



Clinical Implications of thrombotic microvascular angiopathy (TMA) in severe and critical COVID 19 patients

Khadeeja Mustafa, Maryam Murtaza, Shair Ismail, Umar Hassan, Zain Ahmed, Zunaira Naeem, Samrah Saadat, Ayesha Majid, Shehreen Sohail

Introduction:

The severe acute respiratory distress syndrome-associated coronavirus-2 (SARS-CoV-2), etiologic agent of Coronavirus disease 2019 (COVID-19), was initially identified in Wuhan, Hubei, China in mid-December 2019.¹ It was documented to have progressed to a pandemic by the World Health Organization in March 2020, and by early April there were over 1.5 million cases worldwide, with over 90,000 deaths. Organ dysfunction, particularly progressive respiratory failure, kidney injury and a generalized coagulopathy, are associated with the highest mortality.² The lack of prior immunity to COVID-19 has resulted in large numbers of infected patients across the globe and uncertainty regarding management of the

complications that arise in the course of this viral illness³. Progressive respiratory failure is the primary cause of death in the coronavirus disease 2019 (Covid-19) pandemic. Despite widespread interest in the pathophysiology of the disease, relatively little is known about the associated morphologic and molecular changes in the peripheral lung of patients who die from Covid-19.⁴ Many patients with severe COVID-19 present with coagulation abnormalities that mimic other systemic coagulopathies associated with severe infections. Coagulopathy in patients with COVID-

Abstract:

Severe acute respiratory syndrome coronavirus has a great role in causing respiratory illness in humans and has the most important relationship of its spike proteins with host ACE-2 receptors. After entry into the human body, the viral S protein receptor binding domain binds to human ACE-2 receptor. Spike protein is split from the cleavage site along its two subunits S1 and S2 then during this process. S2 subunit release RBD (Receptor- Binding Domain) of S1 mediated to the ACE-2. The RBD of S1 consists of 200 amino acid domains. The sample size was of 350 patients between age 48 years+_2 years. Out of these patients that were screened, 141 were classified as severe and critical stage of the disease and hence serve as subject to this focused study. The effects of this virus on platelets are rather erratic and unusual with slight decrease in the number of platelets. The raised number of d-dimers in blood of patients has been observed resulting in an elevated level of LDH.

Key words: Corona virus, Microvascular angiopathy, Nested case-control, endothelial damage

19 is associated with an increased risk of death. Furthermore, the relevance of COVID-19-coagulation abnormalities are becoming increasingly clear as a substantial proportion of patients with severe COVID-19 develop, sometimes unidentified, venous and arterial thromboembolic complications.⁵. Coronavirus is one of the 124 viruses in Coronaviridae family. It is an enveloped RNA virus. RNA is positive sense. Its glycoprotein envelope has spikes emerging out of it. We have seen that 6 out of 124 types of Coronaviridae family were accused of causing major and minor diseases in humans. Among these 6, 4 types are associated with common cold and flu. While the other two types except these four were seen to be causing severe acute respiratory syndrome often regarded as SARS and middle east respiratory syndrome. With a length ranging from 26 to 136 32 KB, coronavirus is declared to have the longest and broadest genetic material. A large portion of its genome also leads to transcription and translation of important polypeptides for gene expression and multiplication, in addition to its major structural proteins. The natural hosts of SARS-CoV-2 virus are bats. SARS-CoV-2 is a β -coronavirus that has non-segmented and non-enveloped positive SSRNA virus. The different types of this virus affect different species like alpha and beta types infect mammals and gamma and delta affect birds. After the breakdown of this virus total six so far types are found to infect human beings. Alpha-CoV HCoV-229E and HCoV-NL 63 and Beta COV HCoV- HKU1 and HCoV-OC43 these generally have low pathogenicity and give rise to mild respiratory symptoms resembling the common cold but the beta two types of corona virus, SARS-CoV-2 and MERS-CoV cause lethal and deadly respiratory symptoms. Angiotensin- converting enzyme (ACE-2) has the same receptor as the SARS-CoV-2, so it uses this enzyme while infecting humans. According to genomic research SARS-CoV-2 with a genome length of 27 to 32 kb, coronaviruses are the largest among all the RNA viruses, consisting of a non-segmented positive sense RNA. The coronavirus family, Coronaviridae consists of 4 genera; α - β - γ - δ - CoV. There are 6 members of Coronaviridae family that tend to infect humans. Four of them, 2 α - CoV (HCoV-229E and HCoV-NL63) and 2 β -CoV (HCoV-HKU1 and HCoV-OC43) lead to only mild or less severe respiratory infections. The sequenced genome of this virus is 27-32 kb long. The four genera of Coronaviridae family are differentiated by causing diseases in different species. α - CoV (HCoV-229E and HCoV-NL63) and 2 β -CoV (HCoV-HKU1 and HCoV-OC43) cause mild or less severe respiratory infections. The virus gets its crown appearance because of large outwardly ectodomain of spike protein. A short intracellular tail attaches to the spike protein which is basically a single pass transmembrane. The ectodomain

of S protein consists of 3 S1 subunit heads is always responsible for receptor binding and a trimeric S2 subunit stalk usually facilitates membrane fusion, which enables the entry of virus. The spikes of coronavirus act as class I membrane fusion proteins. The main core of receptor binding is always S1 subunit total three in number and present in the ectoderm of spike protein. Membrane fusion is always facilitated by a trimeric S2 subunit. The virus entry in host cell always happens this way. These spike proteins act as class 1 membrane fusion proteins and this protein being metastable repositions its structure for bringing virus and host membranes close to each other. On each side of 3 S1 subunits, the presence of RBD having different subregions and these recognize various receptors distinctly belonging to different viral species. In order to interact with human receptors, the SB domain is used by the corona virus and many other strains associated with the family. The viral and host membranes are brought together by a bundle of six helices appearing from S2 subunit and attaches itself in the host. Human host immune responses have an essential inducer which is mainly the S1 RBD spike. The increased RBD/human ACE-2 (hACE2) binding affinity is responsible by an amino acid substitution (K479N) in the novel corona virus (RBD) for civet to human spillover of SARS-CoV-2. Human to human transfer is most noticeable and is facilitated by mutation that is S48T in SARS-CoV-2 RBD.

Thrombotic micro vascular angiopathy (TMA):

Thrombotic microangiopathy is a clinical disorder driven by activation of endothelial cells. It is characterized by thrombocytopenia and hemolysis with microvascular thrombosis resulting in systemic organ damage. Organ damage in turn leads to life-threatening complications that have to be dealt with effectively in minimum response time⁶. The relation and progression of this medical disorder with the widespread COVID 19 is being established world over for comprehensive management and minimizing mortality.

Methods:

The data has been collected from patients presenting to Mayo Hospital Lahore with various intensities of covid infections. For the sake of reaching conclusion, severe and critical patients will be covered over the course of this study

Study type:

Nested case-control (NCC)

Inclusion criteria:

1. Clinically diagnosed with COVID 19 infection
2. Showed severe or critical intensity of infection
3. Admitted in intensive care unit
4. Lab findings are available
5. Shows signs of organ damage

Exclusion criteria:

Mild to moderate infection patients who were simply kept in isolation and did not require intensive care

Results:

This study was conducted by department of neurology Mayo hospital Lahore and included patients reporting to the emergency and diagnosed with covid infection. Sample size was 350 patients. Mean age is 48 years+₋₂ years. Out of these patients that were screened, 141 were classified as severe and critical stage of the disease and hence will serve as subject to this focused study

Platelets:

The platelets of these patients were analyzed by lab reports and CBCs

Name	<200	<150
Male	13	17
Female	6	8
Total	19	25

Table 1: Platelet count in patients

The effects of this virus on platelets are rather erratic and unusual with the most common observation being a very slight decrease in the number of platelets or a mild thrombocytopenia in majority of the population⁷. In addition to severe and critical patients, slight or no decrease in platelets is also obvious in patients having lesser degree of severity. The drop in numbers is attributed to endothelial activation and the use up of platelets in forming micro thrombi in various places in body⁸.

D-dimers:

Lab reports were analyzed for recording the d-dimers

Patients	Severe	Critical	Total
Patients with raised number of D- dimers	28	30	58

Table 2: Recording of D- dimers

The raised number of d dimers in blood of patients has been observed in a large number of people who have been admitted in intensive care unit. These spikes underline a “hypercoagulation” state seen in these patients⁹. Prevalent medical opinion in this regard has a critical prognostic value as to addition of anti-coagulants in treatment of severe or critical patients under observation to prevent deterioration¹⁰.

LDH:

Lactate dehydrogenase (LDH) is expressed extensively in blood cells and muscles hence an elevated level of LDH is an absolute indication of tissue damage. For reference, the normal value is 140-280 U/L

Patients	>300	>400	>500	Total
Number	Number	Number	Number	Total
Patients	24	7	81	110

Table 3: LDH record of the patients

Increase in the value of LDH is seen in patients of covid infection and is almost invariably high along with other lab perimeters¹². As the disease progresses in severity, there is activation of complement system along with immune response and this in turn leads to endothelial cell dysfunction or damage in blood vessels and organ tissues. In addition to these, LDH is also expressed by erythrocytes and so is a strong indicator of intravascular hemolysis along with endothelial cell damage and multi organ effects^{13,14,15}

Discussion

In the following months where clinicians and medical experts have been kept on toes by the variety of manifestations shown by this novel organism, the clarity and comprehension has grown over time. The lungs are the target organ for COVID-19; patients develop acute lung injury that can progress to respiratory failure, although multiorgan failure can also occur. The initial coagulopathy of COVID-19 presents with prominent elevation of D-dimer³. The most typical finding in patients with COVID-19 and coagulopathy is an increased D-dimer concentration, a relatively modest decrease in platelet count,⁵. The complement system represents the first response of the host immune system to SARS-CoV-2 infection, but there is growing evidence that unrestrained activation of complement induced by the virus in the lungs and other organs plays a major role in acute and chronic inflammation, endothelial cell dysfunction, thrombus formation, and intravascular coagulation, and ultimately contributes to multiple organ failure and death¹¹.

Conclusion:

Along with D-dimer increase and platelets drop, the considerable increase of LDH is hallmark of hemolysis and endothelial damage hence pointing towards hemolytic implications and thrombotic microangiopathy.

Conflict of interest:

All authors declare no conflict of interest.

References:

1. Zhu N., Zhang D., Wang W. A novel coronavirus from patients with pneumonia in China. 2019. *N Engl J Med.* 2020 doi: 10.1056/NEJMoa2001017.
2. Cucinotta D., Vanelli M. WHO declares COVID-19 a Pandemic. *Acta Biomed.* 2020 doi: 10.23750/abm.v91i1.93973.
3. Connors JM, Levy JH. COVID-19 and its implications for thrombosis and anticoagulation. *Blood.* 2020;135(23):2033-2040. doi:10.1182/blood.2020006000
4. Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19 [published online ahead of print, 2020 May 21]. *N Engl J Med.* 2020;10.1056/NEJMoa2015432. doi:10.1056/NEJMoa2015432
5. Levi M, Thachil J, Iba T, Levy JH. Coagulation abnormalities and thrombosis in patients with COVID-19. *Lancet Haematol.* 2020;7(6): e438-e440. doi:10.1016/S2352-3026(20)30145-9
6. Park MH, Caselman N, Ulmer S, Weitz IC. Complement-mediated thrombotic microangiopathy associated with lupus nephritis. *Blood Adv.* 2018;2(16):2090-2094. doi:10.1182/bloodadvances.2018019596
7. The Lancet Haematology. COVID-19 coagulopathy: an evolving story. *Lancet Haematol.* 2020;7(6): e425. doi:10.1016/S2352-3026(20)30151-4
8. Escher R, Breakey N, Lämmle B. Severe COVID-19 infection associated with endothelial activation. *Thromb Res.* 2020; 190:62. doi: 10.1016/j.thromres.2020.04.014

- 9.** Breakey N, Escher R. D-dimer and mortality in COVID-19: a self-fulfilling prophecy or a pathophysiological clue? *Swiss Med Wkly.* 2020;150: w20293. Published 2020 May 26. doi:10.4414/smw.2020.20293

- 10.** Gris JC, Quéré I, Pérez-Martin A, Lefrant JY, Sotto A. Uncertainties on the prognostic value of D-dimers in COVID-19 patients [published online ahead of print, 2020 Apr 28]. *J Thromb Haemost.* 2020;10.1111/jth.14876. doi:10.1111/jth.14876

- 11.** Noris M, Benigni A, Remuzzi G. The case of complement activation in COVID-19 multiorgan impact [published online ahead of print, 2020 May 24]. *Kidney Int.* 2020; S0085-2538(20)30556-1. doi: 10.1016/j.kint.2020.05.013

- 12.** Liu Y, Yang Y, Zhang C, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci.* 2020;63(3):364-374. doi:10.1007/s11427-020-1643-8

- 13.** Shi, J., Li, Y., Zhou, X. et al. Lactate dehydrogenase and susceptibility to deterioration of mild COVID-19 patients: a multicenter nested case-control study. *BMC Med* **18**, 168 (2020).

- 14.** Chen L, Liu HG, Liu W, et al. *Zhonghua Jie He Hu Xi Za Zhi.* 2020;43(3):203-208. doi: 10.3760/cma.j.issn.1001-0939.2020.03.013

- 15.** Kato GJ, McGowan V, Machado RF, et al. Lactate dehydrogenase as a biomarker of hemolysis-associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension, and death in patients with sickle cell disease. *Blood.* 2006;107(6):2279-2285. doi:10.1182/blood-2005-06-2373