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Cytokine Storm in COVID-19

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Abstract: ***COVID-19 pandemic has resulted a reincarnation of the term 'Cytokine storm' The term is mostly used with another term called 'Sepsis'. But the current pandemic has taught us of a 'cytokine storm' much different from 'sepsis'. Though the term 'sepsis' is often used as a broad syndrome, it should be split into smaller and more homogeneous biological groups. Similarly, this theory is applicable for cytokine storm. The present article is a search for the cause of cytokine storm in COVID-19, the analysis of different pathways leading to cytokine storm and finding those candidates at risk of cytokine storm.***

Keywords: COVID-19, Cytokine storm, Sepsis, Hemophagocytic Lymphohistiocytosis, HLH, Macrophage Activation Syndrome, MAS

1. Introduction

COVID-19 pandemic has resulted a reincarnation of the term 'Cytokine storm'. The term was first used in 1993 in relation to Graft versus Host Disease [1, 2]. The term again reappeared in 2005 during Avian H5N1 Influenza virus infection and the recognition of this condition was more since then [3]. The term is mostly used with another term called 'Sepsis'. But the current pandemic has taught us of a 'cytokine storm' much different from 'sepsis'. Though the term 'sepsis' is often used as a broad syndrome, it should be split into smaller and more homogeneous biological groups [4]. Similarly, this theory is applicable for cytokine storm. The present article is a search for the cause of cytokine storm in COVID-19, the analysis of different pathways leading to cytokine storm and finding those candidates at risk of cytokine storm.

2. Cytokine Storm

The cytokine storm is related to excessive presence of cytokines inside the body in response to some pathological insults. The excess cytokines cause damage to the host cells and drive the inflammation out of control. In COVID-19 pneumonia, the aberrant release of pro-inflammatory cytokines lead to lung injury by damaging the epithelial and endothelial barriers, microvascular leakage, alveolar edema and hypoxia [5]. The uncontrolled release of pro-inflammatory factors like IL-6, IL-8, IL-1Beta, GM-CSF, Chemokines like CCL-2, CCL-5, CCL-3, together with Reactive Oxygen Species (ROS) cause ARDS leading to pulmonary fibrosis and death [6]. Once this cytokine storm has started it becomes very difficult to arrest. This is often due to a poor understanding of the underlying pathogenesis which leads to cytokine storm. Often we concentrate more on antibiotics as judging it as sepsis ! But cytokine storm is not equivalent to sepsis and sepsis is not always equivalent to infection. There are some 'sepsis mimicking conditions'.

Hemophagocytic Lymphohistiocytosis (HLH)
In 1984 Risdall reported these conditions which often mimic sepsis [4]. But for decades, and probably till today, this was believed to be extremely rare. Though the current literatures demonstrated a significant portion of sepsis patients are probably suffering from HLH. But before going to the prevalence let's see what is HLH?

What is HLH?
It is a clinical syndrome resulting from immunological hyperactivation of macrophages.. It causes release of various pro-inflammatory cytokines like IL-1, IL-6, IL-8 and more [4]. The histological feature of this condition is activated macrophages phagocyting other immune cells. The condition can be triggered by an infection, malignancy or autoimmune disease. In fact it was first detected in children with autoimmune conditions and also known as Macrophage Activation Syndrome (MAS). So the term HLH and MAS are often used interchangeably. The clinical features of HLH are often very similar to sepsis

The clinical features of HLH [4]
1) Fever
2) Shock, capillary leak syndrome, ARDS
3) Cytopenias
4) Abnormal liver function test , high triglyceride level
5) Disseminate Intravascular Coagulation (DIC)
6) Delirium, seizure
7) Lymphadenopathy, hepato-splenomegaly
8) Very high Ferritin, CRP, Procalcitonin,
9) Multi Organ Failure (MOF)

Differentiating Sepsis vs HLH is a difficult job. Both conditions may be present in same patient or HLH may present as a severe form of sepsis with cytokine storm.

How common is HLH?
Autopsy studies suggest this may be a common condition often missed clinically. Strauss et al, in 2004, found this condition in 35 out of 107 patients- dying in ICU with sepsis, coagulopathy and deranged liver functions [4]. Inai et al in 2014 found HLH in bone marrow of 13 patients out of 18 sepsis patients who died. 4 prospective studies have specifically looked for HLH in the context of sepsis. Francois and Stephan in 1997 evaluated bone marrow on sepsis patients and found 60% of patients had evidence of hemophagocytosis with elevated blood Ferritin levels. Kuwata in 2006 evaluated patients with sepsis for HLH using peripheral blood smear and special stains. They found 40 of the 322 patients had evidence of HLH with increasing mortality in that subgroup and elevated IL-6 levels. 73% of these patients had pneumonia and cause of HLH here was infection. Bentel et al, found this condition in 9 out of 25 patients with H1N1 Influenza in 2011 [4]. In centers with greater awareness of HLH these cases are detected more. So

HLH can be found in considerable subset of sepsis patients who have a very high mortality.

HLH in COVID-19

HLH or MAS has been previously described in SARS-CoV epidemic [7,8]. Similarly in COVID-19 such mechanisms are being hypothesized to exist. Though there was no large scale trials to evaluate the validity of this hypothesis, few autopsy studies initially suggested existance of these condition. In one such study, the researchers observed lymph nodes, spleen and liver of 4 patients who died of cytokine storm [9]. They isolated 2 patients with probable HLH, based on lymphophagocytosis, out of these 4 patients. Other parameters like high Ferritin, increased cytokine profile matching HLH and response to immunomodulator in subset of severe COVID patients, are indirect evidences of presence of HLH. The type of HLH observed in SARS-CoV was designed as secondary HLH or sHLH. The primary HLH or pHLH is an autosomal recessive condition, presenting in children and is typically due to mutations that impair NK cell or Cytotoxic T cell functions [4]. The NK cell and CD8+ T cell mediated killing of the pathogen is hampered due to ineffectiveness of Perforins and granzymes of the host cells [10]. These Perforins and granzymes are secreted by NK cells or CD8 + T cells to kill the pathogen. This leads to more recruitment of T cells with more cytokines and activation of macrophages precipitating MAS or HLH. This condition is often defined as hyper-inflammatory immuno-deficiency state. Though it may sound like oxymoron, in COVID-19, such hyperinflammatory immunodeficient status may exist together.

In COVID-19 pneumonia there is often lymphopenia with robust Interferon suppression. Few data also suggested that severity of COVID-19 may be associated with low IFN Gamma production by CD4+T cells [11]. The SARS-CoV ORF and N proteins have previously shown that they act as antagonist to Interferon pathway [12]. This leads to more T cell recruitment. In addition the NK cell function is defective in COVID-19 [13]. All these are features of pHLH. This loss of first line antiviral defense may activate a second wave of more tissue aggressive immunity with exaggerated IL-6 production along with other cytokines. This often kills the viruses but also destroys the host cells as innocent bystanders. This is called the secondary HLH or sHLH in COVID-19. The distinction between these Primary and secondary HLH is often clinically very difficult, but distinction is required for therapeutic interventions. The main difference between these Primary and Secondary HLH is that the former is associated with viral load and virus induced immune deficiency, while the later is just the immune overshoot. The other differences are as follows :

Table: Differentiating between pHLH and sHLH

Therapy considerations based on COVID-19 MAS-like picture.

	Primary HLH features	Secondary HLH pattern
Immune state	Viral induced immunodeficiency	Normal Immune response/ Hypersensitivity
Viral Load	Persistent Viral Shedding more likely	Initial Viraemia VViral shedding expected to disappear with vigorous T-cells responses to COVID-19 infected cells.
Progression rate	Unclear	Unclear Rapid deterioration 2nd week?
Resolution rate	Slower resolution	May quickly improve on viral load elimination?
CRP	Elevated + +	Elevated + + +
Ferritin	Elevated + +	Elevated + + (possible rapid rise)
Anti-viral therapy	Consider Anti-viral therapy	?
Corticosteroids	Caution (increase viraemia)	consider if viraemia cleared/clearing
Anti-IL-6R and biologics	Less likely to benefit (may cause harm)	More likely benefit
Frequency	Very common	Less common

Reference

IL-6, HLH and COVID-19

Like the primary and secondary status of HLH, confusion is also there with IL-6. It is presently unclear that whether IL-6 levels in COVID-19 is detrimental or beneficial? IL-6 plays an important role in lung repair mechanisms. IL-6 can either suppress or facilitate viral replication [14]. So the timing of administration of IL-6 Receptor Antagonist or anti IL-6 can impact both tissue remodeling or viral replication. It emerges that blocking IL-6 early in the course of COVID-19 with pHLH like state may be detrimental, whereas in sHLH state it may be beneficial. The similar assumption is applicable for steroid.

Atypical HLH

The MAS/ HLH that supervenes in COVID-19 is mostly anatomically compartmentalized in lungs. The other systemic features of HLH are lacking in COVID-19 pneumonia in most of the cases [7]. The Macrophage activation and the coagulopathy are more concentrated in thoracic cavity than being more systematic. So the HLH in COVID-19 is basically a lung centric HLH. The cause is still not known, but more abundant distribution of ACE2 receptors in lungs, which act as entry point receptors for this virus, may be a possible cause.

Viral load, Cytokine storm

The next important question is what determines the occurrence of pHLH or sHLH and subsequent cytokine storm in a COVID patients? Though genetic predisposition with single nucleotide polymorphism in Cytotoxic T lymphocytic pathway is important, the Viral load is another important factor in few of the patients. The rapid and profound viral replication and subsequent cell apoptosis may cause rapid release of cytokines. This phenomenon is called ‘cell pyroptosis’, a pro-inflammatory form of cell apoptosis. The initial studies in large Cohorts of 1145 patients have shown the COVID-19 viral load at the time of presentation is an independent predictor of mortality [15]. Studies in Nanchang, China [16,17] also showed hospitalized patients with severe disease tend to have a high viral load and longer

viral shedding compared to milder patients. The high viral load tends to cause an immunosuppression and pHLH and subsequent T cell trigger also precipitate a sHLH with accompanied cytokine storm. Though all patients with higher viral load are not a candidate for cytokine storm. A recent study have shown that non hospitalised patients had a higher viral load than hospitalized patients and viral load was inversely correlated with disease severity [16]. Though the cohort of the study group was mainly the non hospitalized patients and such retrospective analysis may cause a selection bias. But it is proven now that all patients with high viral loads don't land up in cytokine storm, though patients with cytokine storm initially tend to have high viral loads. So apart from the viral load some other factors like oxidative stress and physiologic reserve in the individual will predict a possible cytokine storm in COVID-19.

Antibody response and cytokine storm

The Antibody response after COVID-19 is not uniform. Patients with milder disease often have poor antibody response and antibody response fades more early in mild or asymptomatic patients, while severe patients mount a stronger antibody response [18]. The appearance of IgM and IgG also occur simultaneously as seen in several studies. The previous SARS-COV in 2002 had shown the role of antibodies against Spike protein (anti S IgG) in promoting the accumulation of of pro-inflammatory monocytes and macrophages in lungs and development of antibody mediated enhanced lung damages [19]. But such evidences are lacking for COVID-19 till date.

The dots

1) Some patients of COVID-19 develop cytokine storm
2) This cytokine storm is not like that seen in sepsis and few patients may also have no relationship between D-Dimer and Ferritin levels.
3) The immunomodulation with IL-6 does not produce benefits in all patients
4) The elderly patients have more chances of cytokine storm and death
5) Younger patients sometimes also land up in cytokine storm
6) The high viral load is often found in severe patients
7) Though high viral loads don't dictate the chances of occurrence of cytokine storm
8) The severe patients often have stronger antibody response than mild patients.

Joining the dots

The underlying mechanism of cytokine storm seen in COVID-19 is HLH/MAS or more specifically lung centric HLH/MAS. The COVID-19 patients show both features of pHLH and sHLH. The high viral load will predict the occurrence of HLH. But all patients with high viral load will not have HLH unless they suffer from increased oxidative stress or genetic polymorphism in Cytotoxic T cell functions. The timing of immunomodulators like steroid or more specifically IL-6 is important. They are useful in sHLH phase and may be detrimental in pHLH phase. Though the clinical distinction between these two phases are not still clear, but increased persistent viral load in RT-PCR or persistent viremia points to pHLH phase. A thorough workup for HLH is mandatory in case of cytokine storm in COVID-19 with rising CRP, Ferritin, Triglyceride and lymphopenia, especially. Those patients having a more D-Dimer response than Ferritin may not be still in HLH phase, but having more damage to pulmonary capillary endothelium and increased coagulopathy. They will be benefitted with Anticoagulation. Whether stronger antibody responses are associated with severe disease or not will also need an observation. So severity in COVID-19 is dictated not by a single factor, it is due to multiple factors like- viral load, HLH, pulmonary endothelial damage, oxidative stress, antibody response, genetic polymorphism and still few probably unknown.

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