Immune-Guided Therapy of COVID-19

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ABSTRACT

Vaccination has been a game changer in our efforts to address the coronavirus disease 2019 (COVID-19) pandemic. However, the disease might still represent a clinical crisis for several more years, in part because of the inevitable emergence of variants capable of evading the preexisting immunity. Drugs affecting viral spread will help curtail transmission, but therapeutics are needed to treat the more severe cases requiring hospitalization. A deep analysis of the evolving immune landscape of COVID-19 suggests that understanding the molecular bases of the distinct clinical stages is paramount if we are to limit the burden of inflammation, which can lead to death in frail individuals, according to age, sex, and comorbidities. Different phases can be defined using immune biomarkers and need specific therapeutic approaches, tailored to the underlying immune contexture.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent for coronavirus disease 2019 (COVID-19), which spread globally and has reached pandemic proportions, affecting more than 323 million individuals and claiming 5.5 millions of lives worldwide, as of January 18, 2022 (1). Because SARS-CoV-2 is a respiratory virus, COVID-19 is mostly recognized as a lung disease, which evolves from mild symptoms (i.e., cough) to acute respiratory distress syndrome (ARDS) leading to lung injury, diffuse alveolar damage, vascular congestion, and focal infiltration of immune cells that can progress to a fatal outcome (2–4). Viral RNA particles have been identified in blood as well as in urine and stool samples, which might explain the extrapulmonary spread and multiorgan manifestations (5, 6).

The clinical manifestations in patients infected with SARS-CoV-2 connote COVID-19 as a complex multisystem syndrome, in which disease exacerbation mostly depends on the induction of both local and systemic immune alterations. The pathology data strongly argue that the events inside organs and tissues of patients with COVID-19 are the relevant beacons to comprehend the pathobiology of organ and body damage (7, 8). It is thus of paramount importance to frame the evolving immune pathogenesis in order to set the ground for a rational, immune-based therapy.

In this review, we discuss the contribution of the immune system to the establishment and evolution of COVID-19 (**Fig. 1**). Details on virus entry, dissemination, and endothelium contribution to the pathology are reviewed elsewhere (9–14).

Immunopathology Background

SARS-CoV2 requires angiotensin-converting enzyme 2 (ACE-2) to infect cells. According to the consensus data set from the human protein atlas (13), ACE-2 has a broad distribution in different organs such as kidney, heart, small intestine, colon, rectum, thyroid gland, gall bladder, liver, testis, and lung. ACE-2 expression and protein levels are generally higher in males, who often present a worse disease than females (14), and are higher in the alveolar type II (AT2) cells of the lungs of older patients (15). SARS-CoV-2 organotropism to renal, gastrointestinal, and neurologic tissue as well as the respiratory tract was suggested by histopathologic studies; multiple-organ failures during COVID-19 could thus, at least partially, depend on the direct cytopathic effect of the virus. However, by *in situ* hybridization, the detection of viral RNA in organs other than lung in autopsy samples is a rare event, with a few bowel localizations, implying that prolonged viral persistence is rare and some positivity by RT-PCR might be related to viral presence in the blood and vessels rather than due to tissue replication (16). At the cellular level, SARS-CoV-2 can directly infect endothelial cells and, among immune cells, preferentially targets mononuclear phagocytes but not lymphocytes (17, 18). An intriguing hypothesis was advanced to describe SARS-CoV-2 pathobiology based on the systematic comparison of pulmonary structure in patients with new-onset SARS-CoV-2–associated respiratory failure and samples from patients with pneumonia induced by other pathogens (19). SARS-CoV-2 initially infects and replicates in nasopharyngeal epithelial cells, due to the higher ACE-2 expression in those cells compared with epithelial cells in the lower airways (20). Indeed, in about 85% of the infected patients, SARS-CoV-2 is restricted to the upper part of the respiratory duct leading to a mild disease characterized by modest symptoms such as fever and dry cough, which normally resolve within 6 to 10 days (21). Some viral particles can reach the alveolar space where they infect alveolar epithelial cells and tissue-resident alveolar macrophages (TRAM). Virus-infected TRAMs acquire a specific transcriptional signature (including genes encoding the chemokines CCL7, CCL8, and CCL13), which determines their ability to recruit and activate memory T cells. T cells release high amounts of IFNγ, fueling the aberrant production of inflammatory mediators by infected TRAMS and ultimately promoting their death (19). Although the relevance of

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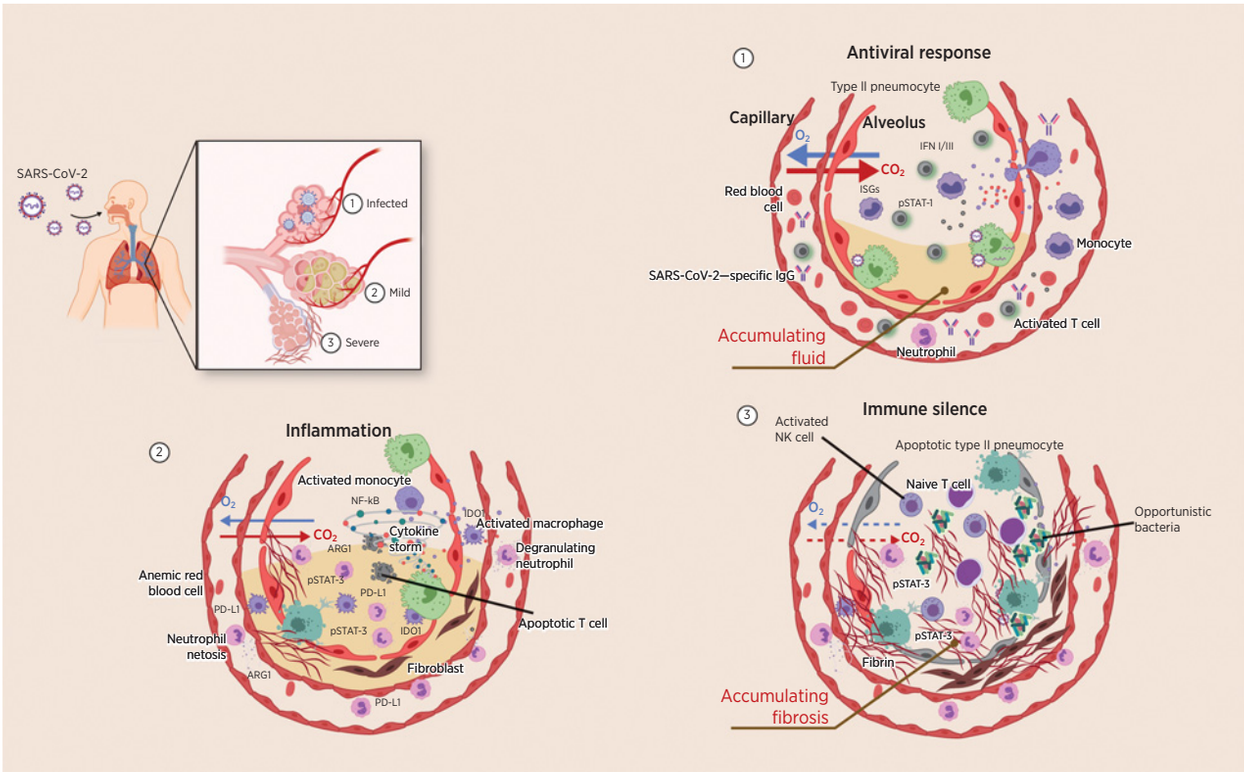

Figure 1.

Evolution of the immune response in lungs following COVID-19 infection. After entering the body through the mouth or nose, SARS-CoV-2 makes its way to the lungs, where it infects epithelial type II pneumocytes expressing ACE through its spike protein. **A,** In response, the immune system confines the area of infection, eliminating alveolar cells (antiviral response) in a process orchestrated by type I/III IFNs, ISGs, and pSTAT1, which requires interplay between activated monocytes and antiviral T cells. Reduced surfactant from alveolar epithelial type II cells, along with increased fluid accumulation in the alveoli, causes reduced gas exchange. **B,** Sustained inflammation, caused by massive secretion of cytokines from activated monocytes/macrophages and T cells (dependent on persistent activation of NF-κB and STAT3), coexists with immunosuppressive mechanisms (e.g., IDO, ARG1, and PD-L1) activated to limit the excessive inflammation. This circuit further contributes to reduce epithelial and endothelial barrier integrity with concomitant increment in pulmonary edema, leukocytes trafficking, reduced gas exchange, activation of coagulation, and deposition of fibrin. **C,** In patients with severe disease, the loss of immunosuppression and the replacement of memory $CD8^+$ T cells with their naïve counterparts establishes a condition of immune silence contributing to superimposed infections and fatal outcome. IFN I/III: interferon type I and III; ISG: interferon-stimulated gene; pSTAT1: signal transducer and activator of transcription 1 phosphorylated; pSTAT3: signal transducer and activator of transcription 3 phosphorylated; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; ARG1: arginase 1; IDO1: indoleamine 2,3-dioxygenase 1; PD-L1: programmed death-ligand 1.

monocytes and macrophages was evident from the pandemic inception, it is not clear how persistent T-cell activation can support the panoply of cytokines released in the course of COVID-19. Several data point to the myeloid cells as ignitors and perpetrators of the hyperinflammation. Indeed, similarly to a "Trojan horse," infected myeloid cells can transfer the virus across neighboring lung regions leading to slow propagation of SARS-CoV-2 throughout the entire pulmonary tree (19). Even if some reports highlight that the viral replication in myeloid cells is abortive (22), RNA-scope analysis indicates the presence of both positive- and negative-strand SARS-CoV-2 transcripts in myeloid cells signifying a productive infection (19). Furthermore, in bronchoalveolar lavage fluid (BALF) from COVID-19 patients, SARS-CoV-2 RNA can be detected in neutrophils and macrophages, where it pairs with an enhanced IFN-stimulated gene (ISG) signature. Although accumulation of virus-specific T cells secreting IFNγ has been linked to a better prognosis (23, 24), other reports have pointed out that the release of IFNγ and TNFα by activated T cells contributes to disease lethality by inducing an inflammatory cell death in myelomonocytic cells termed PANoptosis (25).

By altering cell physiology and bypassing protective antiviral responses, SARS-CoV-2 also promotes a persistent local endothelial cell death in the lung, which alters normal hemostasis and vessel wall permeability, exacerbating the functional organ failure and fueling an intense local inflammation characterized by a massive infiltration of polymorphonuclear cells (PMN) defining a key feature of COVID-19, i.e., the atypical interstitial bilateral pneumonia (26).

Transbronchial cryobiopsies carried out in patients with a moderate COVID-19 interstitial pneumonia revealed early alterations in the pulmonary structure characterized by distortion of the alveolar architecture, hyperplasia of type II pneumocytes, and diffuse vascular modifications (27). In this initial stage, lymphoid and myeloid cell infiltration is scattered, and epithelial cells show an intense expression of phosphorylated STAT3 (pSTAT3), suggesting their initial involvement in the activation of a STAT3-dependent inflammatory process (see further below). This moderate pneumonia displays a disordered angiogenesis characterized by vessels with luminal enlargement and stretched walls. Moreover, in these new blood vessels, endothelial cells show a diffuse expression of both programmed death-ligand 1 (PD-L1) and indoleamine-pyrrole 2,3-dioxygenase 1 (IDO1; ref. 27). The

spatial distribution in discrete regions of interest in lungs supports the direct association among SARS-Cov-2–rich areas with ISG, PD-L1, and IDO by *in situ* RNA hybridization, with a prevalent IDO protein expression in endothelial cells (16).

During the progression of pathology, endothelial cell dysfunction in the pulmonary microvasculature progressively favors loss of vessel barrier integrity and development of procoagulative endothelium, leading to establishment of refractory ARDS, characterized by marked microvascular thrombosis and hemorrhage (28). Additional mechanisms contributing to ARDS depend on the complement system. Postmortem IHC analysis of lung tissues has highlighted high expression of different complement proteins (including MBL, C4, C3, and C5b) in alveolar epithelial and endothelial cells (29). Mechanistically, the nucleocapsid protein of SARS-CoV-2 can indirectly interact with mannose-binding protein associated serine protease 2 (MASP-2) and activate the lectin pathway of the complement system (29). Alternatively, the presence of autoantibodies in patients with COVID-19 (30), as well as the presence of viral antigens at the plasma membrane of infected endothelial cells, could generate immune complexes with circulating virus-specific antibodies capable of activating antibody-dependent cytotoxicity through the classic complement pathway and leading to complement-mediated endotheliopathy, as recently reviewed in (31). A role for the C5a–C5aR1 axis in the pathophysiology of ARDS in COVID-19 is supported by the finding that soluble C5a levels are increased in the blood of patients with severe disease and high expression of C5aR1 receptor, in both blood and pulmonary myeloid cells (32).

Histopathologic findings of organs in patients with COVID-19 after autopsy or endoscopic biopsy pinpoint crucial functional alterations of the gastrointestinal (i.e., liver) and urinary (i.e., kidney) systems mostly associated with vasculitis, epithelial degeneration, and inflammation (6, 33). SARS-CoV-2 might infect lymph node and spleen macrophages and confer on them immunoregulatory properties by promoting lymphocyte killing through an IL6/Fas-dependent pathway (17). A potential role for Fas-mediated apoptosis in systemic lymphopenia in patients with severe COVID-19 is further suggested by the increased number of $Fas^{+}CD25^{+}$ T cells during transition to severe disease (34).

Proteomic profiling of different tissues (lung, spleen liver, heart, kidney, thyroid, and testis) obtained from patients who died from COVID-19 corroborated the presence of a multisystem abnormal inflammation. Roughly 5,000 proteins were found to be significantly dysregulated, including fibrosis markers, inflammation factors, and coagulation system- and angiogenesis-associated proteins (35). In particular, six transcription factors emerged as pervasive drivers of COVID-19 inflammation in multiple tissues: CCAAT/enhancer-binding protein β (c/EPBβ), STAT3, STAT1, nuclear factor κB subunit 2 (NF-κB2), NF-κB p65 (RelA), and transcription factor jun-B (JUNB).

The Evolving Immune Landscape

Early observations indicated that SARS-CoV-2 is sensitive to type I/III IFN pretreatment *in vitro* (36–38), highlighting that these IFNs can effectively limit coronavirus-dependent infection (39, 40). Similar to other coronaviruses, SARS-CoV-2 has evolved several mechanisms to interfere with antiviral immunity such as limiting host pattern-recognition receptor activation (41) and blocking type I/III IFN production (36). Host defects in the IFN response can also contribute to the evolution of COVID-19. Genetic mapping of 659 patients with severe COVID-19 showed an enrichment in a functional deficit of 13 human gene loci that control antiviral IFN signaling, highlighting an association of inborn errors in type I IFN–related genes and signaling, such as *TLR3*, *IRF7*, *IRF9, IFNAR1, STAT1*, with critical COVID-19 (42). Loss-of-function variants of these genes were commonly present in patients with COVID-19 facing a fatal outcome, with some patients presenting autosomal recessive deficiencies in *IRF7* and *IFNAR1*, autosomal dominant *TLR3* and *IRF3*, or new genetic defects in *IRF7* and *IFNAR1/2*. Plasmacytoid dendritic cells (pDC) from patients with autosomal dominant *IRF7* deficiency did not produce type I IFNs in response to SARS-CoV-2 infection, resulting in impaired T-cell responses. These findings, together with the evidence that self-neutralizing Abs against type I IFNs, mainly IFNα2 and IFNω, and particularly in men, contribute for life-threatening COVID-19 (43), further support the crucial role of type I IFN in protective immunity against SARS-CoV-2.

In addition, autoimmune blockade of IFN-dependent antiviral responses was postulated in at least 10% of life-threatening COVID-19 patients, due to the presence of neutralizing autoantibodies toward type I IFN at the onset of critical disease and undetectable levels of serum type I IFN during acute disease (43). Therefore, either virus-mediated or abnormal host IFN response blockade may be overcome by exogenous IFN administration at an early stage of disease. The possible counterregulatory role of type I IFN in inducing higher ACE2 expression, thus providing an advantage for virus entrance, still needs to be clarified as only the truncated form of ACE2, biologically not functional, is induced (44).

Paradoxically, lung tissue obtained at autopsy from patients with COVID-19 that was characterized by an ISG^{high} profile, in the presence of an intact or slightly altered lung morphology, displayed high viral load, excessive levels of proinflammatory cytokines and, overall, identified patients who died early after hospitalization (45). In contrast, an ISG^{low} lung pattern was associated with extensive pulmonary damage, massive accumulation of lung-infiltrating immune cells, and a low viral load and identified patients who underwent a late death (45). Although the study was not a longitudinal analysis and formal proof is still missing, the segregation of patient outcome by ISG signature supports COVID-19 as a multistep disease, which progresses from an ISG^{high} to an ISG^{low} pathology, acquiring peculiar immunologic features during the transition. This pathobiology was confirmed in moderate versus severe COVID-19: a type I IFN signature, high amount of pSTAT-1 and 2 together with high expression of IL10 characterizes moderate illness, whereas severe disease is mainly defined by high expression of IL6, IL8, TNFα, and vascular endothelial growth factor (VEGF), thus strengthening notion that there is a biological transition from a high IFN phase to a relatively low IFN phase in which a broad inflammation prevails (46). It is still an open issue whether SARS-CoV-2 can be a mild (36, 47) or potent (48) inducer of IFN responses; alternatively, the host response might change over time as a consequence of adaptation to persisting immune stress. The high levels of type I IFN in the sera of progressor patients, often coexisting with inflammatory cytokines, and the decline in ISG signature could be a hallmark of COVID-19 pathogenesis evolution that is not shared by severe influenza cases (48–51).

Interestingly, the decrease in ISG^{+} immune cells is influenced by the accumulation of circulating IgG antibodies and immune complexes (52). Patients with severe COVID-19 have higher titers of SARS-CoV-2–specific antibodies and lower viral load compared with individuals with mild disease (52). The *in vitro* blocking of FcRγIIb signaling in PBMCs, cultured in the presence of type I IFN and sera isolated from patients with severe COVID-19, completely restored the ISG signature in treated cells without promoting cell death (52).

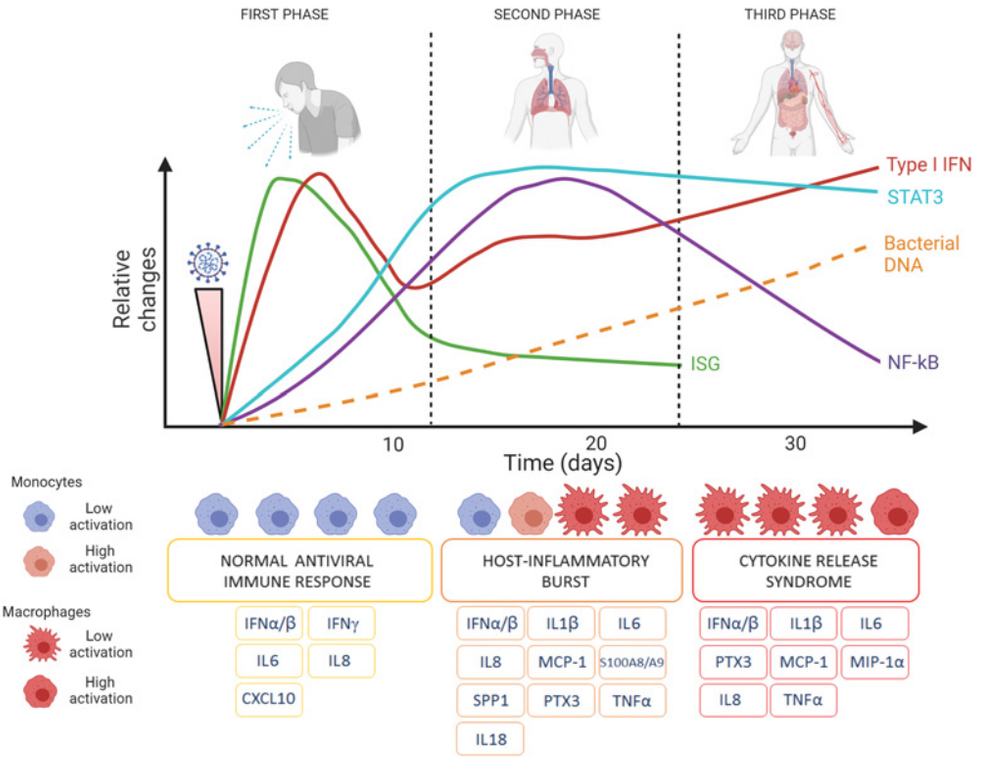

Figure 2.

Time course of antiviral and inflammatory mediators of COVID-19. Following infection of epithelial cells in the nose and mouth (first phase), the virus replicates in epithelial type II pneumocytes (second phase) and disseminates to other tissues and organs (third phase). The immune landscape undergoes a progressive modulation involving different molecular pathways (e.g., type I IFN, STAT3, ISG, and NF-κB) and contributes to disease outcome and progression. Changes in monocyte and macrophage activation during disease progression (e.g., from low activation state in the first phase to high activation state in the third phase) result in qualitative and quantitative alteration in cytokines, chemokines, and other released soluble factors.

Therefore, the contraction in ISG-expressing cells during the severe phase of COVID-19 is partly linked to an exacerbated, antibody-dependent immune reaction.

Type I/III IFN signaling is not a linear cascade but a complex network of cross-talk with other cytokine signaling pathways (53–55). For instance, noncanonical IFN signaling for IFN production sustains ISG stimulation through an IFN/TNF epigenetic coordination leading to pyroptosis and neutrophil extracellular trap (NET)osis, which fuel a massive release of cytokines (53). Moreover, some viral proteins, such as NSP9 and NSP10, directly influence production of proinflammatory mediators (IL6 and IL8) by NF-κB activation, whereas others drive a signaling shift from STAT1- to STAT3-dependent pathways, as reviewed in (56). Therefore, from an immune viewpoint, COVID-19 progresses from a STAT1/ISG/IFN type I/III antiviral immune response, which is sufficient to control disease in a vast majority of infected individuals, to an inflammatory milieu characterized by NF-κB/STAT3 activation, which mirrors some features of cytokine release syndrome (CRS; ref. 57; **Fig. 2**). In particular, pSTAT3 but not pSTAT1 is increased in lymphocytes and myeloid cells circulating in the blood of patients with COVID-19 who have pneumonia (58) and remains constantly elevated throughout disease evolution (59), indicating a persistent activation of this pathway (likely by cytokines such as IL6, IL17, and IL10). High amounts of inflammation inducers (cytokine storm) and the presence of circulating immunoregulatory myeloid cells (immunosuppression) are the hallmarks of this severe stage of disease (**Fig. 2**). Aberrant levels of soluble mediators such as colony-stimulating factors 3 and 2 (CSF3 and CSF2), inflammatory cytokines (IL6, IL1β, TNFα, and VEFG), and chemokines (CCL2 and CXCL1) can promote emergency myelopoiesis (60), leading to the mobilization of myeloid cell subsets with immunosuppressive functions, which are conventionally named myeloid-derived suppressor cells (MDSC; ref. 61). Circulating MDSC frequency increases with COVID-19 aggressiveness (62), likely supporting the progression to lymphopenia in patients by actively inhibiting the expansion and functionality of immune effector cells (i.e., NK cells and T cells; refs. 63–65). The immunosuppressive functions of circulating MDSCs, in particular in the monocytic cell fraction ($CD14^+$ cells), isolated from patients with COVID-19 depend on the expression of arginase 1 (ARG1) and can predict patient outcome (66). The accumulation in patients infected with SARS-CoV-2 of immunosuppressive cells and inflammatory cytokines (i.e., IL6) highlights the existence of similar traits between cancer and COVID-19. For instance, high frequencies of $STAT3^+ARG1^+CD14^+$ immunosuppressive cells have been identified in patients with pancreatic ductal adenocarcinoma (67, 68). In both SARS-CoV-2–infected hACE2 transgenic mice and autopsy samples from the lung of patients with COVID-19, cellular FLICE (FADD-like IL1β-converting enzyme)-inhibitory protein (c-FLIP) is overexpressed in myeloid cells (59). c-FLIP is unique in reprogramming monocytes into immune regulatory elements that display immunosuppressive functions and become a source of proinflammatory cytokines via an NF-κB pathway, which enhances aberrant STAT3 signaling (59, 68). The unrestrained and persistent

immunosuppressive and inflammatory milieu establishes the terminal phase of COVID-19, which is characterized by a fatal immune silence (**Fig. 2**). Indeed, severe disease with a lethal outcome shares some features of sepsis, including marked lymphopenia, an exhausted effector immune response and an increased frequency of immature $CD14^+$ cells (69), which cooperate to generate a state of immune paralysis. This stage of disease is characterized by ineffective host immune responses that lead to an increase in circulating bacterial DNA and presence of secondary infections in the lung, such as HSV-1 and human metapneumovirus superinfection (66, 69–71). The immune landscape in patients whose disease is fatal also comprises a marked contraction in lung effector and memory T cells, and their replacement by naïve T cells not fully armed to counter the pathogens (66, 72), suggesting a critical reset of the lymphoid compartment. Collectively, the immunologic alterations that characterize the terminal phase of COVID-19 evolution can be summarized by the definition of viral sepsis (73), where a classic antiviral immunity is replaced by immunosuppressive and inflammatory immune cells that freeze the immune system generating a strong immune paralysis, unable to protect the host against pathologic insults (**Fig. 2**).

The Immune Correlates of Disease Severity and Fatal Outcome

In an unprecedented effort, numerous studies have attempted to correlate biochemical and immune parameters of COVID-19 evolution, providing important clues about how to design new clinical studies (74, 75). It is increasingly clear that rather than a single biomarker or combination of biomarkers at a single time, the longitudinal assessment of the immune landscape can anticipate the deterioration toward more severe disease. **Table 1** summarizes the immune-related biomarkers that have been associated with either severity or fatal outcome. In summary, multiple studies investigating the immune response to SARS-CoV-2 have examined innate and adaptive immune cells, as well as soluble mediators in the circulation of infected individuals, revealing elevated proinflammatory cytokines (47, 76–80) and alterations in immune-cell composition (48, 66, 81, 82) as the most relevant parameters for patient stratification.

The initial phase of COVID-19 is characterized by high viral replication, which usually resolves within 7 to 8 days (83). As soon as the virus infects the individual, the innate immune response increases within a couple of hours of infection limiting viral replication, recruiting effector cells that eventually will limit viral load and prime the adaptive response of T and B cells (84). Within a week, $CD4^+$ and $CD8^+$ virus-specific T cells with effector functions arise (85); $CD4^+$ T cells are crucial to control primary SARS-CoV-2 infection, activate B cells, and promote the generation of plasma cells, which will produce antibodies after 10 to 15 days (85, 86). It is essential that B and T cells work together to clear rapidly and specifically the virus-infected cells and circulating virions (84). However, several changes in adaptive and innate immunity occur in COVID-19 patients with severe disease and fatal outcome.

Monocytes and macrophages

In severe patients with severe COVID-19, airway macrophages and monocytes exhibit hyperinflammatory signatures, producing long pentraxin 3 (PTX3; ref. 87) and chemokines such as CCL2 and CCL3, and the blood contains aberrant $CD14^+CD16^+CD163^{hi}$HLA-DR^{lo} monocytes expressing the chemokine receptor CCR2 (88) and secreting IL6 (89). Thus, through a feed-forward loop mediated by the CCL2/CCR2 axis, aberrant monocytes from the blood infiltrate the alveolar spaces, perpetuating tissue inflammation and damage. Monocyte-derived alveolar macrophages expressing CD206 and CD163, productively infected by SARS-CoV-2, help propagate the inflammatory status in severe COVID-19 (19). Interestingly, the presence of $CD163^{low}$ macrophages in BALF with a gene signature representative of M1-like macrophages and characterized by the expression of secreted phosphoprotein 1 (SPP1; refs. 19, 23, 66) was predictive of better outcome, further underlining the dynamic changes within the monocyte–macrophage compartment. On the other hand, SPP1 drives the activation of $CD14^+$ monocytes and accumulation of PD-$L1^+$ neutrophils, and high plasma levels of macrophage-derived SPP1 and S100A12 have been demonstrated in severe COVID-19 and predicted the need for transfer to the ICU (90). Another study (88) demonstrated that the airways of individuals with severe-to-fatal COVID-19 exhibit an increased frequency of monocyte-derived macrophages characterized as $CD163^{low}$HLA-DR^{low} and $CD86^{high}$HLA-DR^{high} cells, as well as different blood-derived monocyte subsets expressing $CD163^{high}$HLA-DR^{low}. Future studies will have to solve this apparent contrast, by integrating tissue immune landscape and high dimensional data.

COVID-19 is characterized by a loss of $CD14^{low}CD16^{high}$ nonclassic monocytes (81); in-depth analysis revealed a trend to accumulate inflammatory HLA-$DR^{high}CD11c^{high}$ blood monocytes in patients with mild disease, which is in stark contrast with the increased number of monocytes expressing HLA-DR^{low} $CD11c^{low}$ in patients with severe disease. Particularly, $CD14^+$ monocytes expressing HLA-DRA, HLA-DRB1, and CD83 selectively accumulate in the blood of patients with mild disease, suggesting a scenario in which these monocytes prolong the activation and expansion of antigen-specific T cells, via the contribution of ISGs, such as IFI6 and ISG15 (81). In patients with mild disease, classic monocytes, defined as $CD14^{high}CD16^-$, mostly express activation markers, such as CD38, CD95, and CXCR3; however, the expression of HLA-DR as well as CD69 and CD226, indicative of alternative activation, is heterogeneous. Of note, expression of CD69 and CD226 in classic monocytes promotes diapedesis and tissue infiltration and retention (91). In contrast, patients with severe COVID-19 are characterized by the progressive loss of nonclassic $CD14^-CD16^{high}$ cells, paralleled by the accumulation of HLA-$DR^{low}CD163^{high}CD69^{high}CD226^{high}S100A8/9/12^+CD10^+$ monocytes that also express CD34, a marker commonly associated with an immature phenotype and reminiscent of emergency hematopoiesis (81). These monocytes have the potential to suppress T-cell activation and effector functions, as described in patients with severe, but not fatal, COVID-19 (66, 81, 82). Thus, in the moderate–severe clinical phase of the disease (phase II), macrophages and monocytes can very efficiently cooperate with adaptive immunity, in the severe–critical phase (advanced phase III) they may contribute to a less effective and possibly dangerous response. This appears clinically important when administering therapies aiming to silence hyperinflammation, which could be detrimental in cases of an ongoing "immune silence" (**Fig. 2**). This unresponsive immunotype is quite critical because it might represent ∼20% of patients with COVID-19 (92). Ideally, an effort should be made in phase II to avoid progression to phase III, through a full characterization of prognostic biomarkers in early and advanced phase II.

Polymorphonuclear cells

A high neutrophil-to-lymphocyte ratio and the presence of activated neutrophils, including formation of NETs, have been repeatedly

Table 1. Immune biomarkers of disease severity and mortality in COVID-19.

Biomarkers	Severe disease	Mortality	References
Cytokines, chemokines, and alarmins in blood	• TNFα and IL6 • IL6, IL10, IP10 (CXCL10), IL18 • Multiple type 1, 2, and 3 cytokines + chemokines • S100A8/A9 • SAA1, SAA2, SAA4, SAP/APCS, SERPINA3 • S100A12, SSP1, TNFSF14, and OSM • CCL3 and CCL4 • Impaired IFNα production	• TNFα and IL6 • IL6 and IL10 • IFNα, IL1Rα, CCL2, M-CSF, IL2[a] • GM-CSF and IL1α • PTX3 • sST2, sTNFRSF1A, IL10, and IL15[b]	(47, 48, 60, 66, 69, 72, 74, 76–80, 81, 87, 90, 144, 145, 148, 149, 200)
Cytokines and chemokines in BALF	• CCL2, CCL3, CCL4, TNFβ		(88)
Cells in blood	• $CD14^+HLA\text{-}DR^{lo}CD163^{hi}$ and $CD14^+HLA\text{-}DR^{lo}S100A^{hi}$ monocytes • $CD14^+ARG1^+CD163^+$ immature monocytes • $CD16^{lo}Ki\text{-}67^+$ neutrophils • $CD274^+$ neutrophils • Lymphopenia • $CD95^+CD25^+$ T cells • T-cell apoptosis • $HLA\text{-}DR^+$ $CD38^+$ T cells • Ki-67^+ proliferating T cells • PD-1^+ $TIM3^+$ T cells • Decreased naïve $CD45RA^+CCR7^+$ T cells • Decreased absolute numbers of $CD45RA^-CCR7^+$ and TEMRA $CD8^+$ T cells • High antibody response • Increased B-cell number • Reduced $CD24^+CD38^+$ B cells • Increased plasmablasts • Absence of germinal centers	• $CD14^-CD16^{hi}HLA\text{-}DR^{lo}$ monocytes • $CD86^{lo}HLA\text{-}DR^-CXCR3^+$ plasmablasts • $CD16^{hi}CD66^{lo}$ neutrophils • IFNγ^+ granzyme B^+ Th17 lymphocytes • PMN-MDSCs • Activated NK and γδ T cells	(24, 34, 58, 66, 75, 81, 88, 92, 97, 111, 116, 119, 125, 133, 135, 201, 202)
Cells in BALF	• $CD14^+CD163^+$ monocytes • Increased naïve T cells and reduced $CD4^+$ and $CD8^+$ Trm • $FCN1^{hi}$ monocytes and $IL1B^{hi}$ monocytes		(51, 66, 88)
Immune suppression by MDSC	• Immunosuppression by M-MDSCs in blood[c] • Immunosuppression by PMN-MDSC in blood	• Lack of M-MDSC immune suppression in blood	(62, 65, 66)
Gene signatures	• ISG signature reduced in neutrophils and monocytes of severe patients • ISG signature elevated in myeloid cells of mild–severe patients (including cells in BALs) • Increased type I IFN signature (*IFNAR1*, *JAK1*, *TYK2*) and downregulation of ISG signature in PBMCs • *STAT1/IRF3*, *IL1B*, *TNF*, and type I *IFN* signature in myeloid cells • Interferon-responsive genes in Sars-$Cov2^+$ macrophages and IFNγ in T cells of BALs • Bacterial DNA • *S100A9*, *S100A8*, *CCL3*, *IL1RN*, *TNF*, *IL6*, *CXCL8*, *IFNG* in different blood cells • ATP signaling inflammasome in BALF macrophages	• MS1 monocytes (*RETN*, *ALOX5AP*, *IL1R2*)[d] • Immature neutrophil score (*DEFA1B*, *DEFA1*, *DEFA3*, *LTF*, *S100A8*)	(19, 47, 49–52, 69, 72, 74, 81, 201, 203, 204)
Proteomic and histopathology landmarks in autoptic samples		• NF-κB2, RELA, C/EBPB, STAT1/3, and JUNB in different organs • ISG^{hi} and ISG^{lo} in lung[e]	(35, 45)
Genome-wide association markers	• *OAS1*, *OAS2*, *OAS3*; high *TYK2*, *DPP9*, high *CCR2*, low *IFNAR2* • *SLC6A20, LZTFL1, CCR9, FYCO1, CXCR6*, and *XCR1* • *TLR7*		(124, 205, 206)

Abbreviations: ANXA1, annexin A1; C/EBPB, CCAAT/enhancer-binding protein b; CCR2, CC-chemokine receptor 2; DPP9, dipeptidyl peptidase 9; IFNAR2, IFN receptor subunit 2; ISG, IFN-stimulated genes; JUNB, factor jun-B; MDSC, myeloid-derived suppressor cells; MS1, monocyte state 1; NFKB2, nuclear factor kB (NF-kB) subunit 2; OAS, oligoadenylate synthetase; PTX3, pentraxin 3; PBMC, peripheral blood mononuclear cell; RELA, transcription factor p65; SAA, serum amyloid A; SAP/APCS, serum amyloid P component; SERPINA3, alpha-1-antichymotrypsin; sST2, soluble suppression of tumorigenicity 2 protein; sTNFRSF1A, soluble TNF receptor superfamily member 1A; STAT, signal transducer and activator of transcription; TNFSF14, TNF superfamily member 14; TYK2, tyrosine kinase 2; TLR7, Toll-like receptor 7; Trm, tissue-resident memory.

[a]In the first 12 days from symptom onset.

[b]Throughout the entire hospitalization time.

[c]This parameter might be sex-dependent, as it is more frequent in severe male patients.

[d]This signature is shared among patients with severe sepsis (207).

[e]The ISG signature associated with different time to death and immune landscapes in postmortem autopsies.

linked to the immunopathogenesis of severe COVID-19 (81, 93). Particularly, the presence of $CD16^{int}CD44^{low}CD11b^{int}$ low-density neutrophils (LDN), expressing IL6 and TNFα, is typical of severe COVID-19 and correlates with poor clinical outcome (81, 93, 94). High dimensional data analysis has shed more light on the vast heterogeneity of LDNs. Although all cells express the canonical neutrophil markers CD15 and CD66b, differences in the abundance of CD11b, CD11c, and CD16 were observed among the different subsets. The expression of CD16 alone was sufficient to classify neutrophils as $CD16^{high}$, $CD16^{int}$, or $CD16^{low}$, with the last subset uniquely present in patients with severe disease (81). The proportion of $CD16^{low}$ neutrophils was significantly higher in patients with severe disease. The $CD16^{low}$ LDNs were also positive for Ki-67, which indicates either dysfunctional immature or alternatively activated neutrophils (95, 96). Further, differential gene-expression analysis of LDNs revealed an extensive heterogeneity, with a subgroup expressing various ISGs (*ISG15, RSAD2*, and *IFITM1/3*; ref. 81) and others expressing genes involved in NET formation, mostly *MPO*, *ELANE*, and *PRTN3*. Overall, LDNs express high levels of *S100A8*, *S100A9*, *CD274*, and *ARG1*, although *S100A8* and *S100A9* were equally distributed within the LDN subsets, expression of *CD274* and *ARG1* was mutually exclusive. In patients with severe COVID-19, LDN subsets have an MDSC-like phenotype (PMN-MDSCs defined as $HLA\text{-}DR^{-}CD11b^{+}CD33^{+}CD15^{+}CD14^{-}$ cells), and the expansion of these cells is sustained by the inflammatory milieu (97, 98). Further, these cells were shown to suppress T-cell activation via mechanisms dependent on TGFβ and inducible nitric oxide synthase (iNOS), but not ARG1; of note, their frequency directly correlated with the severity of COVID-19 and levels of IL8 (97). These cells are reminiscent of the immature neutrophils expressing $CD62^{low}$ and $PD\text{-}L1^{high}$ and the immature cluster expressing CEACAM8 and DEFA3 that others have detected in patients with served COVID (81) and (66), respectively. As the disease progresses toward a fatal outcome, LDN-mediated immunosuppression is lost, leading to the development of "immune silence" in which innate immunoregulatory networks are completely abrogated (66).

Similar to the changes seen in the circulation, an increase in neutrophils also occurs in the lungs of patients with COVID-19 (99). PMN infiltration into the alveolar space and neutrophilic mucositis was observed in lung tissue obtained from patients who died from COVID-19, indicating inflammation in the entire lower respiratory tract (100, 101). Transcriptional analysis of BALF from patients with COVID-19 showed high levels of *CXCL2* and *CXCL8*, chemokines that facilitate PMN recruitment to the site of infection (102); an extensive and prolonged activation of these cells can lead to detrimental effects in the lung and result in pneumonia and/or ARDS (103). Activated neutrophils release NETs—extracellular webs of chromatin, microbicidal proteins, and oxidant enzymes—that function to contain infectious agents. However, when not properly regulated, NETs have the potential to propagate inflammation and microvascular thrombosis, as observed in the lungs of patients with ARDS and patients with severe COVID-19. The activation of platelets (104), endothelial cells, and high serum and local levels of IL8 could contribute to NET formation. Sera from patients with COVID-19 have elevated levels of cell-free DNA, myeloperoxidase-DNA (MPO-DNA), and citrullinated histone H3 (Cit-H3), all of which are indicative of NET accumulation; furthermore, these sera could activate resting neutrophils to produce NETs (105). It must be pointed out that, although confirming the presence and heterogeneity of PMNs, single-cell profiling of neutrophils in BALFs did not reveal specific patterns associated with disease severity (51).

Eosinophils can also have a role in COVID-19. Several reports describe eosinopenia in patients with moderate-to-severe COVID-19 (106). Eosinopenia is typically accompanied by reductions in peripheral lymphocyte, platelet, and monocyte counts, as well as elevated levels of C-reactive protein (CRP) and IL6, characteristics that have been observed in patients with COVID-19 (106). The eosinopenia pathophysiology in COVID-19 remains unclear but is likely multifactorial, involving inhibition of eosinophil egress from the bone marrow, blockade of eosinophilopoiesis, reduced expression of chemokine receptors/adhesion factors (107), and/or direct eosinophil apoptosis induced by type I IFN released during the acute infection (108). Importantly, no eosinophil enrichment in pulmonary tissue has been observed in samples from patients with COVID-19 at early stages of disease (109) or in postmortem analyses (110). Therefore, eosinopenia might only be predictive of severe forms of COVID-19.

NK cells

NK cells contribute to the early phases of virus control and regulation of adaptive responses. In COVID-19, NK cells are numerically decreased, especially in patients with severe disease, and show an activated (higher HLA-DR and CD69 expression) phenotype (111–113). In patients with severe disease, it has been reported that there is a reduced frequency of $CD56^{bright}CD16^{+/-}$ NK-cell subsets, with a shift of circulating NK cells toward more mature $CD56^{dim}CD16^{+}KIR^{+}NKG2A^{+}$ and "memory" $KIR^{+}CD57^{+}CD85^{+}$ cells endowed with increased expression of inhibitory NKG2A and KIR molecules (112). These cells were functionally impaired as they displayed decreased cytotoxicity and IFNγ production despite conserved expression of natural cytotoxicity receptors and perforin (112).

T cells

The key role of T cells in COVID-19 has been demonstrated through the characterization of T-cell immunity in patients with COVID-19 at various postinfection stages. SARS-CoV-2–specific memory T cells have been found in most convalescent individuals, including asymptomatic cases and those with undetectable antibody response (86). However, lymphopenia characterizes COVID-19 (114) and can predict disease severity. Patients with severe/critical COVID-19 exhibit 2.1- and 2.2-fold lower absolute numbers in $CD4^{+}$ and $CD8^{+}$ cells, respectively, as compared with patients with moderate disease (115). The reduction in peripheral T cells is particularly prominent within the $CD8^{+}$ T-cell compartment, but it remains unclear whether this is due to these cells trafficking into tissues with ongoing SARS-CoV-2 replication (23), increased elimination of these cells, or preexisting low levels of these cells in individuals who experience severe disease (85). Regarding $CD4^{+}$ lymphopenia, T-cell activation, dysfunction, compartmentalization in the lung or a mix of these events have been advanced as underlying mechanisms. Interestingly, increased T-cell apoptosis is a characteristic of both $CD4^{+}$ and $CD8^{+}$ T-cell compartments (116). The lack of SARS-CoV-2–specific $CD4^{+}$ T cells with a tissue-resident phenotype (117) further support the primary association of T cells with protection rather than immunopathology.

Virus-specific T cells secreting IFNγ associate with a better prognosis (118) and T-cell count and clonal expansion of SARS-CoV-2–specific T cells precede COVID-19 convalescence (119, 120). The key role of the T-cell antiviral response in COVID-19 was demonstrated in animal studies showing that $CD8^{+}$ T-cell depletion abrogates the protection induced by natural infection in rhesus macaques (121). Moreover, $CD8^{+}$ T cells may be important for recovery from COVID-19 in patients with hematologic cancers and impaired humoral response (122). Furthermore, patients with X-linked

agammaglobulinemia did not require oxygen supplementation for COVID-19 pneumonia (123), corroborating the role of the T-cell compartment for the favorable clinical outcome.

Besides quantitative reduction, qualitative perturbations in the T-cell compartment are frequent in patients with severe and critical COVID-19. $CXCR6^+$ T cells are known to traffic to the lung, and its ligand CXCL16 is constitutively expressed in the lung and upregulated during inflammatory responses; the CXCR6–CXCL16 axis is thus associated with severe lung disease and pneumonia. Genome-wide association studies have recently identified the locus 3p21.31 (rs11385942), encompassing the *CXCR6* gene, as a susceptibility locus for severe COVID-19 (124). Results demonstrated that circulating $CD8^+CXCR6^+$ T cells were significantly elevated with inflammation, yet virtually absent in patients with severe COVID-19. Patients with the risk allele have a lower frequency of protective T cells in the lung, therefore increasing the risk of developing severe COVID-19 form (66), which supports the hypothesis that the CXCR6–CXCL16 axis contributes to the immunopathogenesis of severe COVID-19.

An increased frequency of activated T cells, in particular either $HLA\text{-}DR^+$ or $CD38^+$ cells among $CD4^+$ and $CD8^+$ T cells, has been associated with severe disease (92, 116). Moreover, proliferating $Ki\text{-}67^+$ T cells, as well as increased expression of surface markers typical of T-cell exhaustion, such as PD-1 and Tim-3, have been documented (34, 92, 119). PD-1 and Tim-3 expression on T cells could be detected in patients who progressed from prodromal to overtly symptomatic stages and severe COVID-19 (34, 125). The expression was higher in infected patients versus healthy controls, and in ICU patients versus non-ICU patients, in both $CD4^+$ and $CD8^+$ T cells (126). The memory status of T cells in patients with COVID-19 also changes, with reported loss in both naïve ($CD45RA^+CCR7^+$) and effector memory ($CD45RA^-CCR7^-$) $CD4^+$ and $CD8^+$ T cells (127). Naïve T cells were particularly decreased in the blood of severe disease and in elderly (24).

The absolute numbers of central memory ($CD45RA–CCR7^+$) and terminally differentiated effector memory ($CD45RA^+CCR7^-$) $CD8^+$ T cells were reduced in patients with severe disease (116). Among $CD4^+$ cells, T follicular helper (Tfh) cells represent a bridge between T-cell and B-cell responses. Tfh can have direct antiviral functions, but their role in COVID-19 remains controversial. A significant increase was found in recovered patients (128) as well as patients with severe COVID-19 (116).

Perturbation in regulatory T cells (Treg) also correlated with COVID-19 severity. Cytometry and transcriptomic analyses showed that $FoxP3^+$ Tregs were increased in the peripheral blood of patients with severe disease, had higher expression of FoxP3 and overexpressed a range of suppressive effectors, but also proinflammatory molecules like IL32, thus mirroring the behavior reported in cancer (129). This phenotype can lead to a reduced virus clearance and more severe inflammation.

B cells

Plasma cells that form during the acute and convalescent phases of COVID-19 continue to secrete antibodies after the resolution of infection, giving rise to serologic memory. Memory B cells are important to respond quickly to reinfection by generating new high-affinity plasma cells. Long-term protection is achieved through the induction of long-lived plasma cells and memory B cells. Further evidence of the importance of the B-cell response for COVID-19 recovery can be observed in patients with B-cell deficiencies: anti-CD20 therapy can lead to a status of persistent COVID-19 characterized by the presence of high levels of virus-specific and activated T cells (130). The other side of the coin is that following recovery, patients have expanded memory B-cell clones, and those vaccinated after COVID-19 disease recovery, have an even greater antibody response as vaccination supports broader somatic hypermutation and neutralizing activity against a variety-of-concern (VOC) viral isolates (131). This is consistent with the demonstration that there is persistence of long-lived bone marrow plasma cells in mildly infected patients, an essential source of protective antibodies, for up to 11 months (132).

High virus-specific antibody titers correlate with higher *in vitro* SARS-CoV-2 neutralization and inversely correlate with viral load in patients (133–135). However, higher titers have also been associated with more severe clinical cases (133, 135, 136), suggesting that a robust antibody response alone is insufficient to avoid severe disease. Even if neutralizing antibodies are highly predictive of immune protection, and neutralizing titers are important predictors for vaccine efficacy (137), neutralization efficacy declines with time (138), potentially leading to the waning of individual and collective protection. Nonetheless, the frequency of memory B cells remains stable or increases over time, likely contributing to protection against viral infection (139). As stated above, T cells play a prominent role. Indeed, both SARS-CoV-2–specific memory B and T cells display functional hallmarks of antiviral immunity through rapid neutralizing antibodies production, cytokines secretion, and self-expansion upon restimulation (140).

Not enough data on the formation of germinal centers (GC), transient microstructures generated after the provision of help by Tfh to antigen-activated B cells, are available in patients with severe/critical COVID-19. Absence of GCs has been associated with more severe disease (92). In freshly isolated PBMCs, the proportions and absolute numbers of total $CD19^+$ B cells, naïve (IgD^+CD27^-), early transitional T1 and T2 (IgD^+CD27^- $CD10^+CD45RB^-$, respectively), and $CXCR5^+$ follicular ($IgD^+CD27^-CD10^-CD73^+$) cells were profoundly reduced in severely ill patients with COVID-19 with high CRP levels (141). B-cell responses in individuals succumbing to SARS-CoV-2 show a striking absence of GC, associated with a marked reduction in GC B cells; the lack of coordination among adaptive T–B immune responses is likely responsible for this anomaly and might have important consequences, because even robust activation of non-GC type B cells does not give rise to long-lived memory or high-affinity B cells (92). Thus, the lack of GC responses may account for the variable and often low and short-lived antibody responses observed in COVID-19 patients. An additional consequence of this disordered adaptive response is that extrafollicular activation strongly correlated with broad antibody-secreting cell expansion (plasmablasts in particular) and an early production of SARS-CoV-2–specific neutralizing antibodies; yet these patients had severe disease with elevated inflammatory biomarkers, multiorgan failure and death. The increased fraction of B cells lacking CXCR5 and CD21 (DN2 cells) was greatly expanded in patients with severe COVID-19 and associated with IL6 and IP-10 and poor outcome (142).

The dismal outcome in spite of a strong antibody response suggests that coordinated T cell–B cell cooperation is crucial in COVID-19 immunity and that the proinflammatory synthesis by B cells of IFNγ, IL6, CSF2, and TNFα (a cytokine implicated in the lack of germinal center formation) may contribute to the "cytokine storm" (143).

Cytokines, alarmins, and growth factors

The majority of patients with COVID-19 have evidence of lung involvement, with the degree of disease severity often correlated with higher levels of inflammatory markers in the blood (e.g., D-dimers and CRP; refs. 144, 145), increased neutrophil-to-lymphocyte ratio and

levels of cytokines and chemokines, whose expression profile shows similarities to CRS and macrophage-activation syndrome (MAS; ref. 146). In fact, IL6, IL7, TNFα, CCL2, CCL3, and CXCL10 are increased in severe cases, yet IL1β levels appear neither altered in SARS-CoV-2–infected patients nor correlate with life-threatening forms of the disease. Future studies will have to address the discrepancy between IL6 and IL1β levels (e.g., assessing the role of inflammasome activation), but the reduction in circulating T cells has been firmly linked to the increment in serum levels of IL6, TNFα, and IL10 (147). Comparing the concentration of biomarkers across severity groups and relative to healthy donors (HD), several distinct patterns emerged. In particular, concentrations of CCL3, soluble CD163 (sCD163), and CSF1 are elevated in all patients with COVID-19 compared with HDs, regardless of severity. In contrast, CCL2 and CCL4 were elevated selectively in patients with fatal outcome. Ferritin, IL15, CX3CL1, and IL12p70 have been found to be elevated in patients with critical disease who survived but not in those who died. Notably, IL12p40 levels were inversely correlated with disease severity (148). Of interest, also IL18 blood levels emerged along with IL6 as a biomarker of severity, suggesting that the inflammasome pathway could be important in determining the major outcomes (149).

Other growth factors have shown increased expression in patients with COVID-19, including VEGF and hepatocyte growth factor (HGF; ref. 150). Serum VEGF levels were found to be higher in patients with SARS-CoV-2, although they did not differ between those who were admitted to ICU and those who were not. In contrast, serum HGF levels were found to be elevated in patients requiring ICU admission (150).

High levels of plasma calprotectin (an S100A8/A9 stable heterodimer) have been identified as a potential biomarker of SARS-CoV-2–induced emergency myelopoiesis in patients with severe COVID-19 (151). As calprotectin is a TLR4 and RAGE ligand that promotes both NF-κB activation (152) and secretion of multiple inflammatory cytokines like IL6 (153), this mediator might contribute to the CRS affecting patients with severe COVID-19. Interestingly, the S100A8/A9-specific inhibitor paquinimod significantly abrogates the frequency of neutrophils reprogrammed by SARS-CoV-2 in preclinical models, nullifying viral replication and limiting lung damage (154).

Another alarmin associated with COVID-19 evolution is high mobility group box 1 (HMGB1), which is a prototypical damage-associated molecular pattern and a central mediator of lethal inflammation in infection and tissue damage (155). Indeed, severe COVID-19 is characterized by higher plasma levels of HMGB1 (156).

In patients with COVID-19, serum levels of PTX3 positively correlate with disease severity, coagulopathy, and thrombogenesis (157). Interestingly, RNA-seq of blood-derived mononuclear cells and endothelial cells, together with IHC of lung from patients with died from COVID-19, showed that monocytes and macrophages express the highest levels of PTX3, and this emerged as an independent predictor of patient mortality (87).

Rational Therapeutic Intervention According to Immune Phases: The Clinical Trials

The first therapeutic brake in any infection is represented by a specific drug targeting the infectious agent. In COVID-19, viral replication occurs in the first 7 to 8 days. This phase of the disease can be either asymptomatic or with mild symptoms that can be treated at home in the majority of cases. Targeting the inflammasome with colchicine may lead to a decreased rate of hospitalization but does not affect deaths (158). In the first few days, monoclonal antibodies (in combination) directed against epitopes of the S protein can help to stop invasion of the virus (159–162). If patients need to be hospitalized, antiviral treatment is started. For their hypothetical antiviral efficacy, several drugs have been tested in controlled trials (umifenovir, lopinavir/ritonavir, favipiravir, sofosbuvir and daclatasvir, hydroxychloroquine) with no evidence of clinical efficacy (163). Remdesivir, an inhibitor of the viral RNA-dependent, RNA polymerase with *in vitro* inhibitory activity against SARS-CoV-1 and MERS-CoV (Middle East respiratory syndrome), was shown to be clinically useful in hospitalized patients, in the very early phases of the disease [ordinal scale (OS) = 4; refs. 164–169; **Table 2**]. When the disease progresses, virus-induced inflammation prevails and affects the upper airways and lung (major targets) and other anatomical compartments (micro and macrovessels) or organs. Considering the immune response to the virus and the immune cells involved, trials targeting various pathways of the inflammatory cascade have been conducted. When trying to increase the innate immune response, by giving IFNβ1b, a significant fall of ICU admission, though not a significant effect on death rate, was seen in mostly OS-6 patients (170), whereas positive effects on the 28th-day mortality rate was observed mainly in OS-5. These data suggest that early intervention before disease progresses to the most devastating "cytokine storm" could be helpful. In fact, a randomized trial showed that IFNβb1 given to patients with symptom duration less

Table 2. Summary algorithm of trials in hospitalized patients at different phases of the disease.

SARS-CoV-2–infected patients	Approved therapies (FDA or FDA-EUA—emergency use authorization)
Mild COVID-19 disease (PaO2/FiO2) < 300 > 200	Remdesivir (no steroids) (ordinal scale: **5/8**) + Enoxaparin (therapy)
Moderate/severe COVID-19 disease (PaO2/FiO2) < 200 > 100	Tofacitinib (ordinal scale **6/8**) or baricitinib (or anti-IL6) + remdesivir (ordinal scale: **6/8**) plus dexamethasone +Enoxaparin (therapy)
Severe/critical COVID-19 disease (PaO2/FiO2) < 100	Dexamethasone 6 mg/die + anti-IL6 (ordinal scale: **7/8**) + Enoxaparin prophylaxis

Note: BERLIN criteria for ARDS severity: Mild if PaO2/FiO2 <300 > 201; moderate if PaO2/FiO2 > 101< 200; severe if PaO2/FiO2 ≤ 100 (Berlin definition: The ARDS definition task force. Acute respiratory distress syndrome. JAMA 2012; 307: 2526–2533).
NIAID ordinal scale: 1. not hospitalized and no limitations of activities; 2. not hospitalized, with limitation of activities, home oxygen requirement, or both; 3. hospitalized, not requiring supplemental oxygen and no longer requiring ongoing medical care (used if hospitalization was extended for infection control or other nonmedical reasons); 4. hospitalized, not requiring supplemental oxygen but requiring ongoing medical care (related to COVID-19 or to other medical conditions); 5. hospitalized, requiring any supplemental oxygen; 6. hospitalized, requiring noninvasive ventilation or use of high-flow oxygen devices; 7. hospitalized, receiving invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO); and 8. death.

than 7 days (along with lopinavir/ritonavir, and ribavirin) significantly alleviated symptoms and led to clearance of the viral load in all clinical specimens (nasopharyngeal swab, throat saliva, oropharyngeal saliva, and stool) much faster than in patients with symptom duration more than 7 days (163). The most significant advances in reducing mortality were obtained upon targeting "the cytokine storm" in hospitalized patients. In this clinical setting, three major points have emerged:

1. In critical cases, the use of dexamethasone (Dexa) reduced mortality from 40% to 28% in ventilated patients in the ICU with OS-7, significantly less in OS-5 or -6 patients. Dexa, as well as other glucocorticosteroids (GC), has pleiotropic effects. Entering the immune cells, it binds to the glucocorticoid receptor (GR) in the cell cytoplasm, and the ligand-bound GR complex is rapidly translocated into the nucleus where it interacts with NF-κB to block its transcriptional activity, thereby negatively regulating target gene expression, including *IL1*, *IL2*, *IL6*, *IL8*, *TNFA*, and *IFNG*. Importantly, four of these cytokines are linked to COVID-19 severity (148). The results of the RECOVERY trial (171) led to the worldwide adoption of Dexa treatment for patients with OS-7.
2. As previously stated, during the initial phases of COVID-19, JAK1, JAK2, and STAT1 activation is followed by STAT3 phosphorylation. A randomized controlled trial (RCT) with baricitinib (along with Remdesivir) showed reduced progression to mechanical ventilation or mortality, especially in OS-6 (rate ratio, 0.69; 95% CI, 0.50–0.95; ref. 172). These data have been confirmed in the COV-BARRIER RCT, in which baricitinib was compared with the standard of care (SOC), in a large cohort in which 79% of the patients received also Dexa. The 28-day all-cause mortality was 8.1% for baricitinib and 13.1% for placebo, corresponding to a 38.2% reduction in mortality [hazard ratio (HR) 0.57; 95% CI, 0.41–0.78; nominal $P = 0.002$]. The frequency of adverse events, serious adverse events, serious infections, and venous thromboembolic events was similar between the groups. The conclusion of the study was that SOC plus baricitinib and Dexa offers the greatest chance of reducing mortality (173). These results likely derive from the efficacy of baricitinib in modulating inflammatory pathways via JAK1/JAK2 inhibition, and in determining a dose-dependent inhibition of IL6-induced STAT3 phosphorylation (58, 59), as shown in *in vitro* and *ex vivo* studies (58, 174, 175). Baricitinib might also have an antiviral effect by blocking AP2-associated protein kinase 1 (AAK1). Disruption of AAK1 might interrupt the passage of the virus between cells and also the intracellular assembly of virus particles (176, 177). However, although this JAK1/JAK2 inhibitor was also shown to control severe acute respiratory inflammation and lung pathology in macaques infected with SARS-CoV-2, an antiviral effect was not confirmed (178). The efficacy of modulating STAT pathways was further validated by the STOP-COVID trial with the JAK1/JAK3 inhibitor tofacitinib (Tofa; ref. 179). In hospitalized patients with pneumonia, high dose of Tofa in NIAID OS 4–5–6 patients led to a significant drop in 28-day mortality (from 5.5% to 2.8%), while maintaining statistically similar serious adverse events over the course of therapy. As remdesivir was not used in this trial, results strongly argue that the Tofa is critical to control the inflammatory burden in these patients with COVID-19.
3. The inflammatory burden in the third phase of the disease may be so lengthy and devastating that a dramatic percentage of patients still die even when treated with Dexa. The combination of Dexa plus anti-IL6R (tocilizumab, TOCI, or sarilumab, -SARI) in patients requiring organ support (REMAP-CAP trial), when compared with SOC, proved to protect from death with a hazard ratio of 1.61 (95% credible interval, 1.25–2.08; ref. 180). The estimates of the treatment effect in patients treated with glucocorticoids in combination with either TOCI or SARI were greater than the estimates for any intervention on its own, and the estimated interaction between IL6R antagonists and glucocorticoids was additive and slightly in the direction of synergistic. These results were further confirmed in the RECOVERY RCT, in which patients having clear hyperinflammation (CRP ≥ 75 mg/L), were treated with TOCI or SOC and censored after 28 days. The overall death rate was reduced from 42% to 35% ($P = 0.001$), the effect of the combination with glucocorticoids was additive, and importantly the therapeutic intervention revealed more clinical success when symptoms duration was <7 days in males. There was no evidence of an increased infection rate (181).

As IL6 levels are one of the best biomarkers of severity as well as a predictor of mortality, the results of these trials suggest that the inflammatory hurricane in phase III of the disease determines outcome in an important percentage of patients with critical (and severe) disease (182). It is still unclear whether IL6 blockade at peak of disease affects postviral complications such as chronic fatigue, 'brain fog,' anxiety and stress, and long COVID. Long-term extension trials based on targeting IL6R will answer these crucial issues.

The crucial issues are the early categorization of disease severity for each patient, the prompt identification of the disease signature at that stage, and the prompt administration of the optimal therapeutic combination. Along this line, targeting microvessel thrombi and NETosis with an early use of therapeutic heparin in noncritically ill patients has provided evidence of clear-cut clinical benefit in terms of survival and hospital discharge without admission to the ICU (183); instead, in critically ill ICU patients, intermediate prophylactic doses in stage 3 gave similar results in terms of survival to prophylactic doses (184). These findings support a translational approach with therapeutic doses in stage 2 and prophylactic doses in stage 3 (185). The rationale of stopping the cascade of events leading to an early strong activation of the innate immune response had success in the Save-More trial in which targeting IL1 (α and β) in stage 2 reduced severity and mortality (186). Again, the therapeutic strategy and ultimately the outcome will depend upon the best categorization of the patient at hospital admission. **Table 3** summarizes the controlled clinical trials according to the clinical ordinal scale category (NIAID OS).

COVID-19 and cancer

Patients with cancer have an increased risk for developing severe COVID-19 (187), particularly if they are affected by hematologic malignancies (188). The worst outcomes are associated with an impairment of host immunity as a result of either the neoplasia itself or specific cancer therapies, both leading to higher susceptibility to infections, in addition to the presence of comorbidities (189). Anti-CD20, frequently used to treat hematologic cancers, impact the B-cell response and antibody production, with an impairment in anti–SARS-CoV-2 immunity leading to "persistent COVID-19" (130); glucocorticoid, used as a treatment for leukemias/lymphomas or as adjunct therapy in several cancers, may hamper the antiviral immunity, as also observed in rheumatological patients (190). The impact of immunotherapy based on checkpoint inhibitors (e.g., anti–PD-1/PD-1L) is still unclear (191). On the one hand, checkpoint inhibitors may reestablish functional T cells to control SARS-CoV-2 infection/disease;

Table 3. Randomized controlled trials of COVID-19 treatment with indication of the clinical ordinal scale category.

Author (ref.)	Enrolled patients (*N*)	Treatment arm (*N*)	Controls *N* (comparator)	Ordinal scale	Days from symptom onset; median	Primary outcome	Results	Comment
ANTIVIRALS								
Therapy						**Remdesivir**		
Beigel (164)	**1,048**	**532**	**516 (SOC)**	**4 = 138 (13)** **5 = 435 (41)** **6 = 193 (18.2)** **7 = 285 (26.8)**	**9**	**28-day time to recovery**	**RR: 1.29; 95% CI, 1.12–1.49; *P* < 0.001**	**Significant efficacy only in scores 4 and 5**
Spinner (165)[a]	584	384	200 (SOC)	3 = 8 (1) 4 = 483 (83) 5 = 88 (15) 6 = 5 (1)	8	11-day clinical status	5-day OR = 1.65; 95% CI, 1.09–2.48; *P* = 0.02 10-day Wilcoxon rank sum test = 0.18	The proportional odds assumption was not met for 10-day comparison
WHO consortium (166)	5,451	2,743	2,708 (SOC)	4 = 1,325 (24) 5–6 = 3,639 (67) 7 = 487 (9)	NA	28-day mortality	RR = 0.95; 95% CI, 0.81–1.11; *P* = 0.50	Significant efficacy in score 4 to 6; RR = 0.86; 95% CI, 0.67–1.11.
Wang (167)	236	158	78 (SOC)	4 = 3 (1) 5 = 194 (82) 6 = 37 (17) 7 = 1 (0.4)	11	28-day time to clinical improvement	HR = 1.23; 95% CI, 0.87–1.75; *P* = 0.24	28-day 1 category decline HR = 1.34; 95% CI, 0.96–1.86.
Olender (168)	**1,114**	**298**	**816 (SOC)**	**4 = 217 (19)** **5 = 675 (61)** **6 = 153 (14)** **7 = 69 (6)**	**8**	**14-day recovery**	**14-day OR = 2.03; 95 CI, 1.34–3.08, *P* ≤ 0.001**	**Significant efficacy in: score 7 = OR 0.02; 95% CI, 0.01–0.05; *P*: < 0.001 score 6 = OR 0.08; 95% CI, 0.05–0.14; *P*: <0.001**
Goldman (169)	397	200 (5-day treatment)	197 (10-day treatment)	4 = 55 (14) 5 = 220 (55) 6 = 109 (27) 7 = 13 (3)	8	14-day clinical status	14-day difference = 65% vs. 54%; *P* = 0.14	Higher mortality in the 5-day group in score 7: 40% vs. 17%
ANTI-INFLAMMATORY AND IMMUNE THERAPIES								
Therapy						**Colchicine**		
Tardif (158)	4,159	2,075	2,084 (Plac.)	1–3 = 4,159	5	30-day death or hospitalizaton	30-day = 4.6% vs. 6.0%; OR 0.75; 95% CI, 0.57–0.99; *P* = 0.04	30-day hospitalization = OR 0.75; 95% CI, 0.57–0.99 30-day death = 0.56; 95% CI, 0.19–1.66
Therapy						**Dexamethasone**		
RECOVERY Collaborative Group (171)	**6,425**	**2,104**	**4,321 (SOC)**	**4 = 1,535 (24)** **5–6 = 3,883 (60)[b]** **7 = 1,007 (16)**	**8**	**28-day mortality**	**28-day RR = 0.83; 95% CI, 0.75–0.93; *P* ≤ 0.001**	**Score 7, RR = 0.64; 95% CI, 0.51–0.81** **Scores 5–6, RR: 0.82; 95% CI, 0.72–0.94;** **Score 4, RR = 1.19; 95% CI, 0.91–1.55**

(Continued on following page)

Table 3. Randomized controlled trials of COVID-19 treatment with indication of the clinical ordinal scale category. (Cont'd)

Author (ref.)	Enrolled patients (*N*)	Treatment arm (*N*)	Controls *N* (comparator)	Ordinal scale	Days from symptom onset; median	Primary outcome	Results	Comment
Therapy				**Neutralizing monoclonal antibodies**				
ACTIV-3/TICO LY-CoV555 Study Group (159)	314	163	151 (Plac.)	4 = 86 (27) 5 = 180 (57) 6 = 48 (15)	NR	Day 5 ordinal scale improvement	Day 5 = OR 0.85; 95% CI, 0.56–1.29; $P = 0.45$	Time to recovery = no differences
Chen (160)	452	309	143 (Plac.)	2 = 345 (76) 3 = 107 (24)	4	11-day viral clearance Percentage of hospitalizations	11-day difference = −53; 95% CI, −0.98 to −0.08; $P = 0.02$ Hospitalization = 1.6% vs. 6.3%	Only the dose 2,800 mg was effective
Weinreich (161)	275	182	93 (Plac.)	2 = NR 3 = NR	3	7-day time-weighted average change in viral load (log_{10} copies/mL) 29-day attended visits	7-day mean differences from placebo = −0.41; 95% CI, −0.71 to −0.10; 29-day attended visits difference = −3%; 95% CI, −16 to 9	Greatest effect in patients with higher viral load at baseline
Gottlieb (162)	**577**	**309 bamlanivimab**	**112 bamlanivimab + etesevimab 156 (Plac.)**	**1 to 2 = 577**		**11-day viral load change**	**11-day (combination treatment vs. plac.) = −0.57; 95% CI, −1.00 to −0.14; $P = 0.01$**	**Bamlanivimab monotherapy = no significant differences**
Therapy				**Tocilizumab (anti-IL6)**				
Rosas (208)	438	294	144	4 = 15 (3) 5 = 122 (28) 6 = 133 (30) 7 = 168 (38)	11	28-day ordinal scale status	28-day OR = 1.19; 95% CI 0.81 to 1.76; $P = 0.36$	28-day mortality difference = 0.3; 95% CI, −7.6 to 8.2; $P = 0.94$
Salama (209)	**377**	**249**	**128**	**4 = 35 (9) 5 = 242 (64) 6 = 100 (26.5)**	**8**	**28-day intubation or death**	**28-day HR = 0.56; 95% CI, 0.33 to 0.97; $P = 0.04$**	**No improvement of survival**
REMAP-CAP Investigators (180)	**865**	**366 TCZ 48 SARI**	**402 (SOC)**	**5 = 3 (0.3) 6 = 608 (70) 7 = 254 (29)**	**NR**	**21-day number of respiratory support-free days (median)**	**Day 21 TCZ: 10 (IQR −1 to 16); OR = 1.64; 95% CI, 1.25–2.14; SARI: 11 (IQR 0–16); OR = 1.76; 95% CI, 1.17–2.91**	**TCZ + SARI in-hospital mortality 27% vs. controls 36%**
Ely (187)	**101**	**51**	**50 (Plac.)**	**7 = 101 (100)**	**>7 = 93**	**28-day mortality**	**28-day HR = 0.54; 95% CI, 0.31–0.96; $P = 0.030$**	**60-day HR = 0.56; 95% CI, 0.33–0.97; $P = 0.027$**
Therapy				**Baricitinib (anti-JAK 1/2) and tofacitinib (anti-JAK 1/3)**				
Kalil (210)[c]	**1,033**	**515**	**518 (Plac.)**	**4 = 142 (14) 5 = 564 (55) 6 = 216 (21) 7 = 111 (11)**	**8**	**28-day time to recovery**	**28-day RR = 1.16, 95% CI, 1.01–1.32; $P = 0.03$**	**Highest efficacy in ordinal score 6 No differences in ordinal scores 4 and 7**
Marconi (173)	**1,525**	**764**	**765 (Plac.)**	**4 = 186 (12.3) 5 = 962 (63.4) 6 = 370 (24.4)**	**<7 = 253 >7 = 1,265**	**28-day disease progression**	**28-day OR = 0.85; 95% CI, 0.67–1.08; $P = 0.18$**	**28-day 38.2% mortality reduction, HR 0.57; 95% CI, 0.41–0.78; nominal $P = 0.002$**

(Continued on following page)

Table 3. Randomized controlled trials of COVID-19 treatment with indication of the clinical ordinal scale category. (Cont'd)

Author (ref.)	Enrolled patients (*N*)	Treatment arm (*N*)	Controls *N* (comparator)	Ordinal scale	Days from symptom onset; median	Primary outcome	Results	Comment
Guimaraes (179)	**289**	**144**	**145 (SOC)**	**4 = 71 (24.6) 5 = 181 (62.6) 6 = 37 (12.8)**	**10**	**28-day mortality**	**28-day mortality OR = 0.63 95% CI, 0.41–0.97 *P* = 0.04**	**Mortality at day 28 occurred in 2.8% of patients in the tofacitinib group and in 5.5% of patients in the placebo group (hazard ratio, 0.49; 95% CI, 0.15–1.63)**
Therapy				**Anakinra (anti-IL1)**				
Save-More (186)	**594**	**405**	**189 (SOC)**	**4–6 = 594 (100)**	**9**	**Day 28 absolute 3–4 point decrease of WHO-CPS**	**Day 28 = OR 0.36; 95% CI, 0.26–0.50; *P* < 0.001**	**Effective reduction of severity and mortality**
				Anti NETosis therapy (heparin)				
The ATTACC, ACTIV-4a, and REMAP-CAP Investigators (211)	2,231	1,181	1,050 (SOC)	WHO-CPS: 4–6 = 2,231 (100)	NR	Survival to hospital discharge and days free of organ support through 21 days	Day 21 = odds ratio 1.29; 95% credible interval, 1.04–1.61)	Effective reduction of severity, and of mortality

Note: Trials were considered for evaluation only if phase II to IV, prospective, randomized, placebo-controlled, or including properly matched controls. Only trials with more than 100 patients enrolled in the treatment arm or the standard-of-care arm were considered. As primary endpoint, selected trials had one or more of the following clinical outcome measures: the clinical status improvement, hospitalization rate and duration, time to recovery, discharge rate, ICU admission percentage, and mortality. Trials of COVID-19 patients in the early stage of the disease, with mild symptoms, evaluating the reduction of viral load as primary endpoint were also included. Literature search was updated till September 30, 2021.

The trials with the highest amount of supporting data are highlighted in bold.

Currently, the following therapies were approved by the FDA for emergency: remdesivir, dexamethasone, bamlanivimab + etesevimab, baricitinib, tocilizumab.

WHO ordinal scale consisted of the following categories: 0, uninfected; 1, asymptomatic; viral RNA detected; 2, symptomatic; independent; 3, not hospitalized with resumption of normal activities; 2, not hospitalized, but unable to resume normal activities; 3, symptomatic; assistance needed; 4, hospitalized, not requiring oxygen; 5, hospitalized, requiring oxygen by mask or nasal prongs; 6, hospitalized, requiring nasal high-flow oxygen therapy, noninvasive mechanical ventilation, or both; 7, hospitalized, requiring intubation and mechanical ventilation, pO2/FIO2 u150 OR SpO2/FIO2 ≥ 200; 8, hospitalized, requiring mechanical ventilation, (pO2/FIO2 < 150 OR SpO2/FIO2 < 200) OR vasopressors (norepinephrine less than 0.3 μg/kg/min); 9, mechanical ventilation, pO2/FIO2 < 150 AND vasopressors (norepinephrine more than 0.3 μg/kg/min), OR dialysis OR ECMO hospitalized, requiring; 10, dead.

Abbreviations: anti-IL6, anti-interleukin-6; anti-JAK 1/2, anti-Janus kinases 1/2; anti-GM-CSF, anti-granulocyte–macrophage colony-stimulating factor receptor-alpha monoclonal antibody; anti-IL1, anti-interleukin-1; CI, confidence interval; CS, corticosteroids; HR, hazard risk; LPV/RTV, lopinavir/ritonavir; *N*, number; NA, not applicable; NR, not reported; OR, Odds ratio; Plac., placebo; RR, rate ratio; SOC, standard of care; WHO-CPS: World Health Organization Ordinal Clinical Progression Scale.

[a]Patients were randomized to receive remdesivir for 5 or 10 days.

[b]Not specified if low-flux or high-flux requirement.

[c]Treatment arm: baricitinib plus remdesivir, controls: remdesivir.

on the other, T-cell overactivation may be detrimental, contributing to increased hyperinflammation (192). Nonetheless, cancer treatments may limit SARS-CoV-2 infection and disease (193). In particular, therapy based on androgen deprivation downregulates the serine protease TMPRSS2 leading to protection from SARS-CoV-2 infection (194); thoracic radiation may reduce hyperinflammation, which associates with COVID-19 (195); finally, Bruton tyrosine kinase inhibitors may protect COVID-19 patients with B-cell malignancies from severe disease (196).

Few studies are available on the humoral and cellular responses to SARS-CoV-2 in cancer patients. Reports on hematologic malignancies suggest that IgG antibody responses may be qualitatively absent in one third of the patients (197) or quantitatively lower compared with noncancer patients (198). Regarding T-cell responses, an impaired virus-specific T-cell response characterized by T-cell exhaustion and altered functionality is found in patients with solid and hematologic cancers infected with SARS-CoV-2 (193). Moreover, cancer patients with COVID-19 showed significantly lower levels of terminal effector $CD4^+$ T cells, Tregs, Th9, effector NK cells, B cells, intermediate-type monocytes, and monocyte-derived DCs. These immune-cell perturbations were associated with both mild and severe COVID-19 (199).

Conclusions

The partial control of the pandemic with vaccination will certainly change the enormous burden of deaths in all countries; nonetheless, it is a general feeling that the virus (with the emerging VOC) will remain over the next years, possibly as an epidemic infectious disease still leading to hospitalization of some patients, including a fraction entering ICUs with critical pneumonia. In these cases, the proposed algorithm, derived from the several trials published in the last year, will be of pivotal importance to decrease the mortality rate. Data herein discussed on the immunobiology of the disease along its different phases indicate that therapeutic strategies may be of real help to dampen the inflammatory spread, from the lung to other organs. More specifically, targeting IL6, IL1, or more broadly the cytokine cascades with JAK inhibitors has had significant clinical benefit. The accurate definition of the clinical stage and the use of some biomarkers, in particular the degree of lymphopenia, and of eosinopenia, the levels of IL6, PTX3, SPP1, and S100A12, which are critical to characterize the phases of the aggressive pneumonia, could be of help in patient management. Moreover, the immune profiling of patients with COVID-19 may reduce the time and costs of patient hospitalization, as well as allow more refined diagnosis and treatments.

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