Lung Pathogenesis of SARS-CoV-2 Infection

1. **Alveolar phase**
   - Virus infects Type II pneumocytes (AT2) via ACE2 attachment.
   - Alveolar injury to AT2 cells.

2. **Transcytosis from alveolar to pulmonary circulation endothelial cells**
   - Proximity factor between AT2 and endothelial cells (EC) facilitates transcytosis via yet unconfirmed mechanism.

3. **Endothelial injury phase**
   - Pericytes of the pulmonary circulation endothelium have the most abundant ACE2 expression.
   - Virus infects these pericytes after transcytosis from the alveolar space.
   - Virus causes injury and apoptosis of these pericytes.
   - Pericytes act as a "contractile force" maintaining endothelial integrity.
   - Loss of pulmonary circulation endothelial pericytes acts as sites of "laceration" in the endothelium, exposing pro-thrombotic antigens in the endothelium and its basement membrane, prompting platelet activation to pursue physiologic thrombosis to "wall off" the viral pathogen.

4. **Angiotensin II imbalance phase**
   - Normally, pulmonary circulation endothelium harbors ACE, which converts Angiotensin I to II.
   - Normally, pericytes (of pulmonary circulation endothelium) harbor a high expression of ACE2, which function to convert Angiotensin II to (a short half-life) Angiotensin 1-7 locally, to maintain a balanced state locally between a pro-thrombotic, pro-inflammatory Angiotensin II, and an anti-thrombotic, anti-inflammatory Angiotensin 1-7.
   - Loss of pericytes causes an imbalance in this process, leading to a relative excess activity of Angiotensin II in the affected pulmonary circulation, left unopposed by a reduced conversion to Angiotensin 1-7.
   - Unopposed Angiotensin II at the site of "injured" EC may lead to:
     - Angiotensin II pro-inflammatory effect on pulmonary circulation endothelium, leading to increased vascular permeability:
       - Radiographically, this phase corresponds with the initial limited angiocentric interstitial edema (i.e. GGO) on CT without significant vasoconstriction in the smallest pulmonary vessels.
       - Clinically, this phase of injury most likely remains asymptomatic aside from initial alveolar injury symptoms consisting of a few initial days of cough, fever, without any hypoxia.
     - Angiotensin II effect on corresponding alveoli, leading to poor clearance of alveolar fluid, leading to "alveolar edema" and likely contributing to the cough on the few initial days of infection.
     - Angiotensin II effect on vascular smooth muscle cells (SMC), leading to HIF up-regulation in these cells, inducing a higher level of hypoxia-induced pulmonary vascular remodeling.

5. **Immuno-thrombosis phase (protective initial response)**
   - Endothelial injury and Angiotensin II mediated increased vascular permeability leads to platelet activation at the site of injury in the pulmonary circulation.
   - Platelets by interacting with the injured pulmonary endothelium begin a physiologic and protective process of immuno-thrombosis, to "wall off" the viral pathogen, by releasing vasoactive and thrombotic mediators.
   - These vasoactive and thrombotic mediators include platelet-stored granules:
     - Alpha-granules: PDGF, IGF-1, TFGbeta, Platelet Factor 4
     - Dense-granules: 5-HT (serotonin), ADP, ATP, ionized Ca

- This process leads to recruitment of PMNs and other leukocytes to the site of endothelial injury, inducing NETosis as a normal physiologic response to "patch up" the site of endothelial injury and contain the viral pathogen.
- 5-HT release by platelets has been implicated as upstream regulator for expression of IL-6 from vascular smooth muscle cells, among many other inflammatory and cytokine mediators.
- Local effect of 5-HT on pulmonary circulation vasculature includes:
  - Increased vascular permeability (i.e. akin to a "vasodilation") at the site of capillary endothelial injury
  - Vasoconstriction of inflow and outflow to the injury site (i.e. vasoconstriction of pulmonary pre-capillary arteriole via 5-HT and post-capillary venule via 5-HT and platelet-derived histamine).
    - Radiographically, this phase of injury corresponds with the initial vasoconstriction of the smallest pulmonary vessels in the affected lung segment on CT, as well as to the increasing angiocentric interstitial edema at the sites of prior limited interstitial edema.
    - Clinically, this phase of injury likely remains without any significant clinically-detectable burden of dead-space ventilation, and without any significant intrapulmonary shunt development or hypoxia.

6. Progressive immuno-thrombosis phase (feed-forward injurious cycle)

- Among all pro-thrombotic and vasoactive mediators released from platelet granules during the initial immuno-thrombosis phase, 5-HT (serotonin) has a unique feature and set of function in the pulmonary circulation.
- Platelet granules store 95% of the total 5-HT content in the human body.
- Pulmonary circulation endothelium is responsible for clearance of 80-95% of systemic circulating levels of 5-HT (as for instance would occur in non-pulmonary thrombotic events), with liver accounting for at most 15% of this metabolism.
- In SARS-CoV-2 induced lung injury, the pulmonary circulation endothelium, normally the site of systemic 5-HT clearance, is itself the site of injury, dysfunction due to injury, as well as the initial immuno-thrombosis and vasoconstriction, leading to outflow obstruction.
- As the initial protective immuno-thrombosis occurs, 5-HT is released from platelet granules as described above to "wall off" the viral pathogen.
- This released extracellular 5-HT in the injured pulmonary endothelium is unable to be processed and cleared by the dysfunctional local endothelium due to viral injury.
- This inability to clear 5-HT leads to a feed-forward cycle of extracellular 5-HT causing progressive platelet aggregation and leukocyte recruitment at the site of the initial immuno-thrombosis, resulting in a feed-forward cycle of immuno-thrombosis
  - Radiographically, this phase of injury corresponds with progressive vasoconstriction in the smallest vessels on CT, with proximal pre-capillary arteriolar vasodilation due to back-pressure buildup as below.
  - Physiologically, this outflow obstruction in the pulmonary capillaries will result in excess back-pressure buildup, leading to vasodilation of pre-capillary pulmonary arterioles, evident on CTA as progressive vasodilation in the affected lung segments. With adequate back-pressure, eventually Intrapulmonary right to left shunt development occurs in this phase, across an unidentified vascular shunt. This shunt may be formed by recanalization of the bronchopulmonary anastomoses, overpowering the bronchial circulation pressures, shunting the blood from the occluded pulmonary circulation into the bronchial circulation across these anastomoses, back to the pulmonary vein via the bronchopulmonary vein.
  - Biochemically, the excess extra-cellular 5-HT buildup in the local pulmonary circulation, due to the feed-forward immuno-thrombosis and the inability to clear 5-HT, will be funneled via the right to left intrapulmonary shunt into the systemic circulation.
  - Clinically, this phase of injury corresponds with worsening dead-space ventilation due to progressive immuno-thrombosis, as well as with the beginning of a progressive intrapulmonary shunt development and progressive hypoxia.
- Risk factors for a more florid pulmonary circulation immuno-thrombosis includes:
  - Higher platelet-endothelial reactivity

        - Due to higher content of platelet-derived vasoactive and thrombotic mediators
            - Increased with age
            - Increased in male gender
        - Due to higher plasma vWF levels
            - Increased in non-O blood group
    - Higher baseline endothelial dysfunction
        - Diabetes mellitus, chronic kidney disease, hyperlipidemia, hypertension, smoking, aging, obesity
- Children have a baseline healthier state of endothelial function as well as a lower platelet reactivity.
- As stated above, this platelet hyperactivity, florid feed-forward immuno-thrombosis, combined with the development of intrapulmonary vascular shunting not only leads to progressive dead-space ventilation and progressive hypoxia, but also leads to channeling of excess extracellular platelet-derived 5-HT from the pulmonary circulation, shunted unprocessed, into the systemic circulation
- Extracellular systemic 5-HT excess may account for a variety of unusual findings in severe COVID19.

7. Systemic 5-HT mediated phase

- **Tachypnea / Hyperpnea**
    - Tachypnea encountered in severe COVID19 without distress is often seen with an unusual blood gas pattern showing low or normal PaCO2 before the onset of mechanical ventilation.
    - This is unusual as low or normal PaCO2 should not drive a central response to cause tachypnea without distress in these patients.
    - Typically, there is also no evidence of metabolic acidosis, symptoms of pleural irritation, or anxiety to account for this tachypnea without distress.
    - To justify such unusual and unique response, not seen commonly with such frequency in other viral lung infections, on “cytokine storm” is far-fetched and presumptive.
    - Extracellular platelet-derived 5-HT, reaching systemic circulation via the intrapulmonary shunt per above pathway, has been shown in several studies to induce hyperpnea via its action on the peripheral chemoreceptors of the carotid sinus; 5-HT antagonism has shown to suppress this hyperpnea response.

- **Ventilator dyssynchrony**
    - Ventilator dyssynchrony has been encountered often in severe COVID19 lung injury.
    - This may also be accounted for, as well, by the systemic 5-HT mediated hyperpnea as described above.
    - Fentanyl has been shown to provoke “serotonin syndrome” in the literature by inducing an increase in systemic extracellular 5-HT bioavailability. Morphine has been reported to have less risk of such increase in 5-HT. Precedex has been reported to reduce 5-HT action in “serotonin syndrome” and provides better sedation in patients that remain agitated despite Versed and Fentanyl in “serotonin syndrome.”

- **Bronchoconstriction**
    - Dead-space physiology, as outlined above, leads to an increase in PaCO2 and a lowering of pH in the affected local pulmonary circulation.
    - This elevation in PaCO2 and low pH is sensed by the pulmonary neuroepithelial bodies (NEB), which respond via release of 5-HT to cause bronchoconstriction.
    - This physiologic response in dead-space ventilation is designed to reduce V/Q mismatch, by reducing ventilation to the affected lung segments with poor perfusion.

- **Brady / tachycardia**
    - Systemic extracellular 5-HT has been shown to provoke bradycardia and tachycardia.

- **Fever**
    - Systemic extracellular 5-HT has been shown to provoke febrile episodes.

- **Hypotension**
  - Systemic extracellular 5-HT has been implicated to induce hypotension, as well as a mediator to induce shock.

- **Arterial and Venous thrombosis**
  - Systemic extracellular 5-HT along with elevated vasoactive mediators released by the florid pulmonary circulation immuno-thrombosis (i.e. vWF, Fibrinogen, Factor VIII) as described above can lead to widespread and progressive thrombosis in the systemic circulation.

- **"Heparin-resistance"**
  - Pulmonary endothelium is known to be responsible for 50% of total body platelet biogenesis from megakaryocytes.
  - In COVID19 lung injury, the pulmonary endothelium is injured and pro-thrombotic.
  - Additionally, the feed-forward and florid cycle of immuno-thrombosis in the injured pulmonary circulation leads to a large surge of pro-thrombotic factors release from platelets including vWF, Fibrinogen, ADP, and 5-HT.
  - Therefore, it is plausible to expect a large proportion of platelets matured and released by the pulmonary endothelium to exist in a hyperactivated state due to being produced and primed within an activated endothelium in a milieu of abundant thrombotic factors.
  - This process can result in a systemic platelet hyperactivation not unlike what is encountered in HIT (which has a differing origin in an activating anti-PF4 immune complex), leading to arterial and venous thrombosis and "heparin resistance."

- **Diarrhea / Mesenteric ischemia**
  - Systemic extracellular 5-HT has been implicated in non-occlusive mesenteric ischemia.
  - Serotonergic action has been implicated in causing diarrhea.

- **CNS ischemic injury**
  - Systemic extracellular 5-HT has been shown to cause cerebral flow vasoconstriction.
  - Ischemia CVA has been reported due to above cerebral vasoconstriction caused by systemic extracellular 5-HT excess in serotonin syndrome.
  - Role of systemic extracellular 5-HT has been demonstrated in animal models of central sleep apnea.

- **Lung Vascular Remodeling**
  - 5-HT, along with PDGF, are implicated in pulmonary vascular remodeling and angiogenesis.
  - Specifically, pericyte hyperplasia in venules and smooth muscle cell thickening in arterioles of the pulmonary circulation likely occur due to excess extra-cellular trophic factor release (PDGF, serotonin, etc) by platelets in the feed-forward florid immuno-thrombosis phase.

- **Skin**
  - Perniosis (chillblains) has been associated with findings of dermal vessel wall thickening, and among many culprits, platelet aggregation has been implicated in its pathogenesis. Incidentally, pericyte hyperplasia in normal skin biopsy has been shown in COVID19, possibly due to systemic release of trophic factors such as PDGF and 5-HT as postulated above in the feed-forward immuno-thrombosis phase.
  - Acrocyanosis, reported as typically a later finding in SARS-CoV-2 infection, has been shown to be mediated by 5-HT among other factors, and clinical improvement has been noted with 5-HT inhibition in this condition.

- **Pediatric Multisystem Inflammatory Syndrome**
  - In children, platelets are not as hyperactive and endothelium does not harbor yet as much dysfunction at baseline, and this feed-forward cycle of immuno-thrombosis after COVID19 insult resolves spontaneously.
  - However, the pulmonary endothelium nevertheless is injured at least in a few areas due to the viral insult, without causing any discernible symptoms in children, and the pulmonary endothelium remains activated with exposed pro-thrombotic antigens and a hyperactivating behavior.
  - This leads to a subset of platelets released in a "hyperactive" state from the injured pulmonary endothelium. This subset of hyperactive platelets reach systemic capillaries and cause “vasculitic” and "microthrombotic" phenomena, including a disorder resembling Kawasaki disease.
  - Hyperactive platelets and excess serotonin have been implicated in the primary pathophysiology of Kawasaki disease, as hypothesized here as well for COVID19, with ASA and IVIg in Kawasaki established as therapy to target and reduce platelet (and immune complex mediated) hyperactivity.

- **Post-hemorrhagic (i.e. postpartum) lung injury and MSOF**
  - Postpartum hemorrhage expected from, for instance, C-section or other surgical interventions, can trigger platelet release from the lungs, in the context of recent evidence that 50% of platelet biogenesis occurs in the lungs.
  - This release may become a complicating factor in the context of even an asymptomatic SARS-CoV-2 lung infection. Pulmonary arteriole and venule vasoconstriction due to the vasoactive mediators released during the initial protective immuno-thrombosis phase can create an outflow restriction for platelets to exit the pulmonary microvasculature. Upon platelet release from these segments triggered by the hemorrhagic event elsewhere in the body, a physical platelet aggregation due to this microvascular constriction may begin to occur, accelerating an asymptomatic infection to rapidly reach the feed-forward immuno-thrombosis phase with dead-space ventilation and intrapulmonary shunt development.

- **MSOF upon mechanical ventilation and other “physical” forces exerted upon lungs**
  - Edematous lung interstitium may be saturated and “soaked” with excess increasing levels of extra-cellular 5-HT produced in the feed-forward immuno-thrombosis phase of the injury, unable to be taken up by the dysfunctional pulmonary endothelium.
  - Physical movements of the lung, as in sudden position changes, or even the onset of mechanical ventilation, can physically displace extra-cellular 5-HT from the edematous lung interstitium, via the intrapulmonary right to left shunt, into the systemic circulation, and bring about rapid systemic decline, with MSOF, hypotension, tachy/bradycardia, hemodynamic compromise, surge in thrombosis in the pulmonary and systemic circulation, cerebral vasoconstriction, and other described systemic effects.

- **Prolonged Effects during and after “recovery”**
  - It is possible that in the severe COVID19 lung injury with florid immuno-thrombosis (if not prevented by timely and early anticoagulation and anti-thrombotics), the edematous lung interstitium may harbor an accumulation of platelet-derived vasoactive and thrombotic mediators that may take days to weeks to be cleared and processed. This hypothetical prolonged and slow clearance of such factors, in the context of half of the body’s platelet biogenesis occurring in the lungs, may result in a prolonged “platelet hyperactivation syndrome” that may be responsible for the protracted symptoms during the “recovery” phase of severe COVID19 lung injury. These may include a variety of platelet-mediated phenomena as well as neurally-mediated serotonergic phenomena, for instance, autonomic dysfunction, postural orthostatic tachycardia syndrome, tremor, tinnitus, neuropsychiatric manifestations, complex regional pain syndrome, Raynaud’s, acrocyanosis, and a prolonged hyper-coagulable states.

Can this hypothesized vicious feed-forward cycle of immuno-thrombosis mediated by 5-HT and other platelet-activating factors such as histamine and ADP per this proposal be reversed by appropriate antagonists such as 5-HT inhibitors, histamine inhibitors, and ADP inhibitors, particularly if early anticoagulation, anti-thrombotics, and/or EC stabilization are not undertaken in a critical window of time to prevent this feed-forward immuno-thrombosis phase to prevent the hypothesized buildup of excess extracellular platelet activating mediators, predominantly 5-HT?

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