Asian Journal of Immunology

4(2): 11-30, 2020; Article no.AJI.58422

Acute Immune Mediated Lung Injury in COVID 19: A Review

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

Editor(s):
(1) Dr. Cynthia Aracely Alvizo Báez, Autonomous University of Nuevo Leon, Mexico.
Reviewers:
(1) Ritika Gupta, India.
(2) Abioye, Adesoye Idowu, University of Ilorin, Nigeria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/58422

Review Article

Received 01 June 2020
Accepted 16 June 2020
Published 23 June 2020

ABSTRACT

There are many gaps in our present understanding of the of the SARS CoV 2 related matters like its PAMPs,(pathogen associated molecular patterns),antigenic profile, immune evasive mechanisms and also other host related matters, like PRR s(pattern recognizing receptors) and the deranged host defense mechanisms, that cause self-damage. These constraints come in way of accurately delineating the pathogenesis of COVID19 lung disease. Hence is the speculative nature of any concept trying to explain the same. An integrated approach is embarked upon, taking into account the known clinical, radiological, laboratory, and autopsy findings, in search of clues that may suggest a possible mechanism, that explains the underlying lung damage in COVID 19. It is seen that no single mechanism or syndrome could explain fully the pathology and pathogenesis of lung damage in COVID19. Hence, multiple mechanisms consistent with each known facet of the pathology are explored. Thus the inflammatory damage of the alveolar tissue is sought to be explained by the3 complement activation pathways i.e. the alternative pathway, the MBL/Lectin pathway/ and Tissue factor/extrinsic pathway(of the classical complement activation), the contact cascade involving the kallikrein-kinin pathway, and the cytokine mediated pro and anti inflammatory mechanisms. The vascular pathology like hemorrhages and small blood vessel micro-thrombi as observed at autopsy , are viewed from the point of view of simple activation of the coagulation cascade to small vessel vasculitis (leucocytoclastic vasculitis) and coagulative micro angiopathy. Besides, the role of TM-PC-EPCR SYSTEM (Thrombomodulin-Protein C-EPCR System) is explored. The points in favour and against of each of the above are discussed.The central role played by the macrophage polymorphism is focused in the context of the simultaneous presence of

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active inflammation in the lung tissue and the interstetium and healing by interstitial fibrosis, seen in the lungs of COVID 19 patients. The role played by the other humoral and cellular elements of both innate and adaptive immunity is briefly reviewed. The uniqueness and diversified features of COVID 19 lung pathology, suggests two things - that the immune mediated damage seems more probable than could be explained by the viral infectivity and that the pathology seems to stem from a mixture of different underlying and overlapping syndromes. Hence, the author prefers to call all the COVID 19 related features of lung pathology as “Acute immune mediated Lung injury". (AILI) than trying to bunch them under a single syndrome.

Keywords: Pathogen associated proteins (PAMPs); pathogen recognizing receptors (PRRs) complement; cytokines; phagocytosis; antibody; ARDS; immune evasion; capillaritis; microangiopathy.

1. INTRODUCTION

The lung pathology and pathogenesis of covid 19, is elusive and hence remained speculative, till date. Described originally as a ‘pneumonia-like’ condition, similar to one caused by SARS CoV 1 (2003) and MERS CoV, (2011), which are members of the same family of Corona virus. The former was less severe but caused pandemic and the later was 34% more severe,mortality-wise , but remained confined to middle east mostly. The COVID 19, thus resembled more of SARS 1 and hence the virus is named SARS Cov 2. In due course of time, the postscript, ‘like’ is dropped and the syndrome has come to be called ‘COVID 19 pneumonia’, contrary to the facts. The opinions as to the cause of COVID 19 induced lung damage varied from ARDS, SHLH (secondary hemophagocytic lymphohistiocytosis) and Cytokine storm from the clinical point of view and ‘interstitial pneumonia’ from radiological point of view. The autopsy findings raise suspicion of capillarites (small blood vessel vasculitis) to coagulative microangiopathy. It is felt that the clinical as well as laboratory, radiological and autopsy data if taken together sheds some light as to the exact lung pathology and pathogenesis of COVID 19 lung disease. Hence these aspects are briefly reviewed in search of clues to the lung pathology/pathogenesis of COVID 19.

2. CLINICAL DATA

2.1 Pneumonia- Like Condition of COVID 19 vs ARDS

Li, X., Ma, X [1] has elaborately discussed the matter and could make out some differences between the two conditions. Peter G Gibson et al. 2020) [2] have to say " We are familiar with ARDS, however when it occurs as part of COVID-19, it has different features and there remain, unanswered questions". Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S (2020) suggest that among the patients affected, the pneumonia-like process of COVID 19. Unto 20% only develop pneumonia of which, about 3 to 4% develop severe form necessitating ventilator support. ~50% develop hypoxemia by day 8. Severe illness and cytokine release syndrome appear to develop mostly within 5–10 days after the onset of symptoms in susceptible patients. ARDS- like clinical picture is suggested to develop in 42% of patients presenting with COVID-19 pneumonia, and 61-81% of those requiring ICU care [3]. Puah SH et al. report that COVID-19 ARDS follows a predictable time course over days, with median time to incubation period of 8.5 days after onset of symptom in Singaporean patients [4]. Not all COVID 19 patients suffer pneumonia-like syndrome. Even though all of those who develop this very few of them develop severe respiratory distress, necessitating ventilator support. Most of them have comorbid diseases and constitute to the 2 to 3% mortality observed. The autopsy findings which suggested ARDS are - the firm, heavy lung filled with fluid and presence on bits of hyaline membrane in the lungs. Severe illness and cytokine release syndrome appear to develop mostly within 5–10 days after symptom onset in susceptible patients. Not all COVID 19 patients suffer pneumonia like syndrome. Even though those who develop this very few develop severe respiratory distress, necessitating ventilator support. Most of them have comorbid diseases and constitute the 2 to 3% mortality observed. The autopsy findings which suggested ARDS are the firm, heavy lung filled with fluid and presence on bits of hyaline membrane in the lungs. Peter G Gibson et al opine that most of the COVID-19 patients, meet the Berlin definition (2012) for ARDS. All of them have present with an acute clinical emergency, with bilateral opacities in the lung imaging, gas exchange abnormalities, and their disease can't be explained by heart failure or volume overload. The ARDS Berlin criteria defined that for a

patient to be diagnosed as having ARDS, the onset must be within 1 week of a known clinical insult or new or worsening respiratory symptoms. [5], Wang et al. [6] reported median timed onset of ARDS, as 8.0 days, Zhou et al. [7] as 12 days. Chen et al. [8] and Guan et al. [9] did not report the onset of ARDS. The lowest median time reported is 4 days from the onset of first symptom. The main departure from ARDS and SARS CoV2 pneumonia is the preserved lung compliance in face of severe hypoxia, and poor oxygenation without increased work of breathing, which are not seen in ARDS. It is also seen that ARDS developed in some COVID 19 patients after they have been put on ventilator, sparking speculation as to whether such development is due to natural progression of the COVID 19 or ventilator induced. Robba C, Battaglini D Patroniti N [10] et al. suggested 3 phenotypes of patients, basing on their CT findings of lungs in Covid 19 (discussed under imaging features, below). They opine that not all cases of COVID lung involvement resemble ARDS. Gattinoni et al. [11] described two distinct phenotypes, Type L and Type H (Type 1 and Type 2). Type L disease, is characterized by normal lung compliance and gas volume in the presence of hypoxemia. These patients may improve, or they may worsen. About 20% to 30% of patients had or evolved to Type H disease, characterized by worse lung compliance and increased edema and lung weight. "The transition from Type L to Type H may be due to the evolution of the COVID-19 pneumonia on one hand and the injury attributable to high-stress ventilation on the other".

SARS of COVID 2 vs (sHLH)/CYTOKINE Storm: Macrophage activation syndrome (MAS), also known as secondary haemo phagocytic lympho histocytosis (SHLH) or Cytokine storm is distinguished by:

1. Absent haemophagocytosis
2. Lower ferritin levels.
3. Coagulopathy not being due to liver synthesized factors.
4. DIC is seen as a terminal event in some COVID 19 patients only even though hyper-cytokinemia.

2.2 Radiology / Imaging Data

The imaging technics have high sensitivity of about 97% when tested within 5 days of RT-PCR became positive and still can detect the changes as early as 3 days, but 56% are found to be normal when imaged in 2days of patient becoming COVID 19 positive. Thus the sensitivity of imaging the lungs in COVID 19, is a function of time of doing the test. C. Hani M.-P. Revel et al. (2020) extensively reviewed not only the CT findings of COVID 19, but other causes of differential diagnosis of the typical ground glass shadows seen in Imaging of COVID 19 lung [12]. Some of the important radiological findings are presented below. For differential diagnosis the readers may refer to the reference suggested.

1. Bilateral ground glass appearance which are round in 50% cases. They are called COVID balls
2. Areas of focal consolidation.
3. Crazy paving pattern resulting from intralobular reticulations.
4. Late signs: Signs suggesting organizing pneumonia.

1. Linear consolidations
2. Reverse halo sign i.e., areas of ground-glass surrounded by peripheral consolidation.

Study by Salehi et al. revealed that the frequencies of the different CT abnormalities were as follows: GGO (central ground glass opacity) was observed in 88.0% of patients, consolidation in 31.8%, bilateral involvement in 87.5% and peripheral distribution in 76.0% of patients [13]. Guan CS, et al. (2020) assert that “ground-glass opacities may be due to mild oedema of the alveolar septi, hyperplasia of the interstitium, partial filling of airspaces, or a combination of these features. Besides, the crazy-paving pattern may correlate with hyperplasia of interlobular and intralobular interstitia” [14].

The two radiological features still alluding explanation are-

1. Peripheral and subpleural distribution
2. Bilateral lower lobe involvement

Autopsy findings: Xu, L. Shi, Y. Wang, et al. [15] described in detail, the post-mortem findings of COVID 19.

Gross pathology: The lungs are firm and increased in weight and laden with secretions and exudate. Both the surface of lung and the cut section showed, patchy areas of haemorrhage.

Microscopic findings: These can be grouped as cellular, vascular and other features.

The cellular exudate consisted of CD4+ and CD8+ lymphocytes in the lung tissue and interstetium and with some perivascular collection. Also present are platelet cells, 65+ atypical megakaryocytes and desquamated type 2 pneumatocytes showing cytopathic effect. Neutrophils are absent except in the immuno-suppressed case.

Vascular features: Small blood vessels (capillaries) are found to be thickened, with oedematous walls, dilated and showed increased permeability with consequent seepage of plasma into the lung tissue. There is lymphocytic accumulation around the capillaries and micro-throbi inside the capillaries. The bigger pulmonary vessels near hilum are not affected.

2.3 Other Autopsy Findings

1. Focal presence of haemorrhagic. Patches, both in gross appearance and cut section of lung.
2. Focal greyare as of consolidation
3. Presence of clots in the lung tissue.
4. Scattered hyaline membrane and fibrin threads.

3. LABORATORY DATA

The laboratory tests found to be positive in COVID 19 also give some clues as to the underlying mechanisms involved in the pathology and parthenogenesis of COVID 19, if interpreted properly. The involvement of liver, kidney, and heart is indicated by the increase in lab findings pointing in that direction. Probably they represent subclinical involvement of the organs concerned, as overt manifestation of their involvement is seen only in a few of the patients and some in the later phase than earlier phage of the disease evolution. **Leuko-erythroblastic reaction** reported by some is a finding that needs to be investigated.The same is explained as probable involvement of bone marrowis the increased prothrombin time (PT) indicates the involvement of the extrinsic coagulative pathway as the cause of thrombotic phenomena observed in the small blood vessels at autopsy. The presence of D-dimers, FDP, to the underlying thrombolytic (fibrinolytic) process involving capillaries of the lungs. Of interest, is the observation of lymphopenia whereas lymphocytosis is commonly seen in all other viral infections. The lymphopenia is connected to increased severity / mortality in COVID 19 patients. The lab parameters also vary as the disease progresses. The platelets, the neutrophils and CD8+T cells are found to be normal in patients not admitted to ICU, where as neutrophilia and low platelets and CD 8+ T cells counts are seen in ICU pts. The iCU patients have increased risk of complications and mortality. The risk stratification of COVID 19 pneumonia patients has been analyzed by Janusz Jankowski t al. The Lab tests found positive in COVID 19 patients irrespective of their stage of disease, and what they suggest are tabulated and presented in Appendix 3.

4. DISCUSSION

Any envisaged pathogenic mechanism should explain all the clues available from the clinico-pathological data reviewed above. To put it succinctly, such concept has a clear beginning (in the form of virus-host interaction) a clear destination (the pathology elucidated by autopsy findings), a clear source (the normal physiological mechanisms). What is to be clinched is the means by which the normal physiology is turned into pathology in the context of COVID 19. The gaps in the present understanding of the issues involved, leaves no alternative than to ‘hazard a guess ‘as to the actual pathogenesis of the disease.

The viral factors: The genome of SARS CoV 2 encodes four main structural proteins: Spike (S), (which has S1 and S2 sub-units) envelope (E), nucleocapsid (N) and membrane (M) proteins .It is known that the SARS CoV 2 attaches through spike protein to the cell surface negotiate the ACE 2 receptor of the host cell, to gain entry into the body. It is even suggested that by **internalizing the ACE 2 receptor** down regulates its protective lung function, prevailing under normal physiological conditions The difference in degree of severity and infectivity seen between the different countries and within certain states of a country, lead to the conclusion that there are different strains of the COVID 19 virus and that the virus suffered nearly 200 mutations so far. The worldwide distribution belonged to strain types A2 a, A3, B and BB4. The CCMB (Centre for cellular and molecular Biology), Hyderabad, India, found that a different 'cluster of strains’ , named as “Clade A3i", responsible for infection in different states in India, some of which registered more cases / mortality (Maharashtra, Gujarat , Tamil nadu and Delhi) than others. (Vide the pre-publication press note released on 3rd June 2020).

The host factors: The fight between the virus and the defence begins with the virus presenting

its conserved molecular signature expressed on its surface (PAMPs) which the defence cells recognize by special receptors located on their surface (PRRs)

PPRs and PAMPs: PPRs (**pattern recognizing receptors**) are proteins which recognize PAMPs (**pattern associated molecular patterns**), a conserved specific sequence which the pathogens display on their surface (epitopes) for the recognition by the **PRRs** of the effector immune cells The details of the PAMPs and PRRs in case of COVID 19 are still not fully elucidated. Some authors dealing with this aspect, quote the information available on the SARS CoV 1, which holds about 79% homology in antigenic structure with SARS CoV 2. The sofar known PRRs and PAMPs are listed in appendix 1, with the assumption that any of these might be found relevant to the context of Caved 19, by future research. The possible interactive pathway between the PAMPs and PRRs is depicted in Appendix 2.

The recognized viral surface antigens are presented by the antigen presenting cells (ARC) of the effect or immune cellular elements after being processed by MHC class 2 elements. Here also clarity as to details are lacking. This is followed by the humoral or cellular response by both innate and adaptive immunity systems, the sole purpose being destruction and elimination of the virus. Here also gaps in current knowledge is considered as hindrance to the understanding of actual happening in the context of COVID 19 pathogenicity. The virus probably and ingeniously turns the host's defense, against the host itself by various subversive actions, to far then their own survival and multiplication, resulting in immune mediated injury to host tissues The COVID 19 lung pathology should be considered in these lines, as a fallout of the aberrant immune response. The reported damage to the lungs is belied to start within a week or so of the patient becoming symptomatic, the role played by the innate immune mechanisms assume importance, as they first encounter the virus. It is only after a week or so, that the adaptive immune mechanisms come to fore. So, in the later course of the disease, both the immunity mechanisms may supplement or compliment or act independently to sustain the immunological damage. The humoral and cellular components of both system play important roles but some play greater role than the others. So the more important elements are discussed in detail while with due consideration to the subsidiary role played by the other elements, discussion is limited to a brief consideration about them. The discussion is in accordance with the importance of the role each element of both the systems. The discussion is limited only to the destructive immune mechanisms of both the immunity system and nothing will be referred to the protective role they play.

4.1 The Role of Innate Immune System

4.1.1 Role of complement: The alternative pathway:

Joshua M. Thurman and V. Michael Holers, et al. [16] has reviewed this matter:

1. The alternative pathway is capable of auto activation because of a process termed "tick over" of C3 [17].
2. Tick over generates a conformationally altered C3, designated $C_3(H_2O)$.
3. This is capable of binding, the factor B
4. Factor D cleaves Factor B into Ba and Bb.
5. Bb remains bound to $C_3(H_2O)$ to form $C_3(H_2O)Bb$.(fluid-phase C3-convertase).
6. This alternative pathway C3-convertase, although only produced in small amounts, can cleave multiple C3 proteins into C3a and C3b.
7. The complex is believed to be unstable until it binds properd in, a serum protein.
8. The addition of properd in forms the complex C3bBbP, a stable compound.
9. This binds an additional C3b to form alternative pathway C5-convertase.
10. The C5-convertase of the alternative pathway consists of $(C_3b)_2BbP$ (sometimes referred to as C_3b_2Bb).
11. After this step, the complement system follows the same path regardless of the means of activation (alternative, classical, or lectin).
12. C5-convertase cleaves C5 into C5a and C5b.
13. C5b binds sequentially to C6, C7, C8 and then to multiple molecules of C9 to form membrane attack complex. (MAC).

Regulatory proteins that disrupt the complement activation process: Since C3b is free and abundant in the plasma, it can bind to either a host cell or a pathogen surface antigen. Thus, the complement activation can kill a virus infected cell or even the healthy host cells. To prevent the host cell damage, there are several inbuilt inhibitory mechanisms operating under the

physiological conditions. Conversely the virus may subvert the very same mechanisms to sustain its own survival as seen in case of some the virus (vide infra).

4.1.2 Physiological inhibitors of the alternative complement cascade

- Complement Receptor 1 (CR1) or CD35) and DAF (decay accelerating factor also known as CD55) completes with Factor B in binding with C3b on the cell surface and can even remove Bb from an already formed C3bBb complex.
- Complement factor(CF), a plasma protease, prevents the formation of a C3 convertasewhich Cleaves C3b into its inactive form, iC3b. Factor I requires a C3b-binding protein cofactor such as complement factor H, CR1, or Membrane Cofactor of Proteolysis (MCP or CD46) [18].
- Complement Factor H can inhibit the formation of the C3 convertase by competing with factor B for binding to C3C3bl [19] accelerate the decay of the C3 convertase. [20] and act as a cofactor for Factor I-mediated cleavage of C3b [21]. Complement factor H preferentially binds to vertebrate cells (because of affinity for sialic acid residues), allowing preferential protection of host (as opposed to bacterial) cells from complement-mediated damage.
- CFHR5 (Complement Factor H-Related protein [22].

Immune evasion by the virus: The lipid bi-layer membrane of COVID 19 is cited as the cause of failure of host's immune system. Beyond this there is no data as to how actually SAzRScoV2 evades the immune system. Other others have narrowed the analogy with SARS CoV 1 and MERS CoV. The author cites few examples of other virus whose immune evading mechanism was studied and reported. The future research is expected to throw more light on this aspect.

4.1.3 Illustrations of how some virus subvert the defence against self cell destruction

1. Synthesis of new regulatory proteins

illustration: West Nile virus synthesizes two isoforms of NS1 protein to regulate C3. Soluble NS1 increases Factor I-mediated cleavage of C3b to iC3b while the cell surface-bound NS1 decreases deposition of C3b and MAC [23].

2. Incorporation of the host complement regulatory proteins to virions: To evade complement-mediated destruction , human immunodeficiency virus-1 (HIV-1), human T-lymphotropic virus-1 (HTLV-1) and human cytomegalovirus (HCMV) incorporate the complement controlled proteins CD55 and CD59 into their virions to circumvent the complement response [24,25].

3. Prevention of the cell lysis by inhibiting the remainder of the complement cascade

Illustration: HIV enters human CD4+ T cells through complement receptors. HIV gp41 and gp120 proteins activate complement through the classical and lectin pathways, respectively. At the same time, the above two proteins inhibit MAC formation by recruiting Factor H and CD59 to the surface of the virally-infected host cell to abolish complement-mediated lysis [26].

4. Modulate cytokine expression to induce a pro-coagulant state

Illustration: Mediated by IL-1(interleukin) , TNFα,(tumour necrosis factor) and IL-6, Marburg virus, Ebola virus and Hanta virus induce tissue factor expression on the endothelial surface [27,28].

5. Use coagulation factors to enhance viral binding and replication

Illustration: Human species a adenovirus-18 (HAdV-18) and 31(HAdV-31). It is known that the innate immune reaction recruits the alternate complement activation pathway and the adaptive immune response involves the classical complement activation pathway. There is a third, the lectin pathway. The alternate complementary pathway starts with cleaving of C3. The classical pathway and the lectin pathway both converge on production of C3 by a loop.

4.1.4 Complement mediated immune damage

4.1.4.1 Explanation for interstitial pneumonia in lung pathology

1. C3 is cleaved into C3b(catalytic fragment) and C3bi (non catalytic fragment). Further events may follow either of the following pathways.
2. **The C3i pathway:** C3bi is bound to CR 3 (CD11b/CD18), integrin αMβ2) receptor of the macrophage.
3. Affinity modulation of macrophage integrins is sufficient to allow binding of

opsonized particles, but increased diffusion to allow clustering is required in order to activate phagocytosis, which is facilitated by cytokines IL-4, M-CSF, TNF-α and GM-CSF.

C3b pathway: This catalytic fragments continues the cascade of alternate complement activation pathway with ultimate production of MAC Complex which cause the lysis and death of the infected cell including the virus.

The complement receptor activated stimulation will not elicited inflammatory reaction as it cannot recruit the pro-inflammatory cytokines and chemokines. It could be inferred from the polymorphism (see below) exhibited by the alveolar macrophages that the complement mediated perhaps stimulates the alternatively activated M2 type macrophages which are known to be anti inflammatory in response. This could come as handicap to the host because, M2 type stimulation also stimulates the extracellular matrix protein, collagen, which produces interstitial fibrosis as seen in the COVID 19 lung. Thus one of the pathological / radiological feature of interstitial pneumonia/ fibrosis is explained.

Secondly since this inhibits the expression of the inflammatory mediated M1 type macrophages. Absence of inflammatory stimulus may make the pt in whom this pathway acts asymptomatic but yet the lung damage in the form of interstitial fibrosis progresses. It is known that the symptoms of COVID 19 are due to inflammatory response mounted by the body of the host-patient.

Role of Tissue factor(TF)/(extrinsic) pathway in the pathogenesis of COVID 19:

- The increase PT (prothrombin time) and injury to subendothelial tissues by cytokine induced vascular damage implicate a role of this pathway in the coagulopathy seen in COVID 19. Tissue factor(extrinsic) path way:
- Tissue factor/platelet- thromboplastin/ factor3/ CD 135. It is produced by sub endothelial cells and leukocytes. Injury to sub endothelial cells/ platelets releases this factor which initiates the coagulation process. In combination with factor V11, it activates factor X to factor Xa. The (tissue factor pathway inhibitor (TFPI) is a protease present in the ECs inhibits the TF and is a natural anticoagulant.

TF, produced by sub-endothelial cells and leukocytesInjury to sub-endothelial cells/ platelets initiates the coagulation process. In combination with factor V11, it activates factor X to factor Xa. The (**tissue factor pathway inhibitor (TFPI)** is a protease present in the ECs inhibits the TF and is a natural anticoagulant. The TF pathway may be recruited by the cytokine induced damage of the vasculature orInjury to the platelets which consequently release the TF. This is supported by the immature megakaryocytes(indicating rapid turnover/distruction of the platlets) found in the microscopic findings of autopsy.

The role of MBL (mannose binding leptin) / Lectin pathway: The activation of complement via the mannan-binding lectin (MBL) pathway is initiated by the MBL complex consisting of the carbohydrate binding molecule, MBL, two associated serine proteases, MASP-1(mannose-associated serine protease) and MASP-2, and a third protein, MASP-19. When the carbohydrate-recognising heads of MBL bind to specifically arranged mannose residues on the surface of a pathogen, MASP-1 and MASP-2 are activated to cleave complement components C4 and C2 into C4a, C4b, C2a, and C2b. In f, two smaller MBL-associated proteins (MAPs) are found in complex with MBLC4b2a3b, the C3 esterase so formed carries onwards the complement cascade in the same way as the alternative pathway described in detail above. The involvement of the lectin pathology is supported by the finding reported by Cynthia Magro (2020)of deposits of terminal complement components C5b-9 (membrane attack complex), C4d, and mannose binding lectin (MBL)-associated serine protease (MASP)2, in the microvasculature, Leptin is a glycoprotein and the SARS CoV has glycoprotein antigens which may be recognized by the C - Lectin receptors (PPRs) present on the surface of the macrophages and other effector cells ,leading to their activation leptin can modulate the response to an inflammatory challenge by altering production of proinflammatory and anti-inflammatory cytokines and may also affect cytokine signalling by a variety of mechanisms, including induction of SOCS-3.(suppressor of cytokine signalling).

4.1.5 Explanation for vascular changes in lung pathology:

These can be explained by activation of the contact cascade or by cytokine induced damage.

- **Contact cascade**

1) Factor XII (FXII; Hageman factor) of the contact system is proteolyticallycleaved to FXIIa by negatively charged surfaces of damaged cells, and also activated platelet plasma membrane.
2) FXIIainitiates the coagulation cascade leading to clot formation.
3) cleaves prekallikrein to kallikreinfor subsequent release of bradykinin.
4) Through an endothelial G-coupled receptor (bradykinin receptor 1; BKR1), bradykinin induces vasodilation, neutrophil chemotaxis and vascular permeability [29].

- **The cytokine induced Vascular changes in the lungs:** Alexander H. Sprague and Raouf A. Khalil et al. [30] and. Marlies Van de, Wouwer Désiré Colle, Edward M. Conway metal [31] have extensively reviewed this subject matter. Interested readers may refer these articles for full details.

Cytokines are a diverse group of soluble short acting proteins, glycoproteins and peptides produced by various immune cells and vascular cells, activate specific receptors and modulate the functions of many cells and tissues.

- **Cytokines** include **tumour necrosis factors, interleukins, lymphokines, monokines, chemokines, interferons, colony stimulating factors, and transforming growth factors.**
- Cytokines are **produced by macrophages, T cells and monocytes, platelets**, **endothelial cells (ECs**) **and vascular smooth muscle cells (VSMCs)**.
- Cytokines elicit inflammatory response by interacting with specific receptors on various cell types and activate J**AK-STAT, (**Janus kinase/**s**ignal transducers and activators of transcription **NF-κB, and SMAD (**Suppressor of Mothers Against Decapentaplegic. Miscellaneous)**signalling pathway**s leading to **cell adhesion, permeability and apoptosis**
- Cytokines also **interact with mitochondria** to increase the **production of reactive oxygen species**.
- Cytokine-induced **activation** of these pathways in **ECs modifies the production/ activity of vasodilator mediators such nitric oxide, prostacyclin, endothelium-derived hyperpolarizing factor, bradykinin, vasocontractile mediators: endothelin , angiotensin II**.
- Cytokines interact with VSMCs(vascular smooth muscle cells) to activate Ca2+, protein kinase C, Rho-Kinase, and MAPK pathways, which promote cell growth and migration, and VSM reactivity. Cytokines are either proinflammatory or inflammatory.
- **Proinflammatory cytokines:** Produced by activated macrophages, mediate the following effects- Up regulation of **inflammatory reaction by** TNF-α, IL-1, IL-6, IL-12, IL-19, and IFN-β.- Stimulation of **acute phase reactants** TNF-α, IL-1, IL-6, IL-11, IFN-γ, TGF-β.- Chemoattractant such as IL-8, MIP-1α (Macrophage Inflammatory Proteins) ,MIP-1β, **RANTES**(regulated on activation, normal T cell expressed and secreted) PF-4, MCP-1, -2, -3(monocyte chemoattractant protein)
- **Anti-inflammatory cytokines** are involved in the down-regulation of inflammatory reactions.

They include IL-4, IL-10, IL-13, IFN-α, and TGF-β. (Transforming growth factor) The anti-inflammation induced vascular injury results from interaction of the inflammatory cells, the endothelial cells(EC), vascular smooth muscle cells(vsmc) and extra-cellular matrix (ECM).

- ECs are major determinant of vascular tone, leukocyte adhesion, and SMC proliferation. IL1activates T cells;IL-2, which stimulates proliferation of antigen-activated T and B cells; IL-4, IL-5, and IL-6, which stimulate proliferation and differentiation of B cells;
- IFNγ, which activates macrophages; and IL-3, IL-7 TNFα, IFN-γ, IL-8, and MCP-1 influenced tissue factors which initiated coagulation cascade and down-regulated anticoagulant thrombomodulin.
- TNF-α enhanced the endothelial cells to produce anti-fibrinolysis PAI-1 [32]. GM-CSF, which stimulate haematopoiesis. Platelet derived growth factors were the main inducers of MCP-1 gene [33].
- The activated platelets stimulated NF-κB in endothelial cells and enhanced the expression of leukocyte receptors which induced the secretion of MCP-1 and IL-8. Besides, the significant increased expression of lung PAR-1 on pulmonary cells, as fibroblasts, macrophages, epithelial and endothelial cells might

represent another cause for elevated MCP-1 (monocyte chemoattractant protein and IL-8 chemotaxis molecules [34].

Role of Endothelial cells: Under normal conditions, the endothelium maintains a vasodilator, antithrombotic and anti-inflammatory state. For vascular homeostasis, endothelial cells are of utmost importance and they produce a variety of mediators, surface proteins, and autocoids involved in vasomotion, coagulation, and inflammation [35]. Endothelium separates blood clotting factors from exposure to subendothelial prothrombotic extracellular matrix components. Endothelium also expresses vasoactive factors that modulate platelet reactivity, coagulation, fibrinolysis and vascular contractility, all of which contribute to thrombotic formation. Such factors include nitric oxide, prostacyclin, Von Willebrand factor (VWF), thrombomodulin, endothelin, etc. Endothelial cells counteract coagulation by providing tissue factor and thrombin inhibitors and receptors for protein C activation.

Role of TNF alfa and C3: When TNF-α is upregulated, it contributes to changes in coagulation and C3 induction [36]. TNF-α plays a pivotal role in the disruption of macrovascular and microvascular circulation both *in vivo* and *in vitro* [37] and is an important cytokine that can induce both apoptosis and inflammation [38]. In the presence of ROS, there is an increased production of TNF-α and, in turn, TNF-α signalling accentuates oxidative stress [39]. TNF-α up regulation is also associated with a changed coagulation propensity [40]. In short, TNF-α participates in vasodilatation and oedema formation, as well as leukocyte adhesion to the epithelium through expression of adhesion molecules. Furthermore, it regulates blood coagulation, contributes to oxidative stress at sites of inflammation, and indirectly induces fever [41]. TNF-α also plays a central role in the pathogenesis of insulin-resistant metabolic derangements. When TNF-α is upregulated, it contributes to changes in coagulation and C3 induction. TNF-α plays a pivotal role in the disruption of macro vascular and microvascular circulation both *in vivo* and *in vitro* and is an important cytokine that can induce both apoptosis and inflammation. In the presence of ROS, there is an increased production of TNF-α and, in turn, TNF-α signalling accentuates oxidative stress.

TNF-α upregulation is also associated with a changed coagulation propensity. In short, TNF-α participates in vasodilatation and oedema formation, as well as leukocyte adhesion to the epithelium through expression of adhesion molecules. Furthermore, it regulates blood coagulation, contributes to oxidative stress at sites of inflammation, and indirectly induces fever. TNF-α also plays a central role in the pathogenesis of insulin-resistant metabolic derangements. TNF can induce platelet consumption, and platelets do express TNFR1 and TNFR2 [42,43]. TNFR1 expressed on other cells also causes the release of factors with agonist activity for platelets and TNF-α is able to activate platelets through stimulation of the arachidonic acid pathway.

Complement c3: RBCs carry the complement receptor 1 (CR1), also known as C3b/C4b receptor or CD35, on its membrane [44]. Immune complexes, which have reacted with complement and bear C3b fragments also bind to the CR1 on human RBCs, and CR1 on RBCs serves as a transport system for immune complexes in the circulation to prevent immune complex deposition outside the fixed macrophage system [45,46]. Complement also interacts with the surface of activated platelets as well as with other components of the complement system including, C1q, C4, C3 and C9, which bind to activated platelets, [47]. Furthermore, thrombin-activated platelets can actually initiate the complement cascade, [48] and C3a and its derivative C3a-des-Arg, induce platelet activation and aggregation *in vitro* [49]. Platelets express complement receptors C3aR, CR4, as well as a receptor for iC3b and C5a, and the C1q receptors gC1qR and cC1qR on their membranes. cC1qR, in particular, was shown to mediate platelet aggregating and activating effects. Of importance is that platelets may also interact with the complement system *via* proteins that are not considered classical complement receptors, such as P-selectin [50] or GP1bα [51].

TM-PS-EPCR SYSTEM: Marlies Van de WouwDésiré Collen and Edward M. Conway et al extensively reviewed this aspect [52] to which interested readers may refer for more details.

Thrombin-mediates activation of protein C (PC), with Thrombomodulin (TM), acting as a co-faactor. Thrombomodulin, a cell surface-expressed glycoprotein, synthesized by vascular endothelial cells, is critical for PC activation y the thrombin–TM complex is further enhanced ≈20-

fold in vivo when PC is bound to the endothelial cell protein C receptor (EPCR). Platelet factor 4 (PF4) accelerates PC activation by inducing a conformational change that increases its affinity for thrombin–TM complex .Activated PC (APC) is a known natural anticoagulant. APC and EPCR have a role not only in coagulation but also in inflammation also APC suppress further thrombin formation by proteolytic inactivation of the coagulation factorsVa and VIIIa. Along with protein S (PS), APC may also increase fibrinolytic activity, by neutralizing plasminogen activator inhibitor 1 (PAI-1). This results in a hypercoagulable state. Pro-inflammatory cellular effects of coagulation proteases as well as the anti-inflammatory effects of APC/EPCR are mediated by signalling via protease activated receptors PAR on mononuclear cells, endothelial cells, platelets, fibroblast, and smooth muscle cells. The beneficial effects of APC in sepsis are mainly dependent on the PAR-mediated cell-protective properties rather than the anticoagulant protease function on coagulation cofactors FV/Va and FVIII/VIIIa. Protein C, which is activated by thrombin, complexes with endothelial protein C receptor and thrombomodulin and together with protein S forms the activated protein C complex that inactivates activated coagulation factors V and VIII. The receptor PAR-1 is differentially activated by thrombin and the activated protein C/EPCR complex, resulting in antithrombotic and anti-inflammatory effects. Thrombin and vasoactive agents release von Willebr and factor as ultra-large platelet-binding multimers, which are cleaved by ADAMTS13. Platelets can also facilitate leukocyte-endothelium interaction. Platelet activation is prevented by nitric oxide, prostacyclin and exonucleotidases. Thrombin-cleaved ADAMTS disintegration of platelet aggregates while tissue-type plasminogen activator initiates fibrinolysis. Fibrin and products of platelets and inflammatory cells modulate the angiogenic response of endothelial cells and contribute to tissue repair.

Endothelial PARs (Protease activated receptors) participate in the **regulation of vascular tone and permeability**. In endothelial cells, PARs play a key role in promotion vascular barrier function as they provide a positive signals for endothelial adhesion molecules (vascular cell adhesion molecule-1 (VCAM-1), **intercellular adhesion molecule-1(ICAM-1)**, and **E-selectin** [53]. PARs contribute to the pro-inflammatory response. For example **PAR4 induces leukocyte migration** and **PAR2** helps macrophages to **produce cytokines** such as interleukin-8 (IL-8). Activation of PARs alternatively lead to the transactivation of and **signalling** through receptors such as **co-localized PARs, ion channels,** and **toll-like receptors.**

4.1.6 Role of other elements of innate immunity

4.1.6.1 Role of the interferons

Interferons(**IFNs)** are a group of signalling proteins made and released by host cells in response to the presence of several viruses. **Type I interferons** (**IFN**-alpha and **IFN**-beta) are secreted by virus-infected **cells** while **type II,** immune or gamma **interferon** (**IFN**-gamma) is mainly secreted by components of both innate and adaptive immunity T **cells (of adaptive immunity)**, natural killer (NK) **cells** and macrophages (of innate immunity).

IFNγ, a cytokine which is **crucial for innate and adaptive immunity** against many pathogens is produced:

1. ***As a part of the innate immune response:*** Natural killer (NK) Natural killer T (NKT) cells Mucosal epithelial cells, Macrophages Innate lymphoid cells(ILC) produce it .
2. ***As a part of adaptive immunity*:** CD4 Th1 cells, CD8 cytotoxic T lymphocyte (CTL) produce it.

4.1.7 Functions it serves

- Macrophage activation Increases their antigen presentation and lysosome activity.
- Increased expression of class I and class II MHC molecules.
- Increased expression of APCs (antigen-presenting cells) through induction of antigen processing genes, including subunits of the immunoproteasome (MECL1, LMP2, LMP7), TAP and ERAAP and direct upregulation of MHC heavy chains and B2-microglobulin itself.
- Role in macrophagepolymorphism: M1 macrophages are stimulated by interferon (IFN)-γ which secrete proinflammatory cytokines (like TNF-α, IL-1β, IL-12, IL-18)

and the chemokines. (like CCL15, CCL20, CXCL8-11 and CXCL13).

- Antiviral (replicatory), immuno-regulatory, and anti-tumour properties [19].
- Aberrant IFNγ expression causes number or autoimmune diseases.
- Activates inducible nitric oxide synthase (iNOS).
- Induces production of IgG2a and IgG3 from activated plasma B cell.
- Promotes adhesion and binding required for leukocyte migration.
- Primes alveolar macrophages against secondary bacterial infections.

How IFNγ exerts its Cellular responses?

1. It interacts with interferon gamma receptor 1 (IFNGR1) and Interferon gamma receptor 2(IFNGR2).
2. Binding activates JAK-STAT pathway.
3. IFNγ also binds to the glycosaminogly can heparan sulphate (HS) and inhibits its biological activity.
4. Promotes NK cell activity.

IFN-α has a general inflammatory action which skews the immune response towards a Th1 profile, Which leads to induction of classically activated M1 macrophages.(see Macrophage polymorphism below)

1. IFN α Functions

1. IFN-α8 enhances the proliferation of human B cells, and activates NK cells. The subtypes α10 and α2, and α8, are the most efficient NK cell activators.
2. Subtypes α21 and α2 enhance the expression of IFN-gamma inducible protein-10 (IP10),a chemokine, that promotes Th1 inflammatory response. in dendritic cells.
3. IFN-α1 causes increased HLA-II expression and can directly inhibit tumour cell growth *in vitro*.
4. Subtype α2 increases the expression of HLA-I molecules, which correlates with IFN-α-mediated activation of memory CD8 cells and increased catalytic action against virally infected cells and tumour cells (via cytotoxic CD8 cells).

Interferon beta: It is released at the end of an immune attack, blocks the action of gamma **interferon** and helps to reduce inflammation and the body's immune reaction.

Role of other important innate cellular elements:

These are presented in Appendix 4

❖ **Role of adoptive immunity in Covid lung pathology:**

CD8+ T cells and CD 4+ at cells: The autopsy findings considered above, showed that CD8+and CD4+ cells in the lung tissue interstetium. CD 8+ CD8+ T cells directly kill the virus upon stimulation. CD 4+ cells helps to secrete antibodies which have diverse actions.

Ali Ganji et al. [53] have shown that CD8 MFI increased significantly in COVID-19 infected patients ($P < 0.05$), implying increased expression of CD 8+ T cells.

Zheng et al. [54] found that the total numbers of T cells, NK cells and CTLs were reduced in all patients, with severe cases of Covid 19 having significantly lower proportions than those seen in mild cases. CD8+ T and NK cells from COVID-19 patients had increased expression of the inhibitory receptor NKG2A. Furthermore, cells expressing NKG2A had diminished production of CD107a, IFN-γ, IL-2, TNF-α and granzyme B. These findings suggest functional exhaustion of NK and CD8+ T cells and inhibition of antiviral immunity during SARS-CoV-2 infection. It was opined that down regulation of NKG2A may be crucial for disease control.

- **Helper T cells:** Type 1 helper (TH1), cells produce interleukin (IL)-2, gamma-interferon (IFN-gamma) and tumour necrosis factor-beta, (pro-inflammatory). Th1 activate classically activated M1 macrophages. Cells are cytotoxic and hence kill the virus laden cells, when activated. The zCD4+cells helps to stimulate B-cell function.

Type 2 helper (TH2) cells express IL-4, IL-5, IL-6 and IL-10 (anti-inflammatory).

TH2 cells activate alternately activate M2 macrophages.

Role of complement in adaptive immunity: The classical complement pathway is activated by the virus in adaptive immune cells .It takes part in **MAC induced** cell lysis by itself. Also it acts through potentiation loop in producing 3Cb and classical 3C esterase. Its role in complement mediated phagocytosis and complement mediated cytotoxicity, as already seen above.

The central Role of Alveolar Macrophages (AM):

- **Under physiological condition**s the expression of the phagocytic receptor Macrophage 1 antigen **(Mac-1)** keeps the **AMs down-regulated,** to **prevent damage of body tissues** from the activated AMs.
- The adaptive immunity is suppressed through AM's effects on interstitial dendritic cells, B-cells and T-cells.
- The macrophage performs the **phagocytic function,** both **IgG mediated(through Fcy .receptor**)and complement mediated,(**through CR1,CR2 and CR3 receptors) .**
- Can **cause tissue damage** through **PR inflammatory cytokin**es (**mediated by M1 macroph**ages) can exert **anti inflammatory effect** with **anti-inflammatory cytokine**s as well as healing of damaged tissues by fibrosis (through M2 type macrophages.)
- Play role as **antigen presentation cells** (ARC) and **recognizes PAMPs** of the virus with the PRR,on its surface, in conjunction **with MHC class 2 molecules .**
- It controls through, **INOS**(inducible nitric oxide synthase), the **differentiation and maturatio**n of **dendritic cells** through. **Inhibition** of the granulocyte-macrophage colony-stimulating factor (**GM-CSF**)and **TNF-alpha-**mediated mechanisms.
- Through **il 4 and iL10**, it causes the **reduced** production of **metallo-proteinases** (endopeptidases which break down collagen and other extracellular proteins) by human AMs.
- Causes differentiation of naïve CD4-T cells into mature Th2 type cells.
- Il4 **enhances MHC class II antigen** and **Mac-1**(surface receptor as part of innate complement system) expression, thus promoting phagocytosis.
- **Il10 inhibits** the secretion of **pro-inflammatory cytokine**s **TNF-alpha and INF-gamma,** thus **suppressing the proliferation of T-cells, NK cells, and AM**.
- By similar immunomodulation mechanisms to TGF-β. IL-10**reduces the rate of apoptosis**. Indirectly enhancing alveolar macrophage-mediated inhibition of T-cell proliferation. Alveolar macrophages induce expression of the αvβ6 integrins, the cell-surface receptors, activate TGF-β.
- **TGFβ tightly regulates anti-inflammatory activity** by suppressing pro-inflammatory cytokine production, thereby **inhibiting T-lymphocyte function.**
- This **induces a downstream signalling** cascade leading to transcription factors, regulating the **expression of TGF-β target genes**
- **Important inhibitor receptors of AMs include TIM-3, PD-1, CD32b, and CD200R.**

The pathways by which macrophages are activated and deactivated are shown in Table 1 below.

Macrophage polymorphism: The alveolar macrophages (AM) exist as two phenotypes, M1 and M2 macrophages, which have different means of stimulation, express different kinds of cytokines and chemokines and have opposite physiological actions. The M1 macrophages are PR inflammatory and M2 are not only anti inflammatory in function, but also have action on extracellular matrix promoting healing by stimulating collagen fibres, leading to fibrosis. M2 macrophages have 3, M2b and M2c. The salient points of both the phenotypes and the sub-types are shown in Table 3.

Table 1. Activation of AM- sequence of events

1. Binding of PAMPs to TLRs.(PRPs)
2. Actin polymerization (in alveolar macrophages).
3. Suppression of integrin expression
4. Deactivation of TGF-β and the down regulation of the basal phosphorylation level of SMAD 2/3;
5. Activation and detachment of alveolar macrophages from the alveolar epithelial cells
6. Macrophages become primed (by IFN-γ and TNF-α)
7. Phagocytosis and secretion of proinflammatory cytokines (TNF-α and IL-6)
8. The ROS (reactive oxygen species) produced by respiratory burst.
9. Oxidative damage to lung tissue.
10. Positive feedback effect -Enhancement of production of TNF-α by macrophages (step 7).

Table 2. Deactivation of AMs - sequence of events

1. Secretion of IFNγ by activated lymphocytes.
2. Stimulation of the production of matrix metalloproteinase MMP-9 by macrophages.
3. MMP-9 activates latent TGF-β.
4. Reduced expression of αvβ6 integrins on alveolar epithelial cells.
5. Return of the alveolar macrophage to a resting state

** AMs have been reported to produce MMP-9 partly via PGE2-dependent PKA signaling pathways, which are the pathways involved in the inhibition of phagocytosis.*

***Activation of TGF-β is also advantageous because its production stimulates collagen synthesis in interstitial fibroblasts, which is necessary for restoring alveolar wall architecture.*

Table 3. Different polymorphic types of macrophage

Type of macrophage	Stimulated by	Express	Functions
M1 macrophages (Classically activated)	1.LPS 2. Th1 cytokines (IFN-γ, IL-2, IL-12, IL-18 and TNF-β) 3.GM- CGO	(TNF-) α, IL-1, IL-6, and IL-12, and type I interferons (IFN) Th1 cell-attracting chemokines (CCL5 CXCL9 and CXCL10) ((RANTES)	Pro-inflammatory
M2 Macrophages (alternatively activated macrophages (AAMs)	1.Th2 cytokines including (IL-4, IL-5, IL-6, and IL-10) 2. M-CSF	1. Scavenging receptors 2. Mannose and galactose receptors. 3. Secrete high amount of IL-10 4. Express higher levels of the IL-1 decoy receptor and IL-1RA 5. Express the chemokines CCL17, CCL22 and CCL24	1. Anti-inflammatory action 2. Higher phagocytic activity 3.Promotetissue remodelling, 4.Vasculogenesis 5. Tumour progression
M2 a Macrophages	IL-4 and IL-13	1. Upregulate expression of Arginase-1, mannose receptor MRc1 (CD206), 2. Antigen presentation by MHC II systemof IL-10 and TGF-β.	Anti-inflammatory
M2 b Macrophages	immune complexes LPS	IL-1, IL-6, IL-10, TNF-α	Anti-inflammatory
M2 c Macrophages	IL-10, Transforming growth factor beta (TGF-β) and glucocorticoids,	IL-10 and TGFβ	Suppression of inflammatory response

5. SUMMARY

The elusive pathology of COVID 19 lung disease is dueto its protean and varied manifestations. Thus It might resemble from **consolidation** /**pulmonary oedema/ ARDS** or **cytokinestorm** ;**yetit is distinct** from each of these specific syndromes. In fact **COVID lung pathology resemblesa mixture of all theseindividual entities**.Alveolitis due to inflammatory cytokines explains the consolidation finding. Damage to capillaries with consequent seepage of translate explains the pulmonary oedema like component. This in combination with interstitial fibrosis formed due to M2 macrophage 2, with resultant hypoxia might explain the ARDS-like picture. The terminal events of disseminated intravascular coagulation (DIC) and multi organ dysfunction

(MOD), which is a common pathway for severe sepsis or cytokine storm like picture seen in a few COVID 19 patients which contributed to the overall mortality due to COVID 19.The failure to pin point to single pathology, perhaps is due to this fact. Further the simultaneous occurrence of thrombotic and haemorrhage, seen in COVID lung, suggests a range of diseases such as **activation of simple coagulation cascadeand subsequent fibrinolysis**, **small vessel vasculitis** to **thrombotic thrombocytopenia**. Simple coagulation cascade activation/ fibrinolysis, cannot explain either the presence of immune cells like CD8+ and CD4+ cells nor the consolidation/fibrosis component. Small **vessel vasculitis**(capillaritis) / **luecocytoclastic** / **hypersensitive angiitis** is a systemic disease and the specific histological features like **leukocyteclasia** (a process in which the neutrophils are destroyed leading to debris collection) is not demonstrated, in the autopsy findings of COVID 19. Further it is either due to drug induced allergy or idiopathic. Thethhrombotic phenomena are not a feature of small vessel vasculitis. The acute nature and non involvement of medium sized blood vessels and absent fibroid necrosis are points **Wagnersgranulomatosis a non possibility**. The absence of haemoptysis even though, patchyhaemorrhages are seen on gross as well as microscopic autopsy findings indicates that the bleeding is not substantially enough to give rise to clinically haemoptysis which distinguishes COVID lung disease from **Good.**

Pasture's syndrome the haemorrhagic part is perhaps due to purpura as supported by low platelet counts and presence of atypical megakaryocytes seen in the autopsy findings. This takes us to the possibility of **thrombotic thrombocytopenia or coagulative microangiopathy which is a systemic syndrome** unlike the localised pathology as seen in COVID 19. However all coagulative microangiopathy is described in literature in case of fall bladder, but not in case of Lung. If it is true, COVID 19 is the first ever disease to cause such "**localised coagulative microangiopathy**. **Disseminated intravascular coagulation** is a serious condition that may explain both the observed pathological entities, but its protean manifestation are not discernible in routine cases except the seriously terminally ill patients distained for MOD(multiorgan dysfunction). The elusiveness of COVID 19 lung pathogenesis could be due the culmination of more than one pathogenic pathway. The different pathways explaining the interstitial fibrosis and vascular changes already seen bear testimony to this fact. While the effector cells (macrophages, and other phagocytes, Nkcells, NAK cells and dendritic cells etc)do deliver the final blow, nevertheless the role played by other elements of both innate as well as acquired immunity(like antibody classes, opsonins and complement etc) cannot be ignored. It must be emphasized that immunity, innate or acquired can be no less damaging than the protection they offer against the invading pathogens. The system has inbuilt checks and controls, so that the destructive machinery is not directed against the healthy host cells, but are specifically directed against the pathogen or pathogen infected cell.The ingenuity of the pathogen to sabotage these inbuilt safeguards in immune system to farthen its interests, in which process, the fallout of deranged defence mechanisms, trains its guns against host's own tissues, as already discussed above. Thus, foundation for immune mediated injury, in COVID 19 induced lung damage is laid on a firm footing.

6. CONCLUSION

An integrated approach, taking into consideration, all the available clinical, laboratory, radiological and autopsy data lead to arriving at a possible mechanism underlying the lung damage in COVID 19.The pathway of complement activation, contact cascade, the role of the cytokines and the role of various elements of the innate and adaptive immunity are reviewed. The central role played by the macrophage polymorphism, in the pathogenesis and pathology of the COVID19 is stressed. The relevance of ARDS cytokine storm, the small vessel vasculitis, coagulative microangiopathy, disseminated intravascular coagulation and multi organ disfunction in relation to COVID 19 pathology are discussed. It is also shown that no single mechanism could explain the whole gamut of the pathology and pathogenesis of COVID 19 lung damage. The immune mediated damage seems to be more relevant ,rather than the infectivity of the virus. It is obvious that no single entity could explain the inflamatory,fibrotic and vascular features observed in the lung of COVID 19 patients, it seems to be due to a mixture of different overlapping immune mechanisms, resulting in evolution of the unique pathogenic mechanism of COVID 19. It is perhaps justifiable, from the author’s point of view to call the COVID 19 related lung damage as “**Acute immune mediated Lung injury (AILI).**

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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APPENDIX 1 MICROBIAL PATTERN RECOGNITION PROTEINS

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1. **Mannose receptor.**
2. **complement receptors.**
3. **DC-SIGN.**
4. **Toll-like receptors(TLRs).**
5. **Scavenger receptors CD14, and Mac-1**

The PRRs are divided into four families:

1. **Toll-like receptors (TLR)**
2. **Nucleotide-binding oligomerization domain-like receptors (NLR)**
3. **RIG-1 like receptors (RLR)-**
4. **C-type lectin receptors (CLR)**

PAMPs

1. **Glycans**

Lipoglycans such as lipopolysaccharide, a component of the gram- bacteria outer membrane
Peptidoglycans such as bacterial muramyl dipeptide

b-1,3-glucans from the cell wall of various fungi species

2. **Proteins**

bacteria flagellin

APPENDIX 2

Interaction of PRRs and PAMPs:

Step 1.

Ligand recognition/ binding:
PAMPs are recognized and are attached to PRRs.

Step 2. Activation of the kinases and antiviral signalling cascades:

1. TBK1

(TANK-binding kinase 1) 2. MAPK
(Mitogen-activated protein kinases)
3. IKKα and IKKβ.
κB kinase α and β

Step 3.

these kinases phosphorylate and activate
interferon(IFN)-regulatory factors 3 and 7 (IRF3/7), AP-1, NF-κB,
Step 4.These proteins transcriptionally induce the gene expression of
1.type-I IFNs (mainly IFN-α subtypes and IFN-β),

Appendix 3. Laboratory tests reported in COVID 19. Their significance

Laboratory test	Significance
Increased	
ESR	Ac. phase reactants/markers of ac inflammation
CRP (C-reactive protein)	
Ferritin	
LDH. (lactic dehydrogenase)	Liver cell dysfunction
AST(Aspartate transaminase)	
Fibrinogen	
Prothrombin	
hs-cTnT(high sensitivity cardiac troponin)	Maker of cardiocyte damage. Ac MI, myocarditis
PT(Prothrombine time)	Extrinsic coagulation pathway involvement.
(both indicate clotting disorder causing bleed)	
BU/BUN (blood urea nitrogen)	Renal function impairment.
Creatinine	
T8+/T4+ cells	Immune reaction.
Leucopenia /Lymphopenia	Bone marrow insult
Thrombocytopenia	Purpura
Neutrophilia	Secondary infection or complication
Leucoerythroblasticpicture.	Bone marrow involvement in COVID 19.

Appendix 4

Role of other important innate cellular elements

1. Natural killer cells:

They are the counterparts of cells of adaptive immunity.
Functions: They can directly kill the pathogen or through the antibody mediated cell mediated cytotoxicity.

NK cell dependent antibody induced cytotoxicity:

1. NK cell expresses Fcγ receptors - CD16 or FcγRIII.
2. These receptors recognize and bind to the reciprocal portion of antibody, (such as IgG,) which binds to the surface of a pathogen-infected target cell.
3. The NK cell releases cytotoxic factors that cause the death of the target cell through perforin - granzyme pathway.

2. MAIT cells *(Mucosal associated invariant T cells)*

A subset of T cells they display innate, effector-like qualities. AIT cells secrete pro-inflammatory cytokines and alsolyse bacterially-infected cells. Supports the adaptive immune response . They have memory like phenotype.

3. Natural killer T (NKT) cells

A group of T cells that share properties of both T cells and natural killer cells.

. Recognize foreign lipids and glycolipid antigens.

4. Gammadelta T cells (γδ T cells) Subset of T cells -express a unique T-cell receptor (TCR)

-initiation and propagation of immune responses

5. **Innate lymphoid cells (ILCs)**

1. LCs contribute to immunity via
a. secretion of signalling molecules, b. regulation of both innate and adaptive immune cells
2) mucus production in the respiratory tract.
3) Restoration and maintenance of epithelial integrity.
4) secrete IFN-γ in response to viral infection in the lungs .

6. Dendritic cells:

These are professional antigen processing cells. Present antigens to T cells.
MHC class II molecules thereby are critical for the initiation of the antigen-specific immune response.2.

Role of Nab (natural antibodies)

1. Opsonisation
2. Activation of the Complement.

MHC class II molecules: The main function of major histocompatibility complex (MHC) class II molecules is to present processed antigens, which are derived primarily from exogenous sources, to CD4(+)TIymphocytes.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/58422