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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"[1], based on endotheliopathy-associated vascular microthrombotic disease (EA-VMTD)[2]. 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 [3]. 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 [4, 5, 6, 7], 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

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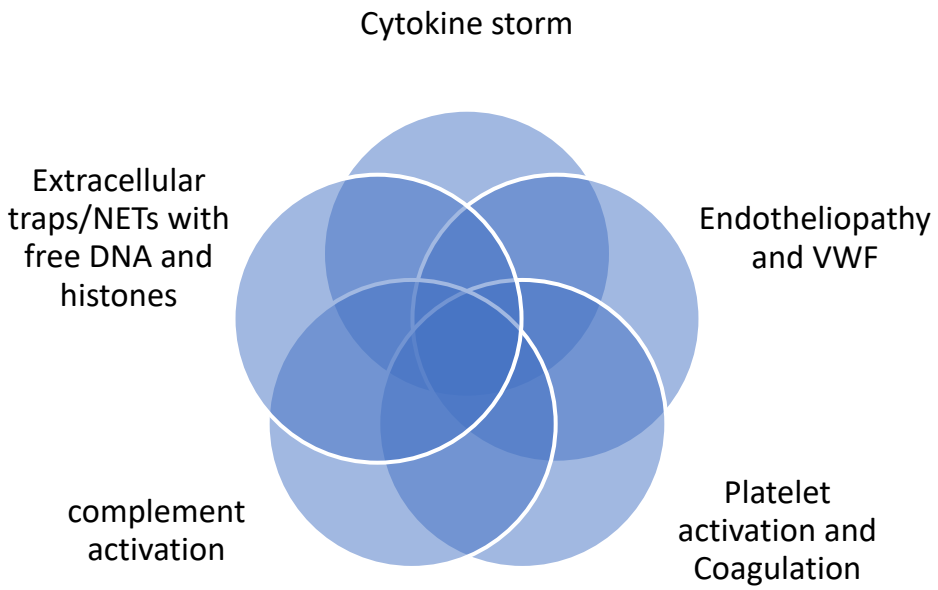
but appear irrelevant in the management of complicated COVID-19 thromboembolic disease. Probable inter-connections of pathophysiology of COVID-19 clot is as below:



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]

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and induces IL-1β secretion. In COVID-19, release of cytokine (TNF-α, IL-1β and other; "cytokine storm"[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[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.[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 [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[17] or from α-granules of platelets (megakaryocytes derived)[18]. Contribution of platelet VWF is normally around 15%[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[20] via multiple mechanisms; for example, thrombin[21], histamine[22] and complement proteins (membrane attack complex) C5b-9[23] release WPB primarily by raising cytosolic Ca^{++}. Epinephrine[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.[25] and some of the COVID-19 related information is discussed here.

ADAMTS13[26] is a zinc containing protease, also known as VWF cleaving protease. ADAMTS13 is an abbreviation for **A D**isintegrin **A**nd **M**etalloproteinase with a **T**hrombo**S**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 [14]. It appears that it is often ignored as the "biomarker of inflammation" or "acute phase reactant"[27] in COVID-19. In fact VWF appears to

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be a vital component of the "clot" in COVID-19. Cytokines IL-8 and TNF-α released in COVID-19 "cytokine storm"[11] upregulate eULVWF release from endothelial WPB and IL-6 inhibits cleaving of eULVWF by ADAMTS13[28]. Histone, one of damage-associated molecular patterns (DAMP) released from NETs stimulates VWF secretion from WPB[29]. Hypoxia upregulates and activates VWF in the lung endothelial cells[30] and in megakaryocytes [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[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[33] and its effects on viral replication (SARS-CoV)[34]. Autophagy also regulates WPB exocytosis[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[35]. NETs induce fibrin generation by autophagy mediated neutrophil tissue factor (TF) via extrinsic pathway[36] [NETs also induce fibrin generation through circulating free DNA (cfDNA) via intrinsic pathway with FXII[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 *in vitro* [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 [41] and also the adverse complications of VWF [42, 43] thus, individuals with high HDL may not progress to severe COVID-19. On the

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other hand, LDL has the opposite effect and contributes to VWF-mediated thrombosis[44].

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*[45]. 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[46] (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[47]. Zinc and calcium ions cooperatively modulate ADAMTS13 and can upregulate its activity by multiple folds *in vitro*[48].

Reactive oxygen species (ROS) released by activated neutrophils/NETs inhibit VWF cleavage by ADAMTS13 by oxidation (hypochlorous acid)[49]. Platelet derived VWF is very resistant to ADAMTS-13 cleavage because of altered glycosylation[50]. Thrombospondin-1 (TSP1) from the α-granules of the activated platelets can block the ADAMTS13 cleavage of VWF up to 70% by competitive inhibition of A2 domain[51].

Neutrophil extracellular traps (NETs), mononuclear cells:

Neutrophil extracellular traps (NETs)[52] 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.

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NETs, coagulation and complement pathways are all interconnected and well-reviewed by Fuchs et al.[53] , de Bont et al.[54] and Kimball et al. [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[56]. A file photo of NETs shown in figure 1 (non COVID-19).

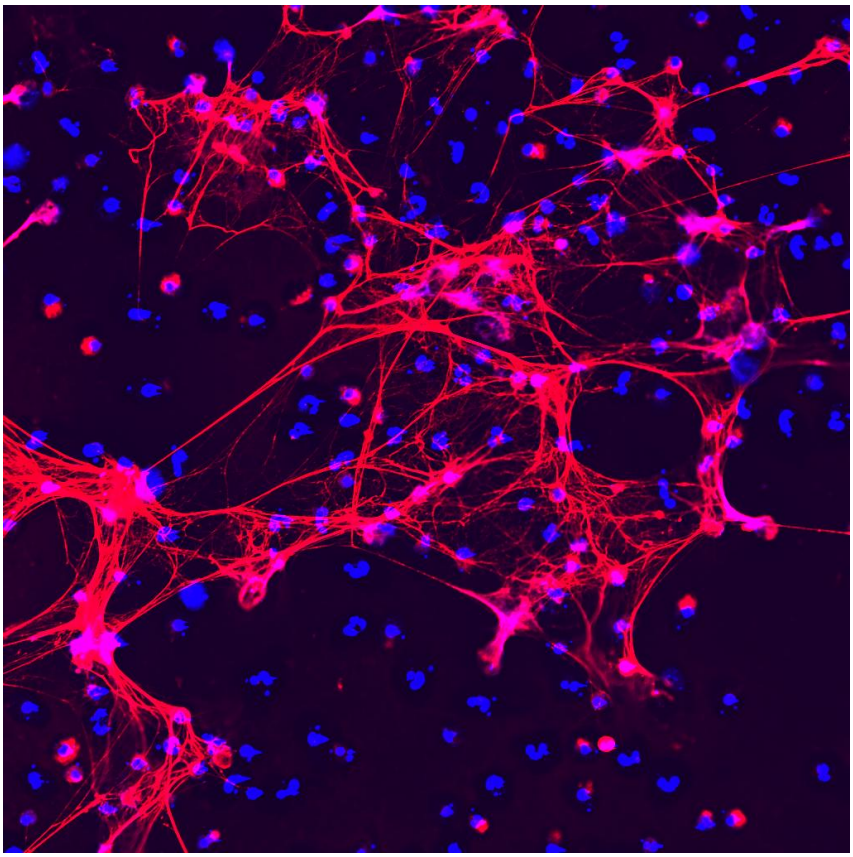

Figure 1. Neutrophil extracellular traps (NETs)(non COVID-19)

Extracellular histones (acting as damage associated molecular patterns DAMP) cause neutrophil margination, endothelial dysfunction, intra-alveolar hemorrhage and macro- and microvascular thrombosis[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

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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) [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[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)[60].

NETs activate platelets via histone and platelets can initiate NETs via TLR4[61(p4)] by presenting high mobility group box 1 (HMGB1)[62].

Human neutrophil peptides (HNP, also called α-defensins) in NETs inhibits proteolytic cleavage of VWF by ADAMTS13[63], promote platelet activation [64], increases endothelial dysfunction[65]. Plasminogen bound to fibrin in the presence of α-defensin is less susceptible to activation by tissue plasminogen activator (tPA)[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 **D**eficiency of **A**denosine **D**e**A**minase **2** referred as DADA2, an autosomal-recessive mutation responsible for systemic vasculitis has been shown to involve NETs induced inflammatory cytokines from macrophages[67]. SARS-CoV-2 related Kawasaki like disease[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 [69], therefore unlikely to develop severe thrombotic disease with SARS-CoV-2 [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.

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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[71]. 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)[72] reported histone (H3 and H4) activated platelets in "platelets rich plasma" can induce thrombin formation *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)[73] reported histone (H4) can "auto-activate" prothrombin *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[74] 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 [75].

Thrombin induces WPB exocytosis from endothelial cells and therefore eULVWF and P-selectin release[21].

Thrombomodulin expression on the endothelial surface is decreased during endotheliopathy resulting in increased leukocyte attachment and also decreased activation of protein C by thrombin[76]. Decreased protein C is expected to make the thrombogenesis worse, especially by histone induced mechanisms.

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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[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[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)[79]. SARS-CoV-2 viral entry process also needs what is called "GM-1 lipid rafts" (GM-1 lipid domain)[80]. These processes are cholesterol dependent and therefore indirectly modulated by plasma apolipoprotein E (ApoE) and low density lipoprotein (LDL) receptors[81]. It is reported that the ApoE ε4/ε4 allele increases the risk of severe COVID-19[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[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[84, 85, 86] and require lysosomal cysteine protease (notable site of pathology involved in Niemann-Pick disease type C1[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[89, 90, 91, 92]. Increased VWF production is also directly associated with increased plasma cholesterol[93].

Role of Megakaryocytes:

Megakaryocytes are usually known as the "producers of platelets"[94] but they also have many other functions. Megakaryocytes are an anti-viral alarm system of the

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body[95]. 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[96]. 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[7].

Megakaryocytes, indirectly, through activated platelets in specific situations, can initiate extracellular traps (ETs)[62] or complement pathways[97] and coagulation[72]. 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 [95], [98]. Unlike Dengue virus (DENV)[99] ,[100], 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"[101]. 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 [102]. 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.

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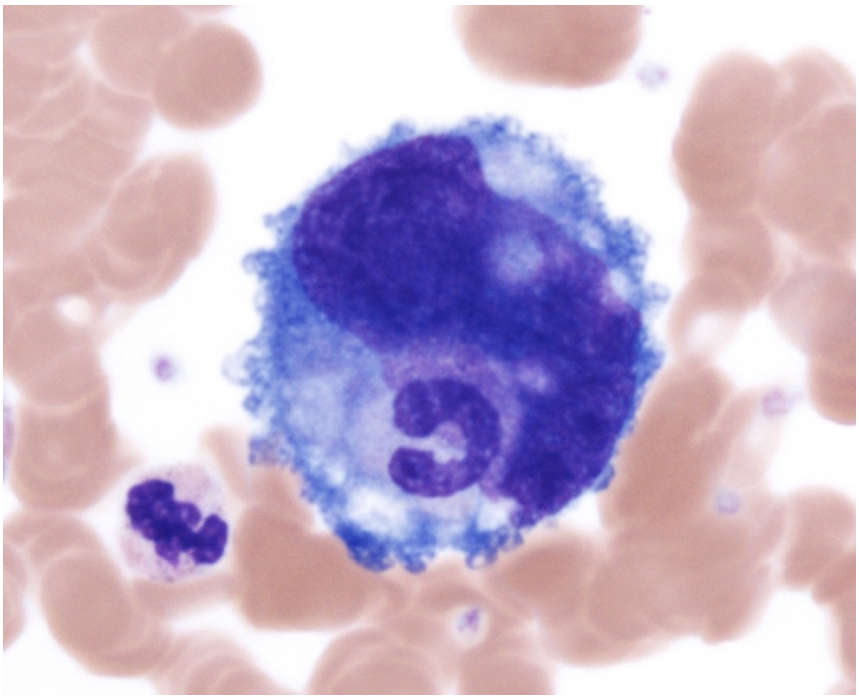

Figure.2 Emperipolesis showing a neutrophil inside a megakaryocyte (Non COVID-19)

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[103]. NETs appear to have directed towards the infected host endothelial cells. Recently published data confirmed SARS-CoV-2 triggering NETs by infecting neutrophils [104]. NETs derived PAD4 may citrullinate ADAMTS13[105] resulting in increased eULVWF attached to the endothelial wall attracting platelet adhesion and causing platelet "decorated" VWF strings[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.

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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)[107] and NETs will cause a meshwork, followed by fibrin deposits resulting in widespread intravascular occlusions as noted in the autopsy[5]. 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[108,53].

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 [109,110,111,112] and destabilize extracellular traps[53] facilitating innate DNase to dissolve the DNA in the NETs. UFH inactivates thrombin (FIIa) which appears to be the

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initiating factor of many adverse pathways in COVID-19 and UFH also deactivates many other clotting factors[113]. UFH also binds to VWF and significantly prevents GP1b receptor mediated platelet adhesion to the VWF strings[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[115]. UFH has many other mechanisms of action including protecting the endothelium[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[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[117] and increased WPB expression in the pulmonary vascular endothelial cells[118] is expected to promote the activation of VWF further. Extravascular megakaryocytes in the lung

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may also be activated since VWF multimers are also secreted into the subendothelial space by WPB.

(3) In addition, the alveolar macrophages (dust cells)[119] 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

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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[120]. 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.

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References:

1. Varatharajah N, Rajah S. Microthrombotic Complications of COVID-19 Are Likely Due to Embolism of Circulating Endothelial Derived Ultralarge Von Willebrand Factor (eULVWF) Decorated-Platelet Strings. *Fed Pr*. 2020;37(6):e1-e2. https://pubmed.ncbi.nlm.nih.gov/24056772

2. Chang JC. TTP-like syndrome: novel concept and molecular pathogenesis of endotheliopathy-associated vascular microthrombotic disease. *Thromb J*. 2018;16:20-20. doi:10.1186/s12959-018-0174-4

3. Goshua G, Pine AB, Meizlish ML, et al. Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. *Lancet Haematol*. Published online June 2020:S2352302620302167. doi:10.1016/S2352-3026(20)30216-7

4. Magro C, Mulvey JJ, Berlin D, et al. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: A report of five cases. *Transl Res J Lab Clin Med*. 2020;220:1-13. doi:10.1016/j.trsl.2020.04.007

5. Fox SE, Akmatbekov A, Harbert JL, Li G, Quincy Brown J, Vander Heide RS. Pulmonary and cardiac pathology in African American patients with COVID-19: an autopsy series from New Orleans. *Lancet Respir Med*. 2020;8(7):681-686. doi:10.1016/S2213-2600(20)30243-5

6. Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. *N Engl J Med*. Published online May 21, 2020. doi:10.1056/NEJMoa2015432

7. Rapkiewicz A, Mai X, Carsons S, et al. Megakaryocytes and platelet-fibrin thrombi characterize multi-organ thrombosis at autopsy in COVID-19: A case series. *EClinicalMedicine*. Published online June 1, 2020:100434. doi:10.1016/j.eclinm.2020.100434

8. Zhang J-M, An J. Cytokines, inflammation, and pain. *Int Anesthesiol Clin*. 2007;45(2):27-37. doi:10.1097/AIA.0b013e318034194e

9. chen yongwen, Feng Z, Diao B, et al. The Novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Directly Decimates Human Spleens and Lymph Nodes. *medRxiv*. Published online January 1, 2020:2020.03.27.20045427. doi:10.1101/2020.03.27.20045427

10. Chen I-Y, Moriyama M, Chang M-F, Ichinohe T. Severe Acute Respiratory Syndrome Coronavirus Viroporin 3a Activates the NLRP3 Inflammasome. *Front Microbiol*. 2019;10:50-50. doi:10.3389/fmicb.2019.00050

11. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet Lond Engl*. 2020;395(10223):497-506. doi:10.1016/S0140-6736(20)30183-5

12. Ye Q, Wang B, Mao J. The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. *J Infect*. 2020;80(6):607-613. doi:10.1016/j.jinf.2020.03.037

13. Vestweber D. How leukocytes cross the vascular endothelium. *Nat Rev Immunol*. 2015;15(11):692-704. doi:10.1038/nri3908

14. Boneu B, Abbal M, Plante J, Bierme R. FACTOR-VIII COMPLEX AND ENDOTHELIAL DAMAGE. *Orig Publ Vol 1 Issue 7922*. 1975;305(7922):1430. doi:10.1016/S0140-6736(75)92650-1

15. Wise RJ, Dorner AJ, Krane M, Pittman DD, Kaufman RJ. The role of von Willebrand factor multimers and propeptide cleavage in binding and stabilization of factor VIII. *J Biol Chem*. 1991;266(32):21948-21955.

16. Sadler JE. BIOCHEMISTRY AND GENETICS OF VON WILLEBRAND FACTOR. *Annu Rev Biochem*. 1998;67(1):395-424. doi:10.1146/annurev.biochem.67.1.395

17. Jaffe EA, Hoyer LW, Nachman RL. Synthesis of von Willebrand factor by cultured human endothelial cells. *Proc Natl Acad Sci U S A*. 1974;71(5):1906-1909. doi:10.1073/pnas.71.5.1906

18. Nachman R, Levine R, Jaffe EA. Synthesis of factor VIII antigen by cultured guinea pig megakaryocytes. *J Clin Invest*. 1977;60(4):914-921. doi:10.1172/JCI108846

19. NICHOLS WL, HULTIN MB, JAMES AH, et al. von Willebrand disease (VWD): evidence-based diagnosis and management guidelines, the National Heart, Lung, and Blood Institute (NHLBI) Expert Panel report (USA)1. *Haemophilia*. 2008;14(2):171-232. doi:10.1111/j.1365-2516.2007.01643.x

20. Valentijn KM, Sadler JE, Valentijn JA, Voorberg J, Eikenboom J. Functional architecture of Weibel-Palade bodies. *Blood*. 2011;117(19):5033-5043. doi:10.1182/blood-2010-09-267492

21. Birch KA, Pober JS, Zavoico GB, Means AR, Ewenstein BM. Calcium/calmodulin transduces thrombin-stimulated secretion: studies in

intact and minimally permeabilized human umbilical vein endothelial cells. *J Cell Biol*. 1992;118(6):1501-1510. doi:10.1083/jcb.118.6.1501

22. Hamilton KK, Sims PJ. Changes in cytosolic Ca2+ associated with von Willebrand factor release in human endothelial cells exposed to histamine. Study of microcarrier cell monolayers using the fluorescent probe indo-1. *J Clin Invest*. 1987;79(2):600-608. doi:10.1172/JCI112853

23. Hattori R, Hamilton KK, McEver RP, Sims PJ. Complement proteins C5b-9 induce secretion of high molecular weight multimers of endothelial von Willebrand factor and translocation of granule membrane protein GMP-140 to the cell surface. *J Biol Chem*. 1989;264(15):9053-9060.

24. Vischer UM, Wollheim CB. Epinephrine Induces Von Willebrand Factor Release From Cultured Endothelial Cells: Involvement of Cyclic AMP-dependent Signalling in Exocytosis. *Thromb Haemost*. Published online June 1997:77(6):1182-1188. doi:10.1055/s-0038-1656135

25. Chen J, Chung DW. Inflammation, von Willebrand factor, and ADAMTS13. *Blood*. 2018;132(2):141-147. doi:10.1182/blood-2018-02-769000

26. Soejima K, Mimura N, Hiroshima M, et al. A Novel Human Metalloprotease Synthesized in the Liver and Secreted into the Blood: Possibly, the von Willebrand Factor—Cleaving Protease?1. *J Biochem (Tokyo)*. 2001;130(4):475-480. doi:10.1093/oxfordjournals.jbchem.a003009

27. Pottinger BE, Read RC, Paleolog EM, Higgins PG, Pearson JD. von Willebrand factor is an acute phase reactant in man. *Thromb Res*. 1989;53(4):387-394. doi:10.1016/0049-3848(89)90317-4

28. Bernardo A, Ball C, Nolasco L, Moake JF, Dong J. Effects of inflammatory cytokines on the release and cleavage of the endothelial cell–derived ultralarge von Willebrand factor multimers under flow. *Blood*. 2004;104(1):100-106. doi:10.1182/blood-2004-01-0107

29. Michels A, Albánez S, Mewburn J, et al. Histones link inflammation and thrombosis through the induction of Weibel–Palade body exocytosis. *J Thromb Haemost*. 2016;14(11):2274-2286. doi:10.1111/jth.13493

30. Mojiri A, Nakhaei-Nejad M, Phan W-L, et al. Hypoxia Results in Upregulation and De Novo Activation of Von Willebrand Factor Expression

in Lung Endothelial Cells. *Arterioscler Thromb Vasc Biol*. 2013;33. doi:10.1161/ATVBAHA.113.301359

31. Singh B, Biswas I, Bhagat S, surya kumari S, Khan G. HMGB1 facilitates hypoxia-induced vWF upregulation through TLR2-MYD88-SP1 pathway. *Eur J Immunol*. 2016;46. doi:10.1002/eji.201646386

32. Deter RL, De Duve C. Influence of glucagon, an inducer of cellular autophagy, on some physical properties of rat liver lysosomes. *J Cell Biol*. 1967;33(2):437-449. doi:10.1083/jcb.33.2.437

33. Carmona-Gutierrez D, Bauer MA, Zimmermann A, et al. Digesting the crisis: autophagy and coronaviruses. *Microb Cell Graz Austria*. 2020;7(5):119-128. doi:10.15698/mic2020.05.715

34. Schneider M, Ackermann K, Stuart M, et al. Severe Acute Respiratory Syndrome Coronavirus Replication Is Severely Impaired by MG132 due to Proteasome-Independent Inhibition of M-Calpain. *J Virol*. 2012;86:10112-10122. doi:10.1128/JVI.01001-12

35. Torisu T, Torisu K, Lee IH, et al. Autophagy regulates endothelial cell processing, maturation and secretion of von Willebrand factor. *Nat Med*. 2013;19(10):1281-1287. doi:https://pubmed.ncbi.nlm.nih.gov/24056772

36. Kambas K, Mitroulis I, Apostolidou E, et al. Autophagy mediates the delivery of thrombogenic tissue factor to neutrophil extracellular traps in human sepsis. *PloS One*. 2012;7(9):e45427-e45427. doi:10.1371/journal.pone.0045427

37. Gould Travis J., Vu Trang T., Swystun Laura L., et al. Neutrophil Extracellular Traps Promote Thrombin Generation Through Platelet-Dependent and Platelet-Independent Mechanisms. *Arterioscler Thromb Vasc Biol*. 2014;34(9):1977-1984. doi:10.1161/ATVBAHA.114.304114

38. Savage B, Sixma JJ, Ruggeri ZM. Functional self-association of von Willebrand factor during platelet adhesion under flow. *Proc Natl Acad Sci U S A*. 2002;99(1):425-430. doi:10.1073/pnas.012459599

39. Ganderton T, Wong JWH, Schroeder C, Hogg PJ. Lateral self-association of VWF involves the Cys2431-Cys2453 disulfide/dithiol in the C2 domain. *Blood*. 2011;118(19):5312-5318. doi:10.1182/blood-2011-06-360297

40. Zheng Y, Chen J, López JA. Flow-driven assembly of VWF fibres and webs in in vitro microvessels. *Nat Commun*. 2015;6:7858-7858. doi:10.1038/ncomms8858

41. Chung DW, Chen J, Ling M, et al. High-density lipoprotein modulates thrombosis by preventing von Willebrand factor self-association and subsequent platelet adhesion. *Blood*. 2016;127(5):637-645. doi:10.1182/blood-2014-09-599530

42. Yuhanna IS, Zhu Y, Cox BE, et al. High-density lipoprotein binding to scavenger receptor-BI activates endothelial nitric oxide synthase. *Nat Med*. 2001;7(7):853-857. doi:10.1038/89986

43. Matsushita K, Morrell CN, Cambien B, et al. Nitric oxide regulates exocytosis by S-nitrosylation of N-ethylmaleimide-sensitive factor. *Cell*. 2003;115(2):139-150. doi:10.1016/s0092-8674(03)00803-1

44. Nussdorfer FD, Taparia N, Feghhi S, St. John AE, López JA, Chung DW. LDL and HDL Have Opposing Effects on VWF Self-Association. *Blood*. 2017;130(Supplement 1):3601-3601. doi:10.1182/blood.V130.Suppl_1.3601.3601

45. Sadler JE. What's new in the diagnosis and pathophysiology of thrombotic thrombocytopenic purpura. *Hematology*. 2015;2015(1):631-636. doi:10.1182/asheducation-2015.1.631

46. Crawley JTB, de Groot R, Xiang Y, Luken BM, Lane DA. Unraveling the scissile bond: how ADAMTS13 recognizes and cleaves von Willebrand factor. *Blood*. 2011;118(12):3212-3221. doi:10.1182/blood-2011-02-306597

47. Claus RA, Bockmeyer CL, Kentouche K, et al. Transcriptional regulation of ADAMTS13. *Thromb Haemost*. 2005;94(1):41-45. doi:10.1160/th04-08-0498

48. Anderson PJ, Kokame K, Sadler JE. Zinc and Calcium Ions Cooperatively Modulate ADAMTS13 Activity. *J Biol Chem*. 2006;281(2):850-857. doi:10.1074/jbc.M504540200

49. Chen J, Fu X, Wang Y, et al. Oxidative modification of von Willebrand factor by neutrophil oxidants inhibits its cleavage by ADAMTS13. *Blood*. 2010;115(3):706-712. doi:10.1182/blood-2009-03-213967

50. McGrath RT, van den Biggelaar M, Byrne B, et al. Altered glycosylation of platelet-derived von Willebrand factor confers resistance to ADAMTS13 proteolysis. *Blood*. 2013;122(25):4107-4110. doi:10.1182/blood-2013-04-496851

51. Wang A, Liu F, Dong N, et al. Thrombospondin-1 and ADAMTS13 competitively bind to VWF A2 and A3 domains in vitro. *Thromb Res*. 2010;126:e260-5. doi:10.1016/j.thromres.2010.07.009

52. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil Extracellular Traps Kill Bacteria. *Science*. 2004;303(5663):1532. doi:10.1126/science.1092385

53. Fuchs TA, Brill A, Duerschmied D, et al. Extracellular DNA traps promote thrombosis. *Proc Natl Acad Sci U S A*. 2010;107(36):15880-15885. doi:10.1073/pnas.1005743107

54. de Bont CM, Boelens WC, Pruijn GJM. NETosis, complement, and coagulation: a triangular relationship. *Cell Mol Immunol*. 2019;16(1):19-27. doi:10.1038/s41423-018-0024-0

55. Kimball AS, Obi AT, Diaz JA, Henke PK. The Emerging Role of NETs in Venous Thrombosis and Immunothrombosis. *Front Immunol*. 2016;7. doi:10.3389/fimmu.2016.00236

56. Yipp BG, Kubes P. NETosis: how vital is it? *Blood*. 2013;122(16):2784-2794. doi:10.1182/blood-2013-04-457671

57. Xu J, Zhang X, Pelayo R, et al. Extracellular histones are major mediators of death in sepsis. *Nat Med*. 2009;15(11):1318-1321. doi:10.1038/nm.2053

58. Muraro SP, De Souza GF, Gallo SW, et al. Respiratory Syncytial Virus induces the classical ROS-dependent NETosis through PAD-4 and necroptosis pathways activation. *Sci Rep*. 2018;8(1):14166-14166. doi:10.1038/s41598-018-32576-y

59. Mohammed RN, Watson HA, Vigar M, et al. L-selectin Is Essential for Delivery of Activated CD8 + T Cells to Virus-Infected Organs for Protective Immunity. *Cell Rep*. 2016;14(4):760-771. doi:10.1016/j.celrep.2015.12.090

60. Lim Y-C, Snapp K, Kansas GS, Camphausen R, Ding H, Luscinskas FW. Important Contributions of P-Selectin Glycoprotein Ligand-1-Mediated Secondary Capture to Human Monocyte Adhesion to P-Selectin, E-Selectin,

and TNF-α-Activated Endothelium Under Flow In Vitro. *J Immunol*. 1998;161(5):2501.

61. Clark SR, Ma AC, Tavener SA, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med*. 2007;13(4):463-469. doi:10.1038/nm1565

62. Maugeri N, Campana L, Gavina M, et al. Activated platelets present high mobility group box 1 to neutrophils, inducing autophagy and promoting the extrusion of neutrophil extracellular traps. *J Thromb Haemost*. 2014;12(12):2074-2088. doi:10.1111/jth.12710

63. Pillai VG, Bao J, Zander CB, et al. Human neutrophil peptides inhibit cleavage of von Willebrand factor by ADAMTS13: a potential link of inflammation to TTP. *Blood*. 2016;128(1):110-119. doi:10.1182/blood-2015-12-688747

64. HORN M, BERTLING A, BRODDE MF, et al. Human neutrophil alpha-defensins induce formation of fibrinogen and thrombospondin-1 amyloid-like structures and activate platelets via glycoprotein IIb/IIIa. *J Thromb Haemost*. 2012;10(4):647-661. doi:10.1111/j.1538-7836.2012.04640.x

65. Kougias P, Chai H, Lin P, Yao Q, Lumsden A, Chen C. Neutrophil antimicrobial peptide α-defensin causes endothelial dysfunction in porcine coronary arteries. *J Vasc Surg*. 2006;43:357-363. doi:10.1016/j.jvs.2005.10.019

66. Higazi A, Ganz T, Karikó K, Cines D. Defensin Modulates Tissue-type Plasminogen Activator and Plasminogen Binding to Fibrin and Endothelial Cells. *J Biol Chem*. 1996;271:17650-17655. doi:10.1074/jbc.271.30.17650

67. Carmona-Rivera C, Khaznadar SS, Shwin KW, et al. Deficiency of adenosine deaminase 2 triggers adenosine-mediated NETosis and TNF production in patients with DADA2. *Blood*. 2019;134(4):395-406. doi:10.1182/blood.2018892752

68. Verdoni L, Mazza A, Gervasoni A, et al. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. *Lancet Lond Engl*. 2020;395(10239):1771-1778. doi:10.1016/S0140-6736(20)31103-X

69. Yost CC, Cody MJ, Harris ES, et al. Impaired neutrophil extracellular trap (NET) formation: a novel innate immune deficiency of human neonates. *Blood*. 2009;113(25):6419-6427. doi:10.1182/blood-2008-07-171629

70. Zeng L, Xia S, Yuan W, et al. Neonatal Early-Onset Infection With SARS-CoV-2 in 33 Neonates Born to Mothers With COVID-19 in Wuhan, China. *JAMA Pediatr*. 2020;174(7):722-725. doi:10.1001/jamapediatrics.2020.0878

71. Brass L. Thrombin and platelet activation. *Chest*. 2003;124:18S-25S. doi:10.1378/chest.124.3_suppl.18S

72. Semeraro F, Ammollo CT, Morrissey JH, et al. Extracellular histones promote thrombin generation through platelet-dependent mechanisms: involvement of platelet TLR2 and TLR4. *Blood*. 2011;118(7):1952-1961. doi:10.1182/blood-2011-03-343061

73. Barranco-Medina S, Pozzi N, Vogt AD, Di Cera E. Histone H4 promotes prothrombin autoactivation. *J Biol Chem*. 2013;288(50):35749-35757. doi:10.1074/jbc.M113.509786

74. Krisinger MJ, Goebeler V, Lu Z, et al. Thrombin generates previously unidentified C5 products that support the terminal complement activation pathway. *Blood*. 2012;120(8):1717-1725. doi:10.1182/blood-2012-02-412080

75. Foley JH, Walton BL, Aleman MM, et al. Complement Activation in Arterial and Venous Thrombosis is Mediated by Plasmin. *EBioMedicine*. 2016;5:175-182. doi:10.1016/j.ebiom.2016.02.011

76. Loghmani H, Conway EM. Exploring traditional and nontraditional roles for thrombomodulin. *Blood*. 2018;132(2):148-158. doi:10.1182/blood-2017-12-768994

77. Zhou P, Yang X-L, Wang X-G, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020;579(7798):270-273. doi:10.1038/s41586-020-2012-7

78. Sheng R, Chen Y, Yung Gee H, et al. Cholesterol modulates cell signaling and protein networking by specifically interacting with PDZ domain-containing scaffold proteins. *Nat Commun*. 2012;3:1249-1249. doi:10.1038/ncomms2221

79. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*. 2020;181(2):271-280.e8. doi:10.1016/j.cell.2020.02.052

80. Karnovsky MJ, Kleinfeld AM, Hoover RL, Klausner RD. The concept of lipid domains in membranes. *J Cell Biol*. 1982;94(1):1-6. doi:10.1083/jcb.94.1.1

81. Wang H, Yuan Z, Pavel MA, Hansen SB. The role of high cholesterol in age-related COVID19 lethality. *bioRxiv*. Published online January 1, 2020:2020.05.09.086249. doi:10.1101/2020.05.09.086249

82. Kuo C-L, Pilling LC, Atkins JL, et al. APOE e4 Genotype Predicts Severe COVID-19 in the UK Biobank Community Cohort. *J Gerontol Ser A*. 2020;(glaa131). doi:10.1093/gerona/glaa131

83. Yin C, Ackermann S, Ma Z, et al. ApoE attenuates unresolvable inflammation by complex formation with activated C1q. *Nat Med*. 2019;25(3):496-506. doi:10.1038/s41591-018-0336-8

84. Brass AL, Huang I-C, Benita Y, et al. The IFITM proteins mediate cellular resistance to influenza A H1N1 virus, West Nile virus, and dengue virus. *Cell*. 2009;139(7):1243-1254. doi:10.1016/j.cell.2009.12.017

85. Amini-Bavil-Olyaee S, Choi YJ, Lee JH, et al. The antiviral effector IFITM3 disrupts intracellular cholesterol homeostasis to block viral entry. *Cell Host Microbe*. 2013;13(4):452-464. doi:10.1016/j.chom.2013.03.006

86. Everitt AR, Clare S, Pertel T, et al. IFITM3 restricts the morbidity and mortality associated with influenza. *Nature*. 2012;484(7395):519-523. doi:10.1038/nature10921

87. Carstea ED, Morris JA, Coleman KG, et al. Niemann-Pick C1 Disease Gene: Homology to Mediators of Cholesterol Homeostasis. *Science*. 1997;277(5323):228. doi:10.1126/science.277.5323.228

88. Sturley S, Marka Z, Márka S, et al. *Insights into Possible COVID-19 Antivirals from a Rare Neurodegenerative Disease: An Executive Summary*.; 2020. doi:10.31219/osf.io/tersc

89. Zhang Y, Qin L, Zhao Y, et al. Interferon-Induced Transmembrane Protein 3 Genetic Variant rs12252-C Associated With Disease Severity in Coronavirus Disease 2019. *J Infect Dis*. 2020;222(1):34-37. doi:10.1093/infdis/jiaa224

90. Zhang Y-H, Zhao Y, Li N, et al. Interferon-induced transmembrane protein-3 genetic variant rs12252-C is associated with severe influenza in Chinese individuals. *Nat Commun*. 2013;4:1418-1418. doi:10.1038/ncomms2433

91. Thevarajan I, Nguyen THO, Koutsakos M, et al. Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19. *Nat Med*. 2020;26(4):453-455. doi:10.1038/s41591-020-0819-2

92. Wang Z, Zhang A, Wan Y, et al. Early hypercytokinemia is associated with interferon-induced transmembrane protein-3 dysfunction and predictive of fatal H7N9 infection. *Proc Natl Acad Sci*. 2014;111(2):769. doi:10.1073/pnas.1321748111

93. De Meyer Guido R. Y., Hoylaerts Marc F., Kockx Mark M., Yamamoto Hiroshi, Herman Arnold G., Bult Hidde. Intimal Deposition of Functional von Willebrand Factor in Atherogenesis. *Arterioscler Thromb Vasc Biol*. 1999;19(10):2524-2534. doi:10.1161/01.ATV.19.10.2524

94. PEASE DC. An Electron Microscopic Study of Red Bone Marrow. *Blood*. 1956;11(6):501-526. doi:10.1182/blood.V11.6.501.501

95. Campbell RA, Schwertz H, Hottz ED, et al. Human megakaryocytes possess intrinsic antiviral immunity through regulated induction of IFITM3. *Blood*. 2019;133(19):2013-2026. doi:10.1182/blood-2018-09-873984

96. Lefrançais E, Ortiz-Muñoz G, Caudrillier A, et al. The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors. *Nature*. 2017;544(7648):105-109. doi:10.1038/nature21706

97. Peerschke EI, Yin W, Ghebrehiwet B. Complement activation on platelets: implications for vascular inflammation and thrombosis. *Mol Immunol*. 2010;47(13):2170-2175. doi:10.1016/j.molimm.2010.05.009

98. Boilard E, Flamand L. The role of the megakaryocyte in immunity has gone viral. *Blood*. 2019;133(19):2001-2002. doi:10.1182/blood-2019-02-900787

99. Vogt MB, Lahon A, Arya RP, Spencer Clinton JL, Rico-Hesse R. Dengue viruses infect human megakaryocytes, with probable clinical consequences.

PLoS Negl Trop Dis. 2019;13(11):e0007837-e0007837. doi:10.1371/journal.pntd.0007837

100. Simon AY, Sutherland MR, Pryzdial ELG. Dengue virus binding and replication by platelets. *Blood*. 2015;126(3):378-385. doi:10.1182/blood-2014-09-598029

101. Cunin P, Bouslama R, Machlus KR, et al. Megakaryocyte emperipolesis mediates membrane transfer from intracytoplasmic neutrophils to platelets. *eLife*. 2019;8:e44031. doi:10.7554/eLife.44031

102. Frydman GH, Tessier SN, Wong KHK, et al. Megakaryocytes contain extranuclear histones and may be a source of platelet-associated histones during sepsis. *Sci Rep*. 2020;10(1):4621-4621. doi:10.1038/s41598-020-61309-3

103. Cros J, Cagnard N, Woollard K, et al. Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. *Immunity*. 2010;33(3):375-386. doi:10.1016/j.immuni.2010.08.012

104. Veras FP, Pontelli M, Silva C, et al. *SARS-CoV-2 Triggered Neutrophil Extracellular Traps (NETs) Mediate COVID-19 Pathology*. Infectious Diseases (except HIV/AIDS); 2020. doi:10.1101/2020.06.08.20125823

105. Sorvillo N, Mizurini DM, Coxon C, et al. Plasma Peptidylarginine Deiminase IV Promotes VWF-Platelet String Formation and Accelerates Thrombosis After Vessel Injury. *Circ Res*. 2019;125(5):507-519. doi:10.1161/CIRCRESAHA.118.314571

106. Dong J, Moake JL, Nolasco L, et al. ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. *Blood*. 2002;100(12):4033-4039. doi:10.1182/blood-2002-05-1401

107. BERNARDO A, BALL C, NOLASCO L, CHOI H, MOAKE JL, DONG JF. Platelets adhered to endothelial cell-bound ultra-large von Willebrand factor strings support leukocyte tethering and rolling under high shear stress. *J Thromb Haemost*. 2005;3(3):562-570. doi:10.1111/j.1538-7836.2005.01122.x

108. Mosher DF. Cross-linking of cold-insoluble globulin by fibrin-stabilizing factor. *J Biol Chem*. 1975;250(16):6614-6621.

109. Wang F, Zhang N, Li B, et al. Heparin defends against the toxicity of circulating histones in sepsis. *Front Biosci Landmark Ed*. 2015;20:1259-1270.

110. Pal PK, Starr T, Gertler MM. Neutralization of heparin by histone and its subfractions. *Thromb Res*. 1983;31(1):69-79. doi:10.1016/0049-3848(83)90008-7

111. Zhu C, Liang Y, Li X, Chen N, Ma X. Unfractionated heparin attenuates histone-mediated cytotoxicity in vitro and prevents intestinal microcirculatory dysfunction in histone-infused rats. *J Trauma Acute Care Surg*. 2019;87(3). https://journals.lww.com/jtrauma/Fulltext/2019/09000/Unfractionated_heparin_attenuates_histone_mediated.20.aspx

112. Iba T, Hashiguchi N, Nagaoka I, Tabe Y, Kadota K, Sato K. Heparins attenuated histone-mediated cytotoxicity in vitro and improved the survival in a rat model of histone-induced organ dysfunction. *Intensive Care Med Exp*. 2015;3(1):36-36. doi:10.1186/s40635-015-0072-z

113. Hirsh J, Warkentin TE, Shaughnessy SG, et al. Heparin and Low-Molecular-Weight Heparin Mechanisms of Action, Pharmacokinetics, Dosing, Monitoring, Efficacy, and Safety. *Chest*. 2001;119(1):64S-94S. doi:10.1378/chest.119.1_suppl.64S

114. Sobel M, McNeill PM, Carlson PL, et al. Heparin inhibition of von Willebrand factor-dependent platelet function in vitro and in vivo. *J Clin Invest*. 1991;87(5):1787-1793. doi:10.1172/JCI115198

115. Hogwood J, Pitchford S, Mulloy B, Page C, Gray E. Heparin and non-anticoagulant heparin attenuate histone-induced inflammatory responses in whole blood. *PloS One*. 2020;15(5):e0233644-e0233644. doi:10.1371/journal.pone.0233644

116. Spiess BD. Heparin: Effects upon the Glycocalyx and Endothelial Cells. *J Extra Corpor Technol*. 2017;49(3):192-197.

117. Dunois-Lardé C, Capron C, Fichelson S, Bauer T, Cramer-Bordé E, Baruch D. Exposure of human megakaryocytes to high shear rates accelerates platelet production. *Blood*. 2009;114(9):1875-1883. doi:10.1182/blood-2009-03-209205

118. Yamamoto K, de Waard V, Fearns C, Loskutoff DJ. Tissue Distribution and Regulation of Murine von Willebrand Factor Gene Expression In Vivo. *Blood*. 1998;92(8):2791-2801. doi:10.1182/blood.V92.8.2791

119. Lambrecht BN. Alveolar Macrophage in the Driver's Seat. *Immunity*. 2006;24(4):366-368. doi:10.1016/j.immuni.2006.03.008

120. He Y, Yang F-Y, Sun E-W. Neutrophil Extracellular Traps in Autoimmune Diseases. *Chin Med J (Engl)*. 2018;131(13):1513-1519. doi:10.4103/0366-6999.235122