrophages to lipid deposit and enhancing local inflammation and plaque instability. IL-1β, IL-6 and TNFα are also produced by CD14++ CD16+ non- classical monocytes activated in atherosclerosis disease, which are strictly correlated with the disease progression; similarly, complement system is associated with atherosclerotic progression, exacerbating inflammatory response, being C3 and C4 serum levels linked to an increased risk for CVD [25, 27, 28].

Increasingly studies have demonstrated the link between high levels of pro-inflammatory cytokines such as IL-6, TNF-α and IL-1β and progression and instability of atherosclerosis plaque pointing out their potential as novel target therapy [25,29-31]. Clinical research is focusing on agents that inhibit IL-1, IL-6 and TNFα pathways in order to reduce risk of coronary heart disease and reduce adverse outcomes after injury [24,25,30].

In the CANTOS trial Ridker et al. compared Canakinumab, a monoclonal antibody targeting IL-1β, with placebo in patients with previous myocardial infarction in order to reduce adverse clinical outcomes. This study shown a reduction in cardiovascular events in patients who received the drug although an increased incidence of fatal infections [32]. In addition, Anakinra (IL-1R antagonist) showed the potential to reduce the inflammatory response in acute myocardial infarction patients [33,34].

For the causal association between IL6R-related pathways and CHD [35], targeting treatment of IL-6 receptor provided a promising therapeutic approach. In this field the impact of Tocilizumab, an IL-6R monoclonal antibody, is currently evaluating in a randomized, placebo-controlled trial [25].

Etanercept, Infliximab and Adalimumab are anti TNF-α antibodies used in rheumatoid arthritis treatment, which demonstrated in this class of patients, significant increase of HDL cholesterol levels and endothelial function improvement [36-39].

Nidorf et al performed successfully a small prospective clinical trial with low-dose colchicine treatment in order to reduce cardiovascular events in patients with stable coronary disease [40] although more research is needed.

The T-helper1 cells type have shown to be the predominant CD4+ effectors in the context of atherosclerosis promoting disease progression due to increasing expression of pro-inflammatory cytokines [41,42]. In

addition, the B2 subsets of B-cells are the main activated in turn exacerbating the adaptive immune response [25].

Several studies suggest also an autoimmune response [43,44] in atherosclerosis with a switch in regulatory T cells from an initial protective phenotype (FoxP3+) into a pathogenic one (RORγt, T-bet, Bcl-6) [45].

This pro-inflammatory and dysregulate state may play a crucial role in increasing host susceptibility to develop "cytokine storm" and worst adverse manifestation of COVID19 due to excessive activation of immunological response. Sars-Cov-2 infection may act as a trigger in these susceptible hosts in which specific inflammation pathways are already activated (Inflammasome, JAK/STAT, NF-κβ pathways) and there is a dysregulation of autoimmune system. Although several studies are needed, this hypothesis may partly explain the severity of infection manifestation in this class of patients.

Effects of Sars-CoV-2 on Cardiovascular System

Bonow et al [46] have postulated hypothesis to explain the documented more susceptibility of patients affected by coronary artery disease (CAD) and risk factors for atherosclerotic cardiovascular disease to develop adverse outcomes and death due to COVID-19 [5,10].

As described in literature for others scenario of acute infections [47], the hyperdynamic circulation caused by COVID-19, in patients with predisposing factors for coronary artery disease , may exacerbated the precarious balance by increasing myocardial oxygen demand resulting in acute coronary events (ACS).

Interestingly, they have speculated that ACS in infected patients may be caused by excessive cytokines raise leading to atherosclerotic plaque instability and rupture [46].

The progression and instability of atherosclerotic plaque is in fact strongly related with raise in plasmatic concentration of IL-6, TNF-α and IL-1β and protease activation leading to plaque rupture and luminal thrombosis due to direct negative effect on plaque protective fibrous cap [26].

Interestingly, Wang et al [48] have highlighted, analyzing infectivity of Sars-CoV-2 on cultured cells, the dependence of adhesion, proteases activity and endocytosis to levels of tissue cholesterol content rather than plasmatic concentration. They have focused on different cells belonging to three macro-groups of population: young, elderly and elderly affected by chronic inflammation. Their results may explain the higher frequency in young to acquire asymptomatic form of COVID-19 and conversely in patients affected by chronic

inflammation, associated to age among others, the more efficiently viral replication leading to worse outcome. These findings support our hypothesis of pre-existing pathogenetic substrate such as atherosclerosis in developing severe COVID-19.

Vice versa, the impact of Sars-CoV-2 on lipid metabolism and progression of atherosclerotic process is confirmed by an observational study conducted to analyze long-term effect of acute Sars-CoV infection [49]. Twenty-five patients were recruited 12 years after recovery due to Sars-CoV in 2003. Metabolomic analysis was performed, showing an altered lipid metabolism with a significantly increased values of free fatty acids, lysophosphatidylcholine, lysophos-phatidylethanolamine and phosphatidylglycerol. Moreover, the 44% of patients were affected by CVD.

Sars-CoV pathogenesis has shown a high similarity if compared with COVID-19, being characterized by an abnormal hyperactivation of immune system leading to excessive amounts of cytokines too [6].

Hyperactivation of pro-inflammatory pattern, mainly characterized by 'cytokines storm' may increase risk of restenosis in patients underwent percutaneous coronary intervention (PCI) with stent implantation due to CHD. As recently reported by Sun et al, pre-operative increased levels of IL-6, TNF-α and IL-23 may predict efficiently risk of restenosis after PCI associated to drug-eluting stents implantation [50].

Besides ACS, cardiovascular system may be involved in patients affected by heart failure, due to hemodynamic decompensation, and in small percentage of cases, acute myocarditis may occur without prior evidence of CVD, due to ACE2 presence on cardiac myocytes, leading potentially to chronic dilated cardiomyopathy [46,51,52].

Hospitalized patients who developed myocardial injury, have been described by Shi et al [53] and Guo et al [54] highlighting similar characteristics. Evidence of myocardial injury was characterized by increased plasmatic levels of high-sensitivity troponin I (TnI).

Clinically, patients with elevation of TnI are older and characterized by a high prevalence of hypertension, diabetes mellitus, CAD and heart failure. A more severe systemic inflammation has been documented including higher plasmatic concentration of leucocytes and CRP among others, leading to a more complicated respiratory picture with higher incidence of ARDS requiring assisted ventilation than in patients without evidence of myocardial injury.

In particular Shi et al [53] have documented on a total population of 416 hospitalized patients studied with confirmed diagnosis of Sars-CoV-2, 82 patients (19,7%) with evidence of myocardial injury, resulting in a mortality rate in this group of 51,2% significantly higher than in patients without elevation of TnI (4,5%). Similarly, Guo et al [54] have studied 187 patients with confirmed laboratory diagnosis, the 27,8% with evidence of myocardial injury characterized by higher in-hospital mortality rate of 57,6% compared with patients not affected (8,9%). Moreover, in this report also patients with pre-existing CVD as comorbidity but without TnI elevation have shown a worse outcome with mortality rate of 13,30%.

Potentially, long-term effects of cardiovascular system involvement mainly due to hemodynamic changes, atherosclerotic progression and resulted increased risk of thrombosis due to COVID-19 may impact directly on left ventricular systolic function and increasing retrograde pressure on right cardiac chambers leading to decompensation. Moreover, increased incidence of deep vein thrombosis (DVT) events due to abnormal blood clotting may cause more pulmonary embolism events and pulmonary hypertension.

Conclusion

The proposed pathogenetic correlation of atherosclerosis and Sars-CoV-2 infection is simplified and summarized in ***Figure 2***.

Atherosclerosis, as chronic inflammatory disease, may cause an ideal substrate to the high viral replication capacity of Sars-CoV-2 in human cells leading hyperactivation of pro-inflammatory pattern due to immune system dysregulated. Probably the level of atherosclerotic progression may influence severity of COVID-19 in susceptible patients, causing different degree of excessive amounts of immune system cells, cytokines among others, mainly involved in the organ damage.

Moreover, the aberrant inflammatory response, as in a vicious circle feeding itself, may lead to atherosclerotic progression increasing risk of instability and rupture.

These hypotheses are sustained also by novel therapeutic approach for atherosclerosis with drugs working mainly against inflammatory intermediary, of which Tocilizumab among others has demonstrated a great efficacy to reduce severe Sars-CoV-2 infection too.

However, methodological studies focused on this topic are necessary to fortify these suggesting.

Acknowledgements

These authors contributed equally to this work.

Funding

The authors received no financial support for the research, authorship and/or publication of this article.

Declaration of conflicting interests

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

References

1. Lauer, S. A., Grantz, K. H., Bi, Q., Jones, F. K., Zheng, Q., Meredith, H. R., et al (2020). The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. *Ann. Intern. Med.*, 172, 577-582.
2. Hu, Z., Song, C., Xu, C., Jin, G., Chen, Y., Xu, X., et al (2020). Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China. *Sci. China Life Sci.*, 1-6.
3. Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Fan, G., et al (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*, *395*(10223), 497-506.
4. Yang, X., Yu, Y., Xu, J., Shu, H., Xia, J., Liu, H., et al (2020). Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Resp. Med*, 8, 475-481.
5. Wu, Z., & McGoogan, J. M. (2020). Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *Jama* 2020; 323(13):1239-1242.
6. Lin, L., Lu, L., Cao, W., Li, T. (2020). Hypothesis for potential pathogenesis of SARS-CoV-2 infection——a review of immune changes in patients with viral pneumonia. *Emerg. Microbes Infect.*, 9(1):727-732.
7. Zhou Y, Fu B, Zheng X, et al. Pathogenic T cells and inflammatory monocytes incite inflammatory storm in severe COVID-19 patients. *Natl Sci Rev*. 2020;nwaa041.
8. Zhang, H., Penninger, J. M., Li, Y., Zhong, N., & Slutsky, A. S. (2020). Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med.*, 1-5.
9. Fu, Y., Cheng, Y., & Wu, Y. (2020). Understanding SARS-CoV-2-mediated inflammatory responses: from mechanisms to potential therapeutic tools. *Virol. Sin.*, 1-6.
10. Zhao, X., Zhang, B., Li, P., Ma, C., Gu, J., Hou, P., et al (2020). Incidence, clinical characteristics and prognostic factor of patients with COVID-19: a systematic review and meta-analysis. *medRxiv*.
11. Wu D, Wu T, Liu Q, Yang Z. The SARS-CoV-2 outbreak: What we know [published online ahead of print, 2020 Mar 12]. *Int J Infect Dis*. 2020;94:44-48.
12. Chen Y, Li L. SARS-CoV-2: virus dynamics and host response. *Lancet Infect Dis*. 2020;20(5):515-516.
13. Cascella, M., Rajnik, M., Cuomo, A., Dulebohn, S. C., Di Napoli, R. (2020). Features, evaluation and treatment coronavirus (COVID-19). In *StatPearls [Internet]*. StatPearls Publishing.
14. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. 2020;8(5):475-481.
15. Verdecchia P, Cavallini C, Spanevello A, Angeli F. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection [published online ahead of print, 2020 Apr 20]. *Eur J Intern Med*. 2020;S0953-6205(20)30151-5.
16. Vinciguerra M, Greco E. Sars-CoV-2 and black population: ACE2 as shield or blade? [published online ahead of print, 2020 May 13]. *Infect Genet Evol*. 2020;104361.
17. van de Veerdonk, F. L., Netea, M. G., van Deuren, M., van der Meer, J. W., de Mast, Q., Brüggemann, R. J., & van der Hoeven, H. (2020). Kallikrein-kinin blockade in patients with COVID-19 to prevent acute respiratory distress syndrome. *Elife*, *9*, e57555.
18. Risitano, A. M., Mastellos, D. C., Huber-Lang, M., Yancopoulou, D., Garlanda, C., Ciceri, F., & Lambris, J. D. (2020). Complement as a target in COVID-19?. *Nat. Rev. Immunol.*, 1-2.
19. Chen, L., Liu, H. G., Liu, W., Liu, J., Liu, K., Shang, J., et al (2020). Analysis of clinical features of 29 patients with 2019 novel coronavirus pneumonia. *Zhonghua jie he he hu xi za zhi= Zhonghua jiehe he huxi zazhi= Chinese J. Tuberc. Resp. Dis.*, *43*, E005-E005.
20. Han, H., Yang, L., Liu, R., Liu, F., Wu, K. L., Li, J., e al (2020). Prominent changes in blood coagulation of patients with SARS-CoV-2 infection. *Clin. Chem. Lab. Med. (CCLM)*, *1*(ahead-of-print).
21. Wolf, D. & Ley, K. Immunity and Inflammation in Atherosclerosis. *Circ. Res.* 124, 315–327 (2019).

22. Taleb, S. Inflammation in atherosclerosis: L'inflammation dans l'athérosclérose. *Arch. Cardiovasc. Dis.* 109, 708–715 (2016).
23. Abbasi J. Cardiovascular Corner—Stable Coronary Artery Disease, An LDL "Vaccine," and Anti-inflammatories. *JAMA*. 2020;323(13):1233–1234.
24. Zhu, Y., Xian, X., Wang, Z., Bi, Y., Chen, Q., Han, X., et al (2018). Research progress on the relationship between atherosclerosis and inflammation. *Biomolecules*, *8*(3), 80.
25. Li, B., Li, W., Li, X. & Zhou, H. Inflammation: A Novel Therapeutic Target/Direction in Atherosclerosis. *Curr. Pharm. Des.* 23, 1216–1227 (2017).
26. Miteva, K., Madonna, R., De Caterina, R., Van Linthout, S. Innate and adaptive immunity in atherosclerosis. *Vascul. Pharmacol.* 107, 67–77 (2018).
27. Wildgruber, M., Aschenbrenner, T., Wendorff, H., Czubba, M., Glinzer, A., Haller, B., et al (2016). The "intermediate" CD14++ CD16+ monocyte subset increases in severe peripheral artery disease in humans. *Sci. Rep.*, *6*(1), 1-8.
28. Hovland, A., Jonasson, L., Garred, P., Yndestad, A., Aukrust, P., Lappegård, K. T., et al (2015). The complement system and toll-like receptors as integrated players in the pathophysiology of atherosclerosis. *Atherosclerosis*, *241*(2), 480-494.
29. Cai, T., Zhang, Y., Ho, Y. L., Link, N., Sun, J., Huang, J., et al (2018). Association of interleukin 6 receptor variant with cardiovascular disease effects of interleukin 6 receptor blocking therapy: a phenome-wide association study. *JAMA Cardiol*, *3*(9), 849-857.
30. Ridker, P. M. & Lüscher, T. F. Anti-inflammatory therapies for cardiovascular disease. *Eur. Heart J.* 35, 1782–91 (2014).
31. Ridker PM. From C-Reactive Protein to Interleukin-6 to Interleukin-1: Moving Upstream To Identify Novel Targets for Atheroprotection. *Circ Res*. 2016;118(1):145-156.
32. Ridker, P. M., Everett, B. M., Thuren, T., MacFadyen, J. G., Chang, W. H., Ballantyne, C., et al (2017). Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N. Engl. J. Med.*, *377*(12), 1119-1131.
33. Abbate, A., Van Tassell, B. W., Biondi-Zoccai, G., Kontos, M. C., Grizzard, J. D., Spillman, D. W., et al (2013). Effects of interleukin-1 blockade with anakinra on adverse cardiac remodeling and heart failure after acute myocardial infarction [from the Virginia Commonwealth University-Anakinra Remodeling Trial (2)(VCU-ART2) pilot study]. *Am. J. Cardiol. 111*(10), 1394-1400.
34. Morton, A. C., Rothman, A. M., Greenwood, J. P., Gunn, J., Chase, A., Clarke, B., Hall, AS., et al (2015). The effect of interleukin-1 receptor antagonist therapy on markers of inflammation in non-ST elevation acute coronary syndromes: the MRC-ILA Heart Study. *Eur. Heart J.*, *36*(6), 377-384.
35. IL6R Genetics Consortium Emerging Risk Factors Collaboration, Sarwar N, Butterworth AS, et al. Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. *Lancet*. 2012;379(9822):1205-1213. doi:10.1016/S0140-6736(11)61931-4
36. Cardillo, C., Schinzari, F., Mores, N., Mettimano, M., Melina, D., Zoli, A., et al (2006). Intravascular tumor necrosis factor α blockade reverses endothelial dysfunction in rheumatoid arthritis. *Clin. Pharmacol. Ther.*, *80*(3), 275-281.
37. Taguchi, H., Nishi, K., Suzuki, T. & Okano, Y. Anti-atherosclerotic effects of etanercept in Rheumatoid Arthritis Patients. *Japanese J. Clin. Immunol.* **35**, 183–7 (2012)
38. Seriolo, B., Paolino, S., Sulli, A., Fasciolo, D. & Cutolo, M. Effects of anti-TNF-α treatment on lipid profile in patients with active rheumatoid arthritis. *Ann. N. Y. Acad. Sci.* **1069**, 414–9 (2006).
39. Tam, L. S., Kitas, G. D. & Gonźlez-gay, M. A. Can suppression of inflammation by anti-TNF prevent progression of subclinical atherosclerosis in inflammatory arthritis? *Rheumatol. (United Kingdom)* **53**, 1108–1119 (2014).
40. Nidorf, S. M., Eikelboom, J. W., Budgeon, C. A. & Thompson, P. L. Low-dose colchicine for secondary prevention of cardiovascular disease. *J. Am. Coll. Cardiol.* **61**, 404–410 (2013).
41. Tabas, I. & Lichtman, A. H. Monocyte-Macrophages and T Cells in Atherosclerosis. *Immunity* **47**, 621–634 (2017).
42. Hedrick, C. C. Lymphocytes in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **35**, 253–257 (2015).
43. Andersen CJ. Impact of Dietary Cholesterol on the Pathophysiology of Infectious and Autoimmune Disease. *Nutrients*. 2018;10(6):764. Published 2018 Jun 13.
44. Sima P, Vannucci L, Vetvicka V. Atherosclerosis as autoimmune disease. *Ann Transl Med*. 2018;6(7):116.

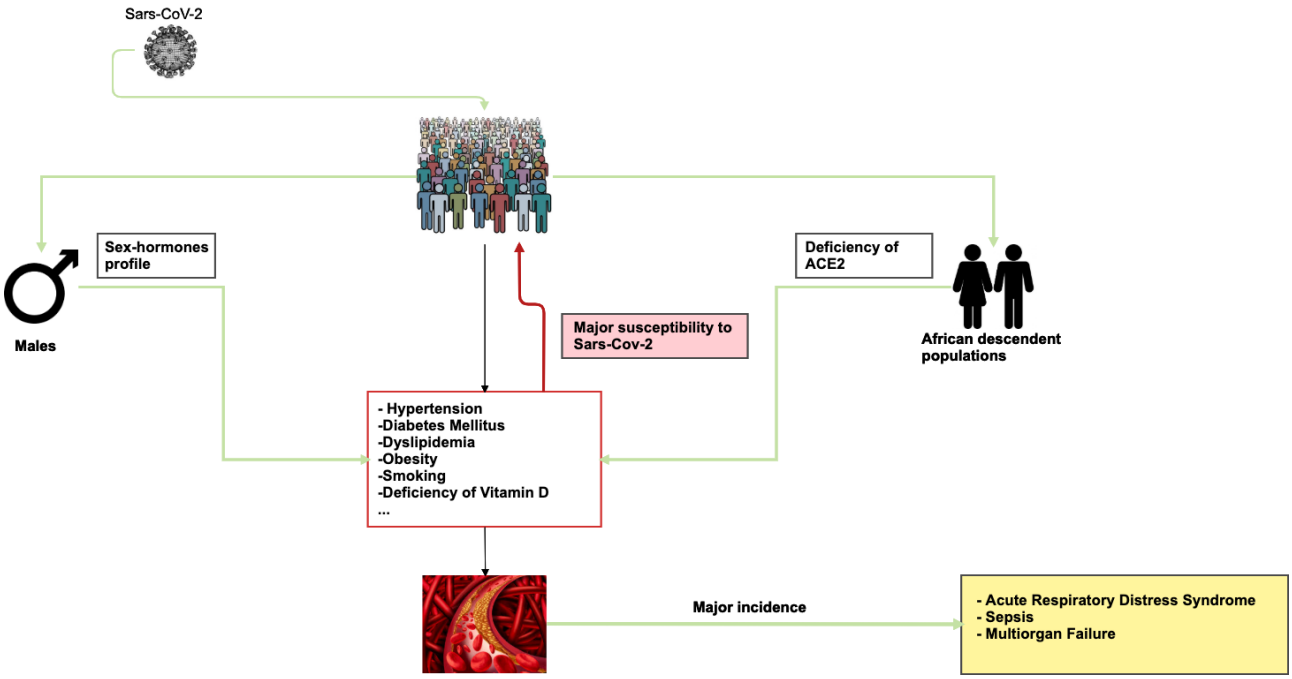
45. Maganto-García, E., Tarrio, M. L., Grabie, N., Bu, D. X., & Lichtman, A. H. (2011). Dynamic changes in regulatory T cells are linked to levels of diet-induced hypercholesterolemia. *Circ.*, *124*(2), 185-195.
46. Bonow, R. O., Fonarow, G. C., O'Gara, P. T., & Yancy, C. W. (2020). Association of Coronavirus Disease 2019 (COVID-19) With Myocardial Injury and Mortality. *JAMA Cardiol.*
47. Smeeth, L., Thomas, S. L., Hall, A. J., Hubbard, R., Farrington, P., & Vallance, P. (2004). Risk of myocardial infarction and stroke after acute infection or vaccination. *N. Engl. J. Med.*, *351*(25), 2611-2618.
48. Wang, H., Yuan, Z., Pavel, M. A., & Hansen, S. (2020). Cholesterol and COVID19 lethality in elderly. *bioRxiv*.
49. Wu, Q., Zhou, L., Sun, X., Yan, Z., Hu, C., Wu, J., et al (2017). Altered lipid metabolism in recovered sars patients twelve years after infection. *Sci. Rep.*, *7*(1), 1-12.
50. Sun, J., Yu, H., Liu, H., Pu, D., Gao, J., Jin, X., et al (2019). Correlation of pre-operative circulating inflammatory cytokines with restenosis and rapid angiographic stenotic progression risk in coronary artery disease patients underwent percutaneous coronary intervention with drug-eluting stents. *J. Clin. Lab. Anal.*, e23108.
51. Chen, C., Zhou, Y., & Wang, D. W. (2020). SARS-CoV-2: a potential novel etiology of fulminant myocarditis. *Herz*, 1-3.
52. Buggey, J., & ElAmm, C. A. (2018). Myocarditis and cardiomyopathy. *Curr. Opin. Cardiol.*, *33*(3), 341-346.
53. Shi S, Qin M, Shen B, et al. Association of Cardiac Injury With Mortality in Hospitalized Patients With COVID-19 in Wuhan, China [published online ahead of print, 2020 Mar 25]. *JAMA Cardiol*. 2020;e200950.
54. Guo T, Fan Y, Chen M, et al. Cardiovascular Implications of Fatal Outcomes of Patients With Coronavirus Disease 2019 (COVID-19) [published online ahead of print, 2020 Mar 27]. *JAMA Cardiol*. 2020;e201017.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 April 2020 doi:10.20944/preprints202004.0430.v1

Peer-reviewed version available at Journal of Clinical Medicine 2020; doi:10.3390/jcm9072095

Figures

Fig.1: Gender and racial difference of infectability by Sars-CoV-2 and susceptibility to acquire severe form of COVID-19 due to the high predisposition for atherosclerosis risk factors.



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 April 2020 doi:10.20944/preprints202004.0430.v1

Peer-reviewed version available at Journal of Clinical Medicine 2020; doi:10.3390/jcm9072095

Fig.2: Proposed pathogenetic correlation between atherosclerosis and Sars-CoV-2