Minireview

Is COVID-19-associated cytokine storm distinct from non-COVID-19 secondary hemophagocytic lymphohistiocytosis?

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Impact Statement

COVID-19 or SARS-CoV-2 infection can lead to severe acute respiratory distress syndrome/pneumonia with features of cytokine storm/cytokine release syndrome (CS/CRS) reminiscent of secondary hemophagocytic lymphohistiocytosis (HLH). Treatment of severely ill patients has included anti-inflammatory drugs targeting various cytokines. However, these cytokines are also essential in eliminating SARS-CoV-2, and blocking cytokine pathways could potentially lead to worse outcomes. Here, we review differences in the immunobiology between non-COVID-19 HLH and COVID-19 CS/CRS, as well as highlight rare cases of COVID-19 actually meeting criteria for HLH. We review recent data supporting the immunologic and genetic distinctiveness of COVID-19 CS/CRS and COVID-19 HLH, which may have implications as to which patients with these disorders respond to anticytokine therapies.

Abstract

Cytokine storm is an umbrella term that describes an inflammatory syndrome characterized by elevated levels of circulating cytokines and hyperactivation of innate and/or adaptive immune cells. One type of cytokine storm is hemophagocytic lymphohistiocytosis (HLH), which can be either primary or secondary. Severe COVID-19-associated pneumonia and acute respiratory distress syndrome (ARDS) can also lead to cytokine storm/cytokine release syndrome (CS/CRS) and, more rarely, meet criteria for the diagnosis of secondary HLH. Here, we review the immunobiology of primary and secondary HLH and examine whether COVID-19-associated CS/CRS can be discriminated from non-COVID-19 secondary HLH. Finally, we review differences in immunobiology between these different entities, which may inform both clinical diagnosis and treatment of patients.

Keywords: Cytokines, immunology/microbiology/virology, immunobiology, inflammation, hemophagocytic lymphohistiocytosis, COVID-19

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Introduction

Cytokine release syndrome (CRS) or cytokine storm (CS) was first described in 1993 as a complication of acute graft versus host disease following allogeneic stem cell transplantation.[1] Since then, the term has been applied to a syndrome characterized by life-threatening systemic hyperinflammation, immune cell activation, and elevated circulating levels of cytokines, which can be triggered by both genetic and acquired disorders.[2] Unfortunately, there is currently no single accepted definition of CRS or CS.[2] In addition, there may be overlap between inflammatory responses deemed pathologically exaggerated versus appropriate, making it difficult to precisely define the CS/CRS entity. Finally, a recognized form of CS/CRS is HLH, which is defined by specific clinical and pathologic criteria and has an immunobiological phenotype that is rapidly emerging from research.[3,4]

Severe COVID-19 pneumonia[5,6] leading to acute respiratory distress syndrome (ARDS)[7] can lead to systemic hyperinflammation[8–11] and CS/CRS.[12] Profiling of cases has previously documented elevated levels of interferon-gamma-induced protein 10 (IP-10), monocyte chemotactic protein 3 (MCP-3), and interleukin-1 receptor antagonist (IL-1ra), which are associated with disease severity and fatality.[13,14] Laboratory parameters such as C-reactive protein (CRP) and ferritin can be markedly elevated, and abnormal liver function and coagulopathy can be seen.[15] Elevation of D-dimer levels and abnormal coagulation profiles consistent with disseminated intravascular coagulation (DIC) are seen and may be indicative of pulmonary microthrombi.[16] Antiviral drugs including remdesivir, approved by the US

Table 1. Classification of histiocytic disorders.

1. Dendritic cell related (most common):
   (a). Langerhans cell histiocytosis.
   (b). Juvenile xanthogranuloma.
   (c). Erdheim–Chester disease.
2. Monocyte-macrophage related:
   (a). Hemophagocytic lymphohistiocytosis:
      (i). Familial (due to gene mutations.)
      (ii). Secondary hemophagocytic syndromes:
         1. Infection-associated.
         2. Malignancy-associated.
         3. Autoimmune-associated (macrophage activating syndrome or MAS).
         4. Other (idiopathic).
3. Malignant histiocytosis:
   (a). Dendritic cell related.
   (b). Histiocytic sarcoma.
   (c). Monocyte-macrophage related.
   (d). Leukemia: monocytic M5A and M5B, myelomonocytic M4, chronic myelomonocytic leukemia.

Table 2. 2004 HLH diagnostic criteria for adults.

The diagnosis of HLH can be established if one of either 1 or 2 below is fulfilled:
1. Presence of HLH-associated mutations (e.g. *PRF1*, *UNC13D*, *STX11*, *STXBP2*, *SH2D1A*, *BIRC4*, *SLC7A7*, *XMEN*, *LYST*, *Rab27A*, *HPS*, *ITK*) together with clinical findings associated with HLH.
2. Fulfill five out of the following eight HLH criteria:
   (a). Fever.
   (b). Splenomegaly.
   (c). Cytopenias in at least two cell lines (hemoglobin <9 g/dL; platelets $<100{,}000$/mL; absolute neutrophil count <1000/mL).
   (d). Hypertriglyceridemia (fasting triglycerides >265 mg/dL) and/or hypofibrinogenemia (fibrinogen <150 mg/dL).
   (e). Hemophagocytosis in bone marrow, spleen, lymph nodes, or liver.
   (f). Low or absent NK cell activity.
   (g). Ferritin >500 ng/mL.
   (h). Elevated soluble IL-2 receptor >2400 U/mL.

HLH: hemophagocytic lymphohistiocytosis; NK: natural killer.

Food and Drug Administration (FDA) in October 2020, are rapidly being developed and tested.[17] To date, however, anti-inflammatory glucocorticoids have been the only treatment leading to improved survival in severe COVID-19, but the survival benefit was only observed in patients requiring supplemental oxygen or exhibiting significant hyperinflammation as measured by CRP levels.[18] Targeted anticytokine therapy to prevent COVID-19 CS/CRS has also led to mixed results in several large-scale, high-profile studies.

We[19] and other investigators[20–23] have also reported on COVID-19 CS/CRS patients who met clinical and pathologic criteria for HLH, with the consensus that such patients are relatively uncommon. In line with these clinical findings, recent data suggest that COVID-19 may lead to a unique type of CS/CRS.[24] In this Mini-Review, we examine the immunobiology of HLH, critique the notion that COVID-19 CS/CRS differs from HLH, and assess whether this distinction may inform treatment for this lethal complication of COVID-19 infection.

HLH and clinical diagnosis

HLH is a highly fatal condition characterized by dysregulated hyperactivation of lymphocytes and macrophages leading to hyperferritinemia, hyperinflammatory cytokine storm, and multiorgan failure.[4] Without treatment, HLH mortality rate approaches 100%.[25] As a disorder of macrophages, HLH is categorized with other histiocytic (dendritic cell or monocyte/macrophage) disorders (Table 1), giving rise to the eponymous name.

Patients with HLH often present with non-specific signs and symptoms such as recurrent fever, cytopenia, transaminitis, or coagulopathy. These findings may be indistinguishable from severe sepsis or multiorgan failure syndrome making the diagnosis of HLH difficult.[26] Most patients initially receive treatment with antimicrobial agents and supportive care. Lack of improvement usually prompts the clinician to perform additional tests such as ferritin, lactate dehydrogenase (LDH), D-dimer, and bone marrow biopsy. Hyperferritinemia with levels greater than 10,000 ng/mL carries over 90% sensitivity for the diagnosis of HLH in children. In the adult population, hyperferritinemia is less sensitive, and other criteria must be met to confirm the diagnosis.[26] Similarly, although the presence of hemophagocytosis in the bone marrow helps with the diagnosis of HLH, its absence does not rule out HLH. If performed early in the disease process, the bone marrow biopsy may not show hemophagocytosis, and it can be seen later in the disease course, sometimes in autopsies. For this reason, bone marrow biopsy is not considered a specific test in the diagnosis of HLH, and a negative finding does not rule out the disease.

There are currently two scoring systems, *Delphi Study*[27] and *HScore*,[28] which can be helpful to include or exclude HLH from the differential diagnosis. Both systems consider an array of laboratory values such as ferritin, LDH, triglycerides, liver enzymes, degree of cytopenia, imaging findings, and bone marrow biopsy results to assess the likelihood of HLH.

Diagnostic criteria

The diagnosis of HLH is based on both clinical and laboratory findings. In 1994, the Histiocyte Society proposed the five initial diagnostic criteria as part of the HLH-94 clinical trial.[29] Updated in HLH-2004, they currently remain the diagnostic standard in the adult population (Table 2). The criteria include fever, hepatosplenomegaly, cytopenia, low natural killer (NK) cell activity, hypertriglyceridemia and/or low fibrinogen, elevated ferritin, increased soluble IL-2 receptor (sIL-2R), and hemophagocytosis on bone marrow biopsy. Five out of eight criteria must be met to establish the diagnosis of HLH. HLH-directed therapy should be initiated if the suspicion remains high despite not meeting all five criteria.[4] Other features supporting an HLH diagnosis include hyperbilirubinemia, hepatomegaly, transaminitis, elevated lactate dehydrogenase (LDH), and D-dimer levels.[4]

Primary versus secondary HLH and special case of macrophage activation syndrome

HLH is broadly categorized into primary (familial) and secondary (acquired) types. Primary HLH, often seen in children, is caused by autosomal recessive (biallelic) mutations affecting immune regulation of lymphocytes and macrophages.[3]

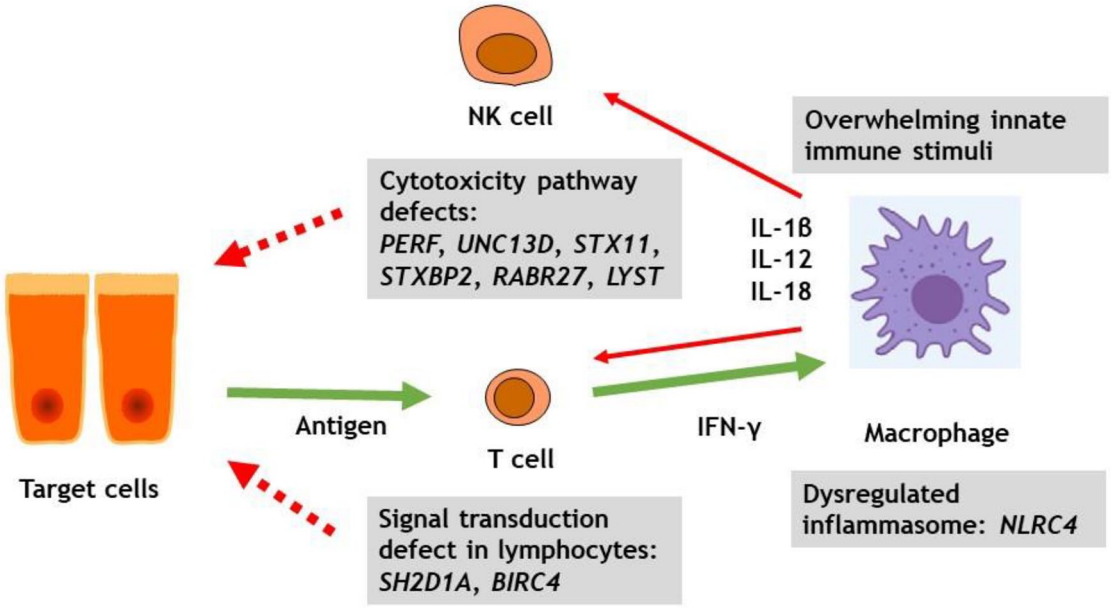

Figure 1. Pathophysiology of HLH. Normally, activated cytotoxic T- and NK-cells create cytotoxic granules that release perforins and granzymes into the immunologic synapse, which enter the cytoplasm of target cells, causing apoptotic cell death. However, genetic mutations causing cytotoxicity or signal transduction defects lead to impaired clearance of pathogen and persistently activated T-cells, which in turn release inflammatory cytokines, particularly interferon-gamma (IFN-γ), resulting in excessive activation of macrophages and end-organ damage. This pathway can also be triggered in secondary HLH by infections, malignancy, and so on. Figure modified with permission.

Secondary HLH, more common in adults, is often triggered by infections, autoimmune conditions, or malignancies in susceptible individuals.[4] Some individuals with apparent secondary HLH may also have occult monoallelic HLH gene mutations.[30] HLH is termed macrophage activation syndrome (MAS) when it is triggered by rheumatologic/autoimmune conditions.[31] Most commonly, MAS has been linked to systemic juvenile idiopathic arthritis (sJIA) but can also be associated with other rheumatologic diseases, such as systemic lupus erythematosus. These distinctions point to underlying differences in the immune state between primary and secondary HLH and MAS.

Immune mechanisms in primary and secondary HLH

All subtypes of HLH share a common terminal pathway that leads to unregulated activation of macrophages and T-lymphocytes culminating in fatal cytokine storm and end-organ damage (Figure 1). However, the immune mechanisms in primary and secondary HLH and MAS are complex and influenced by the underlying state of the immune system.

In immunocompetent individuals, NK-cells and cytotoxic T-cells kill infected cells via a process that is mediated by a perforin-dependent cytotoxic pathway.[32] Functional cytotoxic T- and NK-cells make cytotoxic granules that contain perforins and granzymes.[32] When these cells are activated by infected cells, the perforin and granzyme granules contained in the T-cells are released into the synapse.[32] Through the action of the perforins, granzymes enter the cytoplasm of the target cell and cause apoptotic cell death. Once the target cells are eliminated, down regulation of the immune response follows. Patients with familial HLH have mutations in genes (e.g. *PRF1, UNC13D, STX11, STXBP2, SH2D1A, BIRC4, SLC7A7, XMEN, LYST, Rab27A, HPS*, and *ITK*) that result in decreased or absent perforin[3,32] (Table 3). Therefore, in familial HLH, activated cytotoxic cells are unable to eliminate infected cells and antigen presenting cells, leading to persistent stimulation of macrophages and T-cells. This process can rapidly progress to severe cytokine storm, a hyperinflammatory state with resultant end-organ damage, and death without prompt treatment.[3]

The pathophysiology of secondary HLH, seen in adult patients, is not well-understood. However, many etiologies have been implicated. The most common triggers include infections, malignancies, rheumatologic disorders, immune deficiency syndromes, and immunotherapies.

Immunobiology of primary versus secondary HLH versus sepsis

Several recent reports have clarified the immunobiology of pediatric and adult HLH and sepsis, summarized in Figure 2.

Carvelli *et al.*[30] analyzed the immunologic and genetic features of 68 adults with secondary HLH. In contrast to primary (pediatric) HLH, NK-cells from adults with secondary HLH exhibited an activated phenotype and normal cytotoxic capacity but also greatly decreased NK-cell numbers and interferon-gamma (IFN-γ) production. Whereas primary HLH is a monogenic disorder associated with loss of lymphocyte cytotoxicity, about half of the adult secondary HLH patients harbored 1 or more germline variants of uncertain significance (VUS) in an HLH-associated gene, but none harbored a pathogenic variant. These data suggested that adult secondary HLH NK cells become energized but exhausted and can no longer properly respond to stimuli or control the

Table 3. Known genetic mutations in primary HLH.

Disease	Gene	Protein	Lab findings
Familial HLH2	*PFR1*	Perforin	Decreased/absent perforin expression
Familial HLH3	*UNC13D*	Munc13-4	Low CD107a
Familial HLH4	*STX11*	Syntaxin11	Low CD107a
Familial HLH5	*STXBP2*	Munc18-2	Low CD107a
X-linked lymphoproliferative disorder type I	*SH2D1A*	SAP protein	Decreased SAP expression
X-linked lymphoproliferative disorder type II	*BIRC4*	XIAP	Low XIAP expression
Lysinuric protein intolerance	*SLC7A7*	Y + LAT-1	
X-linked immunodeficiency with magnesium defect, EBV infection, and neoplasia (XMEN)	*XMEN*	MagT1	
Chediak–Higashi syndrome	*LYST*	Lyst	Low CD107a
Griscelli syndrome type II	*RAB27A*	Rab27A	Low CD107a
Hermansky–Pudlak syndrome	*HPS*	AP3	Low CD107a
NLRC4 mutation	*NLRC4*	NLRC family protein	High IL-18 levels
CD 27 deficiency	*CD27*	CD27	
ITK deficiency	*ITK*	IL-2 inducible T-cell kinase	

HLH: hemophagocytic lymphohistiocytosis.

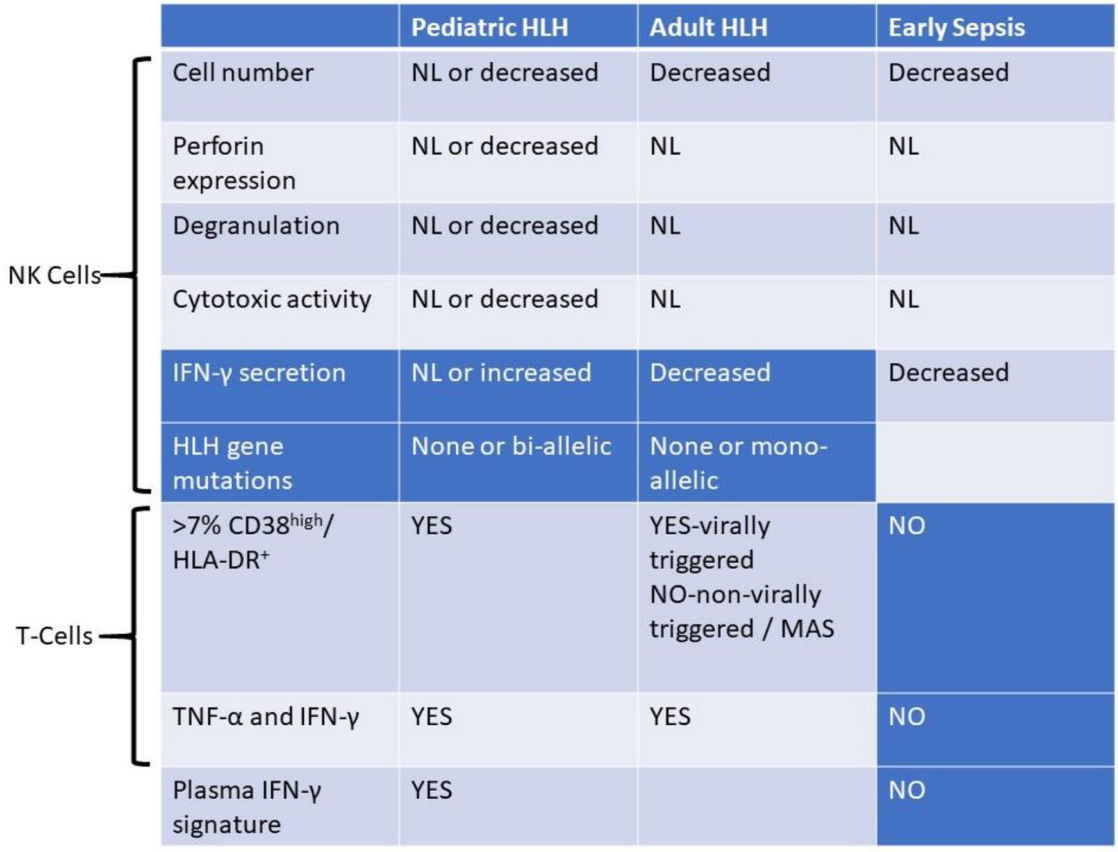

Figure 2. Immunobiology of NK-cells and T-cells in pediatric HLH, adult HLH, or sepsis.
Blue shading indicates differences between HLH and sepsis.
HLH: hemophagocytic lymphohistiocytosis.

immune response. This pattern of NK-cell function in adult secondary HLH resembles that seen in sepsis.

In contrast, Chaturvedi *et al.*[33] compared the T-cell phenotype in patients with primary or secondary HLH versus patients with sepsis. They found that, in HLH, there was expansion of a subset of peripheral blood T-cells that express CD38 and HLA-DR. This profile was most striking for $CD8^+$ T-cells, which also exhibited an effector memory phenotype and type 1 cytokine bias. The authors concluded that despite previously recognized phenotypic overlap between HLH and sepsis, the two could be readily distinguished from each other according to T-cell activation profile. Analogous to the

Chaturvedi *et al.* report, Lin *et al.*[34] compared concentrations of inflammatory plasma proteins in 40 patients with pediatric HLH to 47 patients with severe sepsis or systemic inflammatory response syndrome (SIRS). Significant differences were found in HLH plasma compared with sepsis/SIRS, including increased IFN-γ-regulated chemokines CXCL9, CXCL10, and CXCL11. Gene expression in $CD8^+$ T-cells and activated monocytes from blood was also enriched for IFN-γ pathway signatures in peripheral blood cells from patients with HLH compared with sepsis/SIRS. This study demonstrated that IFN-γ signaling is uniquely elevated in HLH, corresponding with the findings from Chaturvedi *et al.*

Finally, novel biomarkers may be useful in the diagnosis of HLH. A retrospective study by Cui *et al.*[35] showed that the serum levels of soluble CD163 (sCD163) and ferritin were significantly elevated in pediatric patients with sepsis-associated HLH when compared with septic patients without HLH.

Discriminating COVID-19-associated cytokine storm from non-COVID-19 secondary HLH

Emerging observations from groups around the world,[20] including our own,[19] suggest that COVID-19-associated CS/CRS appears to be distinct from non-COVID-19 adult secondary HLH. Most patients with COVID-19 CS/CRS do not fulfill classical or HScore diagnostic criteria for HLH.[19,20] This may be because of absence of classical criteria such as cytopenia or splenomegaly or hypofibrinogenemia. Only modestly elevated levels of IL-6, CRP, and ferritin are typically seen in COVID-19-associated CS/CRS, which corresponds with the infrequency of COVID-19-associated secondary HLH. It is possible that the relatively lower cytokine levels in COVID-19-associated CS/CRS may be related to the profound lymphopenia seen in these patients. In agreement with these clinical observations, a recent analysis of serum biomarkers from 30 COVID-19 patients versus 50 non-COVID-19 secondary HLH patients was able to clearly distinguish COVID-19 from secondary HLH, reporting strongly reduced levels of soluble Fas ligand during COVID-19 infection versus pronounced activation of the IL-18–IFN-γ axis, increased serum levels of IL-1 receptor antagonist, intercellular adhesion molecule 1, and IL-8 in patients with non-COVID-19 secondary HLH.[24]

However, rare COVID-19 patients do fulfill classical HLH-2004 diagnostic criteria and/or have an *HScore* suggesting high probability of disease.[19,21,22] Here, the role of unrecognized genetic sequence variants leading to HLH susceptibility needs further study, as these may identify a population at particular risk for COVID-19 secondary HLH. For example, Luo *et al.*[36] reported an association between four primary immunodeficiency (*UNC13D, AP3B1, RNF168*, and *DHX58*) gene variants with COVID-19-associated CS/CRS. The total percentage of COVID-19 patients with variants in *UNC13D* or *AP3B1* (Hermansky–Pudlak syndrome-2), two typical HLH genes, was dramatically higher in the high-level cytokine group than in the low-level group. Thus, germline variants in *UNC13D* and *AP3B1* were associated with the development of cytokine storm and fatal outcomes in COVID-19. In agreement with this line of investigation, some investigators[37] have suggested that the immune mechanism in COVID-19-associated HLH may be related to virally induced immunosuppression and NK-cell dysfunction that bears greater similarity to primary than secondary HLH.

Treatment of COVID-19 CS/CRS and COVID-19 secondary HLH

In this final section, we review some anti-inflammatory therapies[38,39] for both COVID-19 CS/CRS and COVID-19 secondary HLH. In both entities, experimental treatment options for COVID-19 infection have been informed by those used to treat classical HLH and other forms of cytokine storm. One of the key questions is whether COVID-19 CS/CRS or the more uncommon COVID-19 secondary HLH (fully meeting criteria for HLH) respond to drugs targeting various cytokines or cytokine pathways, such as anakinra or tocilizumab. A corollary question is whether these drugs are more likely to work in patients exhibiting a greater degree of hyperinflammation,[8] as for example, might be present in COVID-19 secondary HLH.

Analysis of recent trials with the IL-1 receptor antagonist drugs has hinted at the possible importance of patient selection dependent upon the degree of hyperinflammation as measured by biomarkers. Kyriazopoulou *et al.*[40] reported improved survival and shortened hospital stay in COVID-19 patients treated with anakinra, which blocks signaling of both IL-1α and IL-1β. Nearly 600 patients with COVID-19 pneumonia at risk for respiratory failure, as defined by an elevated serum level of soluble urokinase-type plasminogen activator receptor (suPAR), were randomly assigned to receive either anakinra or placebo (along with standard of care) early during hospitalization, before they needed mechanical ventilation. Selection for patients with elevated suPAR level may have identified not only those with excess inflammation but also those with organ damage secondary to COVID-19-associated thrombosis. In contrast to this positive study, a recent randomized, double-blind, placebo-controlled trial of canakinumab, a monoclonal antibody to IL-1β only, failed to demonstrate a significantly greater likelihood of survival in 454 patients with severe COVID-19 pneumonia.[41] Unlike the Kyriazopoulou study, only an elevated level of CRP or ferritin was required for study entry. One interpretation of these studies may be that the selection of patients by suPAR levels rather than by CRP or ferritin levels better defined those patients with COVID-19 pneumonia most likely to benefit from IL-1 blockade.

A further example of patient selection may be discrimination between patients with COVID-19 CS/CRS from those with COVID-19 fully meeting criteria for secondary HLH and therefore expected to exhibit greater degrees of hyperinflammation. To our knowledge, there have only been scattered reports of cytokine targeting treatments in patients fully meeting criteria for secondary HLH. One early report by Dimopoulos *et al.*[21] suggested favorable responses to anakinra in eight patients with COVID-19 secondary HLH as diagnosed by HScore. Blockade of the IFN-γ pathway is another potential treatment for COVID-19 CS/CRS. Emapalumab, an anti-IFN-γ antibody, is approved by the

US FDA for the treatment of refractory primary HLH. Based on possible similarities between COVID-19 HLH and primary HLH, emapalumab may represent a specific therapy for COVID-19 HLH. Although there has been a multicenter study investigating the efficacy of emapalumab in combination with anakinra in severe COVID-19 patients, further investigations may be needed to evaluate its efficacy in the COVID-19 HLH subset.

Severe COVID-19 patients have also been treated with monoclonal antibodies to either IL-6 (tocilizumab, sarilumab, and levilimab) or the IL-6 receptor (siltuximab, clazakizumab, sirukumab, and olokizumab). Non-blinded, randomized studies of IL-6 blockade have generally supported a survival benefit, which, unfortunately, was not confirmed in blinded, placebo-controlled trials.[42] For example, EMPACTA, a placebo-controlled trial that randomized 249 severe COVID-19 patients to tocilizumab or placebo showed a reduced likelihood of progression to mechanical ventilation or death with tocilizumab but no survival benefit.[43] Another phase 3 randomized, placebo-controlled trial with levilimab in severely ill COVID-19 patients showed a sustained clinical improvement rate and decreased frequency of intensive care unit (ICU) hospitalization, but again failed to confirm a survival benefit due to low statistical power.[44] It should be noted that these studies measured different endpoints, had diverse patient selection criteria, and varied concomitant regimens, which may have confounded survival benefits.

Other targets of interest include Janus kinase (JAK) and Bruton's tyrosine kinase (BTK),[45] key regulators in the production of multiple cytokines and chemokines including tumor necrosis factor (TNF)-α, IL-6, IL-10, and MCP-1. JAK inhibition targets multiple inflammatory cytokines that use common signaling pathways employed by shared cytokine receptors. Several observational studies have reported that the JAK inhibitor, baricitinib reduced inflammatory cytokines, improved lymphocyte recovery and lung function.[46] Furthermore, baricitinib may play a particular role in treating patients with moderate disease requiring oxygen support but not mechanical ventilation or extracorporeal membrane oxygenation (ECMO).[47] Another JAK inhibitor, ruxolitinib, was shown to be effective in reducing cytokine levels, improving lymphocyte count recovery, leading to faster clinical improvement.[48] Finally, a randomized, placebo-controlled trial of tofacitinib led to improved COVID-19 survival, even in the presence of concurrent glucocorticoid treatment.[49] Similarly, BTK signaling is important in the activation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome, resulting in maturation and secretion of IL-1β. A prospective observational study by Roschewski *et al*.[50] demonstrated that BTK inhibition with acalabrutinib reduced the inflammatory biomarkers to normal levels in most of 19 hospitalized patients with severe COVID-19. A randomized phase 3 trial is currently underway to assess the survival benefit of BTK blockade in this patient population.

In summary, both COVID-19 CS/CRS and HLH are hyperinflammation syndromes that lead to end-organ damage but via different immunobiologic mechanisms. COVID-19 CS/CRS patients have markedly low levels of Fas ligand, whereas HLH patients display pronounced activation of the IL-18–IFN-γ axis.[24] COVID-19 patients who meet HLH criteria tend to have germline variants[36] in HLH genes *UNC13D* and *AP3B1* and may bear similarities to primary HLH. Treatment of COVID-19 CS/CRS with anticytokine agents thus far has led to mixed results. Since cytokines are essential in eliminating SARS-COV-2, blocking the cytokine pathway could potentially lead to worse outcomes. Thus, selecting the right subgroups of patients for anticytokine agents at the right time is extremely important. Currently, there is no clear consensus as to which inflammatory biomarkers identify appropriate COVID-19 CS/CRS patients for anticytokine therapy. Elevated suPAR levels, in addition to CRP and ferritin, appear important when selecting patients for treatment with anakinra.[40] Studies with inhibitors of JAK or BTK also have yielded encouraging results, and randomized phase 3 trials are ongoing. We expect to see continued improvements in outcomes with concerted effort across the COVID-19 CS/CRS arena, improved biomarker profiling and patient selection.

AUTHOR' CONTRIBUTIONS

J.M.L. and J.C. wrote and edited the manuscript.

DECLARATION OF CONFLICTING INTERESTS

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