COVID-19 CLOT: What is it? Why in the lungs? Extracellular histone, "auto-activation" of prothrombin, emperipolesis, megakaryocytes, "self-association" of Von Willebrand factor and beyond

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Abstract:

COVID-19 thromboembolic disease has brought all of us back to the drawing board. In COVID-19, pre-existing activated endothelium with increased Von Willebrand factor (VWF), low density lipoprotein (LDL) promoting “self-association” and “sticking” of long VWF strings to the vascular endothelial wall, suppressed ADAMTS13 cleavage of VWF, hypoxia induced upregulation and activation of VWF, fibrous network from neutrophil extracellular traps (NETs) with free DNA and histone, all appear to be initiating the thrombogenesis. Worsening complement activation, cytokine storm and resulting endothelial destruction, unregulated thrombogenesis leads to vascular occlusions and hypoxia. At this stage, the presence of abundant extracellular DNA, histone and α-defensins appears worse than the SARS-CoV-2 itself. Previously observed in vitro mechanisms like histone “auto-activating” prothrombin, histone activated platelets generating thrombin without FXII, thrombin and plasmin cleaving complement C5 appears highly likely in COVID-19. Megakaryocytes are actively producing platelets in the lungs and appear to play a major role in thrombogenesis of COVID-19 raising suspicion of emperipolesis. This focused review is a compilation of my observations in relation to the pathophysiology of the intravascular environment, mainly in COVID-19 lungs. Pathophysiology based clinical trials are paramount in reducing morbidity and mortality in COVID-19.

Key words: Megakaryocyte, IFITM3, VWF, ADAMTS13, emperipolesis, self-association, unfractionated heparin (UFH), histone, NETs, Thrombin

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Introduction:

COVID-19 caused by the virus SARS-CoV-2 was characterized as a pandemic by the World Health Organization (WHO) on March 11, 2020. Understanding of the pathophysiology of thrombotic complications of COVID-19 are still evolving. Previously (published on May 15, 2020), in a letter to the editor of *Federal Practitioner*, we have highlighted that microthrombosis of COVID-19 are likely due to embolism of circulating ultra-large Von Willebrand factor (eULVWF) decorated platelet strings secondary to “COVID-19 induced endotheliopathy”, based on endotheliopathy-associated vascular microthrombotic disease (EA-VMTD). Many recent articles have concluded that “COVID-19 induced endotheliopathy” as the reason for the thrombotic complications and also reported multifold increases in plasma VWF. This author’s review of published data from pre (mostly SARS-CoV and MERS-CoV) and current COVID-19 era, it is very interesting to observe the multiple vital functions played by the megakaryocytes and its role in “clot” formation along with platelets and VWF (*in vitro, ex vivo* and *in vivo*). Though many alternate or additional pathways are involved, this review is a focused account of this author’s observations during literature review of likely mechanisms leading to the serious thrombotic complications associated with COVID-19. Given the grave pandemic situation, the intention of this review article is to bring this author’s observation to the medical community. These observations are based on my internal medicine background and in no way do I intend to claim to be an expert in COVID-19, hematology or thrombosis nor am I making any management recommendations in COVID-19.

COVID-19 Clot:

Currently available autopsy reports of COVID-19 lung, intravascular thrombus mainly consist of rich platelets aggregates, megakaryocytes actively producing platelets, neutrophils/ mononuclear cells and fibrin. Complement deposition with C5b-9 (Membrane attack complex, MAC), C4b (classical pathway) and also extensive endothelial injury are also noted. These findings are consistent with involvement of extensive platelet adhesions and aggregation over VWF/NETs strings, neutrophil extracellular traps (NETs/NETosis; mononuclear extracellular traps: ETs/ETosis will be referred as NETs in this article), complement activation with fibrin deposition. The initiating process of this thrombogenesis is debatable.
but appear irrelevant in the management of complicated COVID-19 thromboembolic disease. Probable inter-connections of pathophysiology of COVID-19 clot is as below:

![Diagram](https://example.com/diagram.png)

In complicated COVID-19, all the above mechanisms are in a vicious circle. It appears that many “non-traditional” pathways are involved in pathophysiology of COVID-19 clot. To understand the thrombogenesis described as reviewed in the autopsy, a brief account of likely mechanisms are discussed here.

**Cytokine storm and thrombogenesis:**

Cytokines are small proteins secreted by cells (mainly T helper cells and macrophages), also have many specific names, and serve as a communication between cells (signaling) for further action\(^8\). Cytokines are likened to be the “social media” of the body connecting and communicating between cells.

It is reported SARS-CoV-2 can infect\(^9\) tissue-resident macrophages or “dust cells” of the lungs which can initiate the release of cytokine. Another study demonstrated, SARS-CoV spike protein viroporin 3a activates the nucleotide-binding domain like receptor protein 3 (NLRP3) inflammasome of macrophages\(^10\).
and induces IL-1β secretion. In COVID-19, release of cytokine (TNF-α, IL-1β and other; “cytokine storm”\textsuperscript{11, 12}) initiates E-selectin on the endothelial cell wall allowing attachment of ligands from monocyte, and dendritic cells thereby allowing entry to the infected site (alveoli) by paracellular or transcellular migration\textsuperscript{13}. Further details are discussed in respective associated items in this article.

**Von Willebrand factor and endothelial activation:**

“Thus if VIII Ag is an indicator of endothelial damage, it may be possibly a contributory factor of thrombosis in arterial disease”

- Boneu et al.\textsuperscript{14} *Lancet*, June 28, 1975.

Plasma glycoprotein VWF helps in primary hemostasis of vascular injury by collagen recognition and platelet adhesion and is also the carrier protein for the coagulation factor VIII\textsuperscript{15, 16}. This is comparable to the string in the “beads and string” of a necklace where the beads represent platelets. Von Willebrand factor is secreted mainly from Weibel-Palade bodies (WPB) of endothelial cells\textsuperscript{17} or from α-granules of platelets (megakaryocytes derived)\textsuperscript{18}. Contribution of platelet VWF is normally around 15%\textsuperscript{19} but in the COVID-19 situation, individual contributions as the result of endotheliopathy and active platelet production with activation are unknown. WPB exocytosis from the endothelial cells unloads mainly the eULVWF and P-selectin\textsuperscript{20} via multiple mechanisms; for example, thrombin\textsuperscript{21}, histamine\textsuperscript{22} and complement proteins (membrane attack complex) C5b-9\textsuperscript{23} release WPB primarily by raising cytosolic Ca\textsuperscript{2+}. Epinephrine\textsuperscript{24} releases WPB by increasing cytosolic cAMP (is it a valid reason to avoid related medications in COVID-19 shock?). The behavior of circulating VWF including platelet derived VWF in shear stress is extremely complex, well-reviewed by Chen et al.\textsuperscript{25} and some of the COVID-19 related information is discussed here.

ADAMTS13\textsuperscript{26} is a zinc containing protease, also known as VWF cleaving protease. ADAMTS13 is an abbreviation for A D integrin A nd M etalloproteinase with a T hromboS pondin type 1 motif, member 13. The significant risk factors for the progression of COVID-19 from asymptomatic stage to complicated stage appears to be multifactorial. The main risk appears to be the pre-existing “inflamed” plasma membranes or the so-called “activated endothelium” due to various reasons. The hallmark of endothelial activation is increased plasma VWF\textsuperscript{14}. It appears that it is often ignored as the “biomarker of inflammation” or “acute phase reactant”\textsuperscript{27} in COVID-19. In fact VWF appears to
be a vital component of the “clot” in COVID-19. Cytokines IL-8 and TNF-α released in COVID-19 “cytokine storm”\textsuperscript{11} upregulate eULVWF release from endothelial WPB and IL-6 inhibits cleaving of eULVWF by ADAMTS13\textsuperscript{28}. Histone, one of damage-associated molecular patterns (DAMP) released from NETs stimulates VWF secretion from WPB\textsuperscript{29}. Hypoxia upregulates and activates VWF in the lung endothelial cells\textsuperscript{30} and in megakaryocytes \textsuperscript{31}. This author’s opinion is that, in COVID-19, the arterial oxygen saturation or content in the systemic circulation may be within normal limits but a specific pulmonary small vessel may have low oxygen content secondary to partial thrombotic occlusion; one can expect that there should be a significant thrombotic occlusive complications already in the pulmonary circulation by the time a COVID-19 patient develops symptomatic systemic arterial hypoxia. This may be the reason for the rapid deterioration of clinical status in COVID-19 when clinical hypoxia sets in.

**Autophagy:**

Autophagy\textsuperscript{32} is the name for the intracellular “waste removal process” but appears to be involved in many other pathways. There is debate about autophagy involvement in the intracellular SARS-CoV-2 viral cycle\textsuperscript{33} and its effects on viral replication (SARS-CoV)\textsuperscript{34}. Autophagy also regulates WPB exocytosis\textsuperscript{35} and given the increase in the VWF in COVID-19, one might speculate that the intracellular SARS-CoV-2 may upregulate WPB exocytosis via autophagy. Of note, inhibiting autophagy (WPB release) and the resulting increased bleeding time is one of the mechanisms of action of the antimalarial drug chloroquine\textsuperscript{35}. NETs induce fibrin generation by autophagy mediated neutrophil tissue factor (TF) via extrinsic pathway\textsuperscript{36} [NETs also induce fibrin generation through circulating free DNA (cfDNA) via intrinsic pathway with FXII\textsuperscript{37}].

**Lateral self-association of circulating VWF:**

Two or more circulating VWF multimers adhere together in the circulation in a process called lateral self-association, enabling the strings to stick to the vascular wall causing long “meshwork” sometimes as long as 5 cm \textit{in vitro}\textsuperscript{38, 39, 40(p)}. This appears to be the devastating phenomenon in thrombotic complications in COVID-19. It is reported that high density lipoprotein (HDL) prevent WPB release of VWF, self-association of VWF \textsuperscript{41} and also the adverse complications of VWF \textsuperscript{42, 43} thus, individuals with high HDL may not progress to severe COVID-19. On the
other hand, LDL has the opposite effect and contributes to VWF-mediated thrombosis\(^4^4\).

**ADAMTS13 and issues with VWF cleaving:**

Proteolytic cleavage processes in the plasma are extremely complex and happen only when “conformational position” exposes the particular domain. For example, for VWF cleaving, a particular domain (A2) of VWF should be in “open” conformation, not blocked by another external agent, and also the ADAMTS13 should be in open conformation. Shear stress (blood flow) and many other factors will also affect this process.

It is well known that low plasma ADAMTS13 activity level is associated with severe disease like in thrombotic thrombocytopenic purpura (TTP) but also has some limitations with the activity level testing *in vitro*\(^4^5\). This author’s opinion is that the normal ADAMTS13 activity may not be “normal” in COVID-19 coagulopathy because of the conformational status of circulating VWF\(^4^6\) (A2 domain/ cleaving site not exposed) or it is competitively blocked by many other mechanisms (see below). The inflammatory response and coagulation is a natural host defense mechanism and therefore ADAMTS13 must not be upregulated in acute clinical states, and in fact none of these clinical conditions or the inflammatory stimuli are noted to upregulate ADAMTS13\(^4^7\). Zinc and calcium ions cooperatively modulate ADAMTS13 and can upregulate its activity by multiple folds *in vitro*\(^4^8\).

Reactive oxygen species (ROS) released by activated neutrophils/NETs inhibit VWF cleavage by ADAMTS13 by oxidation (hypochlorous acid)\(^4^9\). Platelet derived VWF is very resistant to ADAMTS-13 cleavage because of altered glycosylation\(^5^0\). Thrombospondin-1 (TSP1) from the \(\alpha\)-granules of the activated platelets can block the ADAMTS13 cleavage of VWF up to 70% by competitive inhibition of A2 domain\(^5^1\).

**Neutrophil extracellular traps (NETs), mononuclear cells:**

Neutrophil extracellular traps (NETs)\(^5^2\) is a process where neutrophils put out the content of the nucleus like a “net” outside the plasma membrane to capture and kill the “foreign” organism.
NETs, coagulation and complement pathways are all interconnected and well-reviewed by Fuchs et al.\textsuperscript{53}, de Bont et al.\textsuperscript{54} and Kimball et al.\textsuperscript{55}. This author’s observation of the role of the NETs and it’s likely relationship in COVID-19 are mentioned here. NETs, mainly consist of nuclear material, namely DNA, histone and elastase\textsuperscript{56}. A file photo of NETs shown in figure 1 (non COVID-19).

![Figure 1. Neutrophil extracellular traps (NETs)(non COVID-19)](https://www.flickr.com/photos/132318516@N08/35988820593)

Extracellular histones (acting as damage associated molecular patterns DAMP) cause neutrophil margination, endothelial dysfunction, intra-alveolar hemorrhage and macro- and microvascular thrombosis\textsuperscript{57}, which appears to be the classic pathology noted in COVID-19 per review. This author’s observation is that plasma extracellular histone and DNA accumulation in COVID-19 may be worse than that of SARS-CoV-2 virus itself. NETs form a fibrous network inside the vasculature and form the base for the scaffolding for the thrombosis.
Respiratory syncytial virus (RSV) induce reactive oxygen species (ROS) dependent NETs target host cells which are infected with the virus through peptidyl arginine deiminase type IV (PAD4)\textsuperscript{58} i.e. neutrophil may be able to identify and attack virus infected cells. It appears that SARS-CoV-2 may be able to initiate the similar process. Activated T cells enters the virus infected area in a L-selectin dependent manner\textsuperscript{59}. Monocytes adhered to the endothelium via E-selectin, recruits other monocytes like a “piggyback” (L-selectin on the flowing monocyte binds to the PSGL-1 on the endothelium adhered monocyte)\textsuperscript{60}.

NETs activate platelets via histone and platelets can initiate NETs via TLR4\textsuperscript{61(p4)} by presenting high mobility group box 1 (HMGB1)\textsuperscript{62}.

Human neutrophil peptides (HNP, also called $\alpha$-defensins) in NETs inhibits proteolytic cleavage of VWF by ADAMTS13\textsuperscript{63}, promote platelet activation\textsuperscript{64}, increases endothelial dysfunction\textsuperscript{65}. Plasminogen bound to fibrin in the presence of $\alpha$-defensin is less susceptable to activation by tissue plasminogen activator (tPA)\textsuperscript{66}. All these measures are likely to worsen the COVID-19 thrombogenesis.

**Kawasaki-like disease in SARS-COV-2 and NETs.**

It is very interesting to note that the Deficiency of Adenosine DeAminase 2 referred as DADA2, an autosomal-recessive mutation responsible for systemic vasculitis has been shown to involve NETs induced inflammatory cytokines from macrophages\textsuperscript{67}. SARS-CoV-2 related Kawasaki like disease\textsuperscript{68} in young children may be DADA2 related or those children are probably unable to mount full blown NETs to cause vascular occlusions. This is further supported by the fact that the newborns and neonates are unable to mount NETs\textsuperscript{69}, therefore unlikely to develop severe thrombotic disease with SARS-CoV-2\textsuperscript{70} in this author’s view.

**Complement activation:**

The name complement means it complements other mechanisms in host defense. There are many “traditional” and non-traditional pathways to activate complement proteins. Those are discussed in respective associated items in this article.
Coagulation and possible non-traditional pathways to generate thrombin (FIIa) and possible consequences:

The “traditional” pathways (intrinsic/contact and extrinsic) in the coagulation cascade merge at factor X to make activated factor Xa. Factor Xa cleaves prothrombin to thrombin. Thrombin is a major activator of platelets and plays a major role in hemostasis\(^1\). It has been reported that there are few “non-traditional” mechanisms where thrombin can be generated directly. This author is interested only in those mechanisms affecting the cascade after Xa since fractionated heparin (LMWH which inactivate Xa) yet to be proven to be effective in preventing the fibrin formation in COVID-19.

Semeraro et al.(2011)\(^2\) reported histone (H3 and H4) activated platelets in “platelets rich plasma” can induce thrombin formation \textit{in vitro} (without FXII) by at least partly using toll like receptors (TLR2, TLR4); also reported antiplatelet agents markedly reduce thrombin generation whereas DNA enhanced histone dependent thrombin generation. It should be noted, in COVID-19, the lungs should be in a state comparable to “platelet rich plasma” with extracellular histone and DNA. Could histone loaded (see emperipolesis) platelets be able to directly convert prothrombin to thrombin?

Barranco-Medina et al.(2013)\(^3\) reported histone (H4) can “auto-activate” prothrombin \textit{in vitro} but also noted, thrombin degraded histone in the test environment. Again it should be noted that in COVID-19, there will be ample of histone (H4) available in the lungs from extensive NETs.

Thrombin can cleave the complement protein C5 at an unusual highly conserved site (R947) forming a membrane attack complex (MAC) C5b\(_T\)-9\(^4\) which is more lytic, resulting in worsening endotheliopathy. It is also reported that plasmin can cleave complement protein C5 to C5a and C5b forming the “normal” MAC \(^5\).

Thrombin induces WPB exocytosis from endothelial cells and therefore eULVWF and P-selectin release\(^2\).

Thrombomodulin expression on the endothelial surface is decreased during endotheliopathy resulting in increased leukocyte attachment and also decreased activation of protein C by thrombin\(^6\). Decreased protein C is expected to make the thrombogenesis worse, especially by histone induced mechanisms.
DNA, Histone and intranuclear/ intracellular components:

DNA, Histone and intranuclear/ intracellular components are released into the plasma during NETs, endothelial injury and in cell death. These components are discussed with the other associated items in this article. In COVID-19, the amount or concentration of these components are expected to be high mainly in the pulmonary vessels secondary to NETs.

Role of Cholesterol:

The SARS-CoV-2 virus enters the respiratory tract and attaches to the angiotensin-converting enzyme 2 (ACE2) receptor of the type II alveolar cell wall for entry into the cell\textsuperscript{77}. This does not appear to be a straightforward process. Cholesterol rich domains (CRD) or rafts of the plasma membrane play crucial roles in cell signaling\textsuperscript{78} and is needed for this process. For the endocytic entry (one of two possible pathways) of the virus particle into the cell, the SARS-CoV-2 virus has to be attached to the ACE2 receptor on the cell wall and the viral spike (S) protein has to be primed by transmembrane protease serine 2 (TMPRSS2)\textsuperscript{79}. SARS-CoV-2 viral entry process also needs what is called “GM-1 lipid rafts” (GM-1 lipid domain)\textsuperscript{80}. These processes are cholesterol dependent and therefore indirectly modulated by plasma apolipoprotein E (ApoE) and low density lipoprotein (LDL) receptors\textsuperscript{81}. It is reported that the ApoE $\varepsilon4/\varepsilon4$ allele increases the risk of severe COVID-19\textsuperscript{82}. It is worth mentioning that ApoE is also directly involved in the immune response via classic complement pathway; ApoE-C1q complex attenuates inflammation via reducing C5\textsuperscript{83}.

The cellular cholesterol homeostasis is modulated by interferon-inducible transmembrane protein 3 (IFITM3) which is also the mechanism of cellular immunity against viruses by cells including megakaryocytes\textsuperscript{84, 85, 86} and require lysosomal cysteine protease (notable site of pathology involved in Niemann-Pick disease type C1\textsuperscript{87, 88}). The IFITM3 gene, single-nucleotide polymorphism (SNP) rs12252, CC homozygote variant is associated with severe illness in COVID-19 and other viral diseases\textsuperscript{89, 90, 91, 92}. Increased VWF production is also directly associated with increased plasma cholesterol\textsuperscript{93}.

Role of Megakaryocytes:

Megakaryocytes are usually known as the “producers of platelets”\textsuperscript{94} but they also have many other functions. Megakaryocytes are an anti-viral alarm system of the
Lungs are a major site of platelets production contributing for about 50% of the total production and also mature and immature megakaryocytes, haematopoietic progenitors are present in the extravascular spaces of the lungs. Based on autopsy findings, in complicated COVID-19, megakaryocytes are reported to be actively producing platelets in the lung and are involved in the vascular thrombosis.

Megakaryocytes, indirectly, through activated platelets in specific situations, can initiate extracellular traps (ETs) or complement pathways and coagulation. As with other viral infections, upon presentation of the SARS-CoV-2 virus or IL-1β (many other stimuli), megakaryocytes are expected to secrete type 1 interferons and upregulate IFITM3 in the nearby cells and signals the platelets to be “alert” and prevent the viral entry. Unlike Dengue virus (DENV), intracellular SARS-CoV-2 in megakaryocytes or platelets are not reported (not infected) and therefore unlikely to cause significant thrombocytopenia.

**Emperipolesis:**

Megakaryocytes also participate in a cell-in-cell interaction with neutrophils termed as emperipolesis where neutrophils enter the megakaryocytes cytoplasm, execute reciprocal transfer of the membrane and cellular components (will be transferred to the platelets later) and exit, quoted as “angry neutrophils make angry platelets.” A file photo of emperipolesis is shown in figure 2 (non COVID-19).

Recently released report confirmed the presence of membrane and cellular histone in platelets and megakaryocytes during sepsis. Though emperipolesis may explain this observation, this needs further validation especially in COVID-19. It is unclear how emperipolesis and exchange of cellular material will be regulated when a neutrophil is infected with SARS-CoV-2. It would be interesting to see if the megakaryocytes produce “custom made” platelets and neutrophils specifically to target SARS-CoV-2 in COVID-19.
Intravascular and endothelial entry of SARS-CoV-2 and effects:

SARS-CoV-2 enters the vascular endothelial cells by attaching to the ACE2 receptor as described above. The viral antigen recognition by nonclassical monocytes (CD14dim) is expected to release potent cytokine TNF-α, IL-1β and CCL3 via proinflammatory pathway\textsuperscript{103}. NETs appear to have directed towards the infected host endothelial cells. Recently published data confirmed SARS-CoV-2 triggering NETs by infecting neutrophils \textsuperscript{104}. NETs derived PAD4 may citrullinate ADAMTS13\textsuperscript{105} resulting in increased eULVWF attached to the endothelial wall attracting platelet adhesion and causing platelet “decorated” VWF strings\textsuperscript{106}. It is unsure if vitronectin (regulate MAC) gets citrullinated and inactivated and therefore complement activation may proceed unchecked.

Role of platelets:

Since platelet is probably the major or one of the major players in COVID-19, it is mentioned in the respective associated items to make it easy to follow.
Discussion:

Covid-19 has awakened the medical community to think differently in relation to host defense, inflammation, immune response and treatment options.

COVID-19 clot formation:

It appears in COVID-19, activated platelets adhere to the VWF strings that are attached to the endothelial wall and aggregation follows. Mononuclear cells are recruited by the platelets (via P-selectin) and NETs will cause a meshwork, followed by fibrin deposits resulting in widespread intravascular occlusions as noted in the autopsy. On the other hand, NETs may initiate this process. Apart from the blood cells, these intravascular “mesh of clot” scaffolding are expected to contain VWF, ETs (extracellular DNA and histones), fibrinogen/fibrin and a clot stabilizing structural protein called fibronectin.

These VWF strings comes from three sources

1. eULVWF attached to endothelial wall upon secretion from WPB
2. Attached to the endothelial wall later by “self-association” of circulating VWF multimers
3. Embolization of preformed VWF strings from proximal vasculature secondary to the shear stress.

As mentioned above, these VWF strings in the “clot” appear to be resistant to ADAMTS13 cleavage (mostly competitive inhibition); also the plasminogen bound to fibrin in this clot is less susceptible to tPA.

Unfractionated heparin and its advantages:

Unfractionated heparin (UFH) has known multiple non-anticoagulation mechanisms of action over low molecular weight heparin (LMWH) and in this author’s view, this may be an advantage especially in COVID-19 thrombosis. For example, even though LMWH can bind to histone, many studies are done with UFH; UFH can bind to histone and have a protective effect against cell death and destabilize extracellular traps facilitating innate DNase to dissolve the DNA in the NETs. UFH inactivates thrombin (FIIa) which appears to be the
initiating factor of many adverse pathways in COVID-19 and UFH also deactivates many other clotting factors\textsuperscript{113}. UFH also binds to VWF and significantly prevents GP1b receptor-mediated platelet adhesion to the VWF strings\textsuperscript{114}. Further, recently released study by Hogwood et al. highlighted that the non-anticoagulant heparins (selectively desulfated heparins) can attenuate pro-inflammatory effects and complement activation of histones\textsuperscript{115}. UFH has many other mechanisms of action including protecting the endothelium\textsuperscript{116} and those are not discussed here in detail. In this author’s view, it may be beneficial to use an anticoagulant targeting coagulation pathway after activated FXa in COVID-19.

In this author’s view, it appears that in COVID-19, instead of giving combination of multiple medications like recombinant CD59 to protect the endothelium, vitronectin to regulate complement pathway, a VWF inhibitor, a non UFH anticoagulant (LMWH or oral agents like warfarin, and novel oral anticoagulants), activated protein C or anti-histone antibody etc., one may argue to give just UFH which have all the effects of the above medications and importantly block thrombin and its effects. It appears that, if primary hemostasis is targeted early in the COVID-19, anticoagulation (targeting fibrin formation in this case) may not be necessary since the disease may not progress. This is based on the reported finding showing histone induced thrombin generation (dependent on ADP) is dramatically inhibited by certain antiplatelet agents\textsuperscript{72}. In this author’s opinion, the advantages of UFH warrants an urgent clinical trial to evaluate the advantages in complicated COVID-19.

**Why the lungs?**

After reviewing hundreds of articles, this author believes that the thrombotic complications specifically targeting the lung (tropism) in COVID-19 are likely due to (not limited to):

(1) Lung is the major organ where megakaryocytes reside and actively produce 50\% of platelets rendering it a highly prothrombotic or “platelet rich plasma” environment.

(2) Megakaryocytes can be activated to produce platelets by rolling on VWF (GP1b) under shear stress\textsuperscript{117} and increased WPB expression in the pulmonary vascular endothelial cells\textsuperscript{118} is expected to promote the activation of VWF further. Extravascular megakaryocytes in the lung
may also be activated since VWF multimers are also secreted into the subendothelial space by WPB.

(3) In addition, the alveolar macrophages (dust cells) can easily be infected by SARS-CoV-2 (may be before even entering the alveolar cells) and initiate the cytokine storm.

(4) Pulmonary artery circulation is expected to carry blood with significantly lower oxygen content in complicated COVID-19 which in turn upregulates and activates VWF.

(5) Lung is the “filter” of the pulmonary circulation and therefore VWF/platelet/NETs/fibrin strings detached from the endothelial cells of the venous system results in pulmonary embolism.

Conclusion:

This author concludes that the COVID-19 thromboembolic complications appears to be a syndrome of extreme activity of all host defense mechanisms simultaneously as discussed above. Megakaryocytes appear to play a vital role in host defense but in COVID-19, it also takes part in an unintended life threatening complication leading to unregulated intravascular thrombosis. Most of the functions of megakaryocytes are mediated by the platelets and its contents and hence megakaryocytes appear directly responsible for the intravascular thrombotic complications in COVID-19. It is unclear if platelets are “custom made angry platelets” for COVID-19 with intracellular histones by neutrophils via emperipolesis and if so, in the view of this author, this may be the etiology for the complications.

Severe COVID-19 induced endotheliopathy appears as an exacerbation of pre-existing endotheliopathy or the so called “endothelial activation”. The underlying associated pre-existing medical conditions has increased VWF as a biomarker of pre-existing endotheliopathy.

Finally, since SARS-CoV-2 virus will be cleared by the host’s innate immune mechanisms, the major question is, can the host survive the progression of thrombosis and resulting hypoxia until that time. Based on this author’s review, platelets decorated VWF strings, extracellular traps and coagulation can cause obstruction of the blood vessels individually or in combination. Apart from
targeting the progression of the “clot” by anticoagulation, this author’s opinion is that the treatment of the COVID-19 thrombotic complications should also include measures against VWF self-association, platelet aggregation, overcoming ADAMTS13 resistance, histone and also destabilizing extracellular trap network. It appears, plasma LDL cholesterol is a major player in the progression of the COVID-19 complications and thus acute LDL reducing measures and or HDL infusion may be beneficial. As mentioned above, direct thrombin blocking activity and non-anticoagulation effects of UFH appear to have a huge clinical advantage in the treatment of COVID-19 thrombotic disease. In addition, non-anticoagulant unfractionated heparins may be helpful in patients who are at high risk of bleeding. Pathophysiology based clinical trials are needed to validate the above findings in COVID-19 and paramount in reducing morbidity and mortality.

**Post-recovery from COVID-19 complications:**

NETs are implicated in the pathogenesis of autoimmune diseases. In normal hosts, NETs are removed by the macrophages whereas its functions are affected in COVID-19, likely impairing the NETs clearing. It appears to this author that prolonged exposure of the host’s own intracellular materials including the DNA in complicated COVID-19 may result in a plethora of autoimmune diseases in the future.

Acknowledgement: I would like to thank all the researchers who have done extensive work enabling me to do this focused review. I apologize if I have failed to credit any of the contents mentioned above, because of the space constraint.

Disclaimer: *The views expressed in this article are those of the author and do not necessarily reflect the position or policy of the Department of Veterans Affairs, the United States government or the West Virginia University School of Medicine.*

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