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Review

The contributory role of lymphocyte subsets, pathophysiology of lymphopenia and its implication as prognostic and therapeutic opportunity in COVID-19

Mahda Delshad [a,1], Naeimeh Tavakolinia [b,1], Atieh Pourbagheri-Sigaroodi [c], Ava Safaroghli-Azar [c], Nader Bagheri [d], Davood Bashash [c,*]

[a] Department of Laboratory Sciences, School of Allied Medical Sciences, Zanjan University of Medical Sciences, Zanjan, Iran
[b] Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[c] Department of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[d] Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran

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ABSTRACT

The incidence of the novel coronavirus disease (COVID-19) outbreak caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has brought daunting complications for people as well as physicians around the world. An ever-increasing number of studies investigating the characteristics of the disease, day by day, is shedding light on a new feature of the virus with the hope that eventually these efforts lead to the proper treatment. SARS-CoV-2 activates antiviral immune responses, but in addition may overproduce pro-inflammatory cytokines, causing uncontrolled inflammatory responses in patients with severe COVID-19. This condition may lead to lymphopenia and lymphocyte dysfunction, which in turn, predispose patients to further infections, septic shock, and severe multiple organ dysfunction. Therefore, accurate knowledge in this issue is important to guide clinical management of the disease and the development of new therapeutic strategies in patients with COVID-19. In this review, we provide a piece of valuable information about the alteration of each subtype of lymphocytes and important prognostic factors associated with these cells. Moreover, through discussing the lymphopenia pathophysiology and debating some of the most recent lymphocyte- or lymphopenia-related treatment strategies in COVID-19 patients, we tried to brightening the foreseeable future for COVID-19 patients, especially those with severe disease.

Abbreviations: ACE2, Angiotensin-converting enzyme 2; JAK, Janus kinase; ADE, Antibody-dependent enhancement; MAPK, Mitogen-activated protein kinase; AHF, Acute heart failure; MHC-II, Major histocompatibility complex-class II; AID, Activation-induced cytidine deaminase; MSCs, Mesenchymal stem cells; AIM assays, Analysis of activation induced marker; NK cell, Natural killer cell; ALC, Atypical lymphocytes; NKG2A, Natural killer G2A; ARDS, Acute respiratory distress syndrome; NKG2D, Natural killer G2D; BALF, Broncho alveolar lavage fluid; NLR, Neutrophil to lymphocyte ratio; Bcl-6, B cell lymphoma 6; PB, Peripheral blood; CAR-NK, Chimeric antigen receptor-engineered NK cell; PD-1, Programmed cell death protein 1; CM T cell, Central memory T cells; PLR, Platelet to lymphocyte ratio; COVID-19, Coronavirus disease-19; RBD, Receptor-binding domain; CRP, C-reactive protein; SARS-CoV, Severe acute respiratory syndrome coronavirus; cTFH, Circulating follicular helper T cells; sCD25, Soluble CD25 (interleukin-2 receptor); DI, Dengue infection; SGLT2, Sodium-glucose cotransporter-2; EM T cell, Effector memory T cells; SOS1, Son of sevenless homolog 1; FACS, Fluorescence-activated cell sorting; SP, Spike protein; FasL, Fas ligand; STAT, Signal transducer and activator of transcription; FoxP3, Forkhead box P3; TCR, T cell receptor; GrA, Granzyme A; TFH, T follicular helper cells; GrB, Granzyme B; TH1, T helper type 1; ICS, Intracellular cytokine staining; TIM-3, T cell immunoglobulin and mucin domain-3; ICU, Intensive care unit; TLM, Time to lymphocyte model; Ig, Immunoglobulin; TNF-α, Tumor necrosis factor-α; IL, Interleukin; Tregs, Regulatory T cells; IL-2Ra, Interleukin-2 receptor alpha; WHO, World health organization.

* Corresponding author at: Department of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
E-mail address: D.bashash@sbmu.ac.ir (D. Bashash).
[1] These authors contributed equally to this work.

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1. Introduction

December 2019 will never be forgotten in the history of medicine, when an outbreak of pneumonia caused by the novel coronavirus (severe acute respiratory syndrome coronavirus 2; SARS-CoV-2) sooner or later prompted the World Health Organization (WHO) to issue a public health warning emergency. Pneumonia, acute respiratory distress syndrome (ARDS), and multiple organ failure are the most important challenges that the virus induces in infected individuals. This is, in fact, the host immune responses against SARS-CoV-2 that holds a respectable share in the occurrence of these symptoms, rather than the virulence characteristics of the virus. Although many studies have blamed severe activation of the immune system and excessive production of pro-inflammatory cytokines –known as cytokine storm– to be the main cause of serious complications, this is lymphopenia and lymphocyte dysfunction that, at least partly, enforce disease progression in such way. The attention to the importance of lymphocytes in the pathogenesis of COVID-19 has been also increased by the recent disclosure that the virus-induced cytokine storm may be a compensatory mechanism adopted by the host immune system to cover the lack of lymphocytes and their exhausted phenotype. Since lymphocytes are the main immune cells battling with the beast of rapidly-evolving viruses, especially SARS-CoV-2, it comes as no surprise to assume that any abnormality in the frequency, specificity, function, and durability of these cells may contribute to the progression of COVID-19 toward an unfavorable outcome. All in all and taking advantage of these facts, it seems that the more accurate knowledge we gain about the role of lymphocytes in the pathogenesis of the disease, there would be the more ray of hope for the management and the treatment of the disease in the near future. In the present review, we discussed the role of each subtype of lymphocytes, including B cells, T cells, and NK cells, in the pathogenesis of COVID-19. We also provided brief information about the association between the lymphocytes and the disease prognosis, pathophysiology for lymphopenia, and finally the lymphocyte- or lymphopenia-related treatment strategies in COVID-19 patients, especially those with severe disease.

2. Lymphocytes in COVID-19

From the first identification of COVID-19, one of the clinical manifestations that was highlighted in patients was the remarkable reduction in the number of peripheral lymphocytes. It was even more highlighted in severe cases, as the percentage of lymphocytes in this group dropped to lower than 20% [1]. The results of the flow cytometric analysis showed that SARS-CoV-2 may significantly decrease the number of lymphocyte subsets, including $CD4^+$ T cells, $CD8^+$ T cells, NK cells, and B cells [2–5]. Notably, $CD8^+$ T cells seem to undergo a more significant reduction as compared to other subsets, especially in COVID-19 patients with a severe condition [6]. In another study, Qin et al. also indicated that reduction in the number of lymphocytes is not restricted only to the effector lymphocytes, as the number of memory T cells ($CD3^+CD4^+CD45RO^+$) showed a remarkable reduction in severe disease, an event which suggested that why COVID-19 patients could not, at least in some cases, develop immunity against future infection with the virus [4]. The following parts of this paper try to gather evidence that takes a broad overview of the effects of COVD-19 on different subtypes of lymphocytes.

2.1. B lymphocytes in COVID-19

The impact of SARS-CoV-2 infection on plasmablasts (PBs) and memory B cells was first reported in a study conducted by Mathew et al. It became evident that while SARS-CoV-2 has no adverse effect on naïve B cells, it could significantly reduce the population of both class-switched (IgD^-CD27^+) and non–class-switched (IgD^+CD27^+) memory B cells. Conversely, the number of $CD27^-IgD^-$ B cells, $CD27^+CD38^+$ PBs, and also KI67 expression in B cells was increased in the patients' groups as compared to the control group [7]. The same results also announced by Wen et al. who succeeded to identify four B cell subpopulations in COVID-19 patients using scRNA-seq: naïve B cells that express CD19, CD20 (MS4A1), IGHD, IGHM, IL4R, and TCL1A; memory B cells, which are detected by the expression of CD27, CD38, and IGHG; immature B cells (B3) that express CD19 and CD20; and plasma cells (B4) expressing high levels of XBP1 and MZB1. Their results suggested that while the percentage of naïve B cells was reduced during COVID-19, the number of plasma cells was increased in patients. Additionally, unlike the B cells isolated from healthy donors, those B cells harvested from recovered patients displayed an elevation in the expression of S100A8, IGLL5, SSR3, IGHA1, XBP1, and MZB1, which are known as activation-related genes [8]. Vassallo et al. also reported that there is an association between higher $CD10^+$ B lymphocyte levels and the development of a mild type of COVID-19 disease [9].

Humoral responses in COVID-19 are often of limited durability, as seen with other human coronavirus epidemics. In an interesting study, Pillai et al. showed that while the germinal centers in either lymph nodes or spleen are destroyed and the number of Bcl-6^+ B cells diminished significantly, the number of AID^+ B cells remained unchanged. These data provide valuable supports for why antibody responses are restricted in COVID-19 infection. In fact, dysfunction of Bcl-6^+ T follicular helper (TFH) cells and disturbed humoral immune responses at the early stage of the disease may be responsible for the rapid distribution of the virus into vital organs. In a plain word, the differentiation arrest of Bcl-6^+ TFH cells in the germinal center may result in loss of this zone in lymphoid organs and accumulation of non-germinal center-derived activated B cells [10].

2.1.1. The role of antibodies in COVID-19

The humoral immune responses against enveloped viruses include the production of a wide range of immunoglobulins (Ig), foremost IgM, IgG3, IgG1, and IgA, which are mainly specific to the glycoproteins and the nucleoproteins of the envelope. Although the presence of antibodies to the nucleoproteins is representative of the infection, there are no enough studies suggesting how they precisely hamper the replication of the virus [11,12]. Fourteen days after the onset of symptoms, IgM and IgG against spike protein (SP) of the SARS-CoV-2 envelope are detectable in the majority of COVID-19 patients. The results of the *in vitro* analysis suggested that these antibodies, which mostly recognize the receptor-binding domain (RBD) of the SP, could neutralize the replicative capacity of the virus and act as neutralizing antibodies [13–15].

In a study conducted on a total of 222 patients, the authors found enhanced IgM levels at the early stage with this notion that its level was higher in patients with the severe form of the disease. Also, high levels of IgG were detected in severe cases of COVID-19 but it was increased at the late stage [16]. Since IgG levels were higher in the critically ill patients, the high titer of this antibody is mostly considered to be a worse prognostic factor [16]. However, it should be noted that antibody responses are different in each individual and the early peak level of antibodies could not be an indicator of a better outcome, as some severe cases had a high concentration of Abs at the early stages of the disease [14]. Inline, Lee et al. examined SARS-CoV-1 IgG concentrations and suggested that patients who seroconverted earlier in the course of the disease progressed to a more aggressive type of the disease [17]. Zhang et al. also suggested that patients with a high level of IgG were more prone to develop a more severe type of disease as compared to those with a lower level [16]. They indicated that probably antibody-dependent enhancement (ADE) –that is a mechanism in which binding of a virus to sub-neutralizing or cross-reactive non-neutralizing antiviral antibodies reinforces its entry, and subsequently, enhances virus replication– may be responsible for this phenomenon [18,19]. Aside from protective functions, it seems that the produced IgG against SARS-CoV-2 SP can boost the infection of immune cells and undesirably enhance the immunopathogenesis of COVID-19. To provide a better overview, we designed a schematic figure representing the roles of B cells in COVID-19

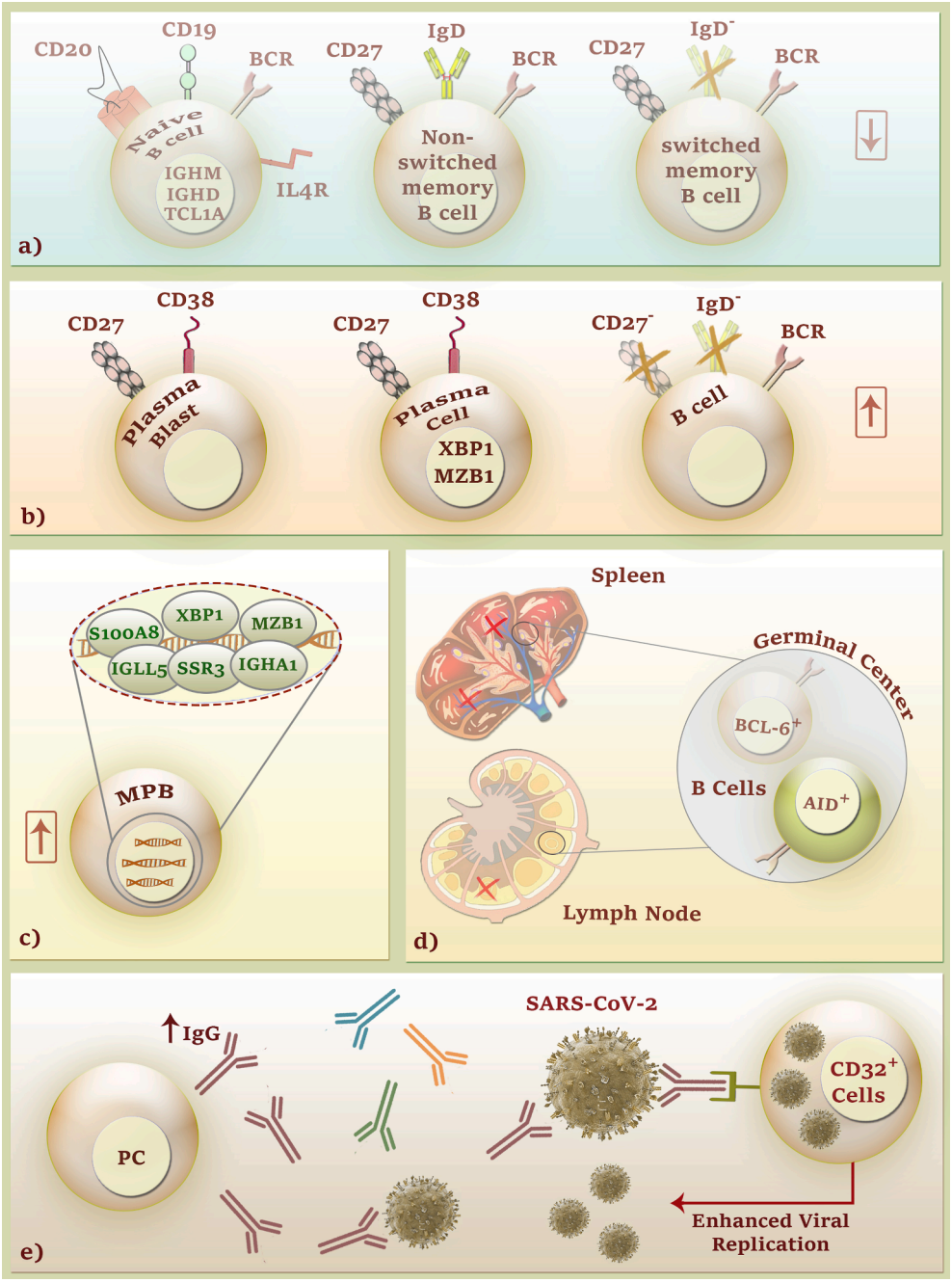

Fig. 1. A glance at the effect of SARS-CoV-2 on B lymphocytes. While infection with SARS-CoV-2 decreases the number of both naïve and memory B cells (switched and non-switched) **(a)**, the percentage of plasmablasts, plasma cells, and $IgD^-/CD27^-$ B cells is increased noticeably in COVID-19 patients **(b)**. Moreover, B cell activation-related genes, such as S100A8, IGLL5, SSR3, IGHA1, XBP1, and MZB1 were mainly expressed in the memory B cells and plasma cells (MPBs) **(c)**. There is also evidence of prominent loss of germinal centers in the lymph nodes and spleen coupled with the depletion of $Bcl\text{-}6^+$ B cells but the maintenance of AID^+ counterparts in acute COVID-19, which in turn, lead to reduced durability of humoral responses **(d)**. Due to the elevation in the amount of sub-neutralizing or cross-reactive non-neutralizing antiviral antibodies especially IgG, a mechanism entitled antibody-dependent enhancement (ADE) happens by which the pathogenesis of COVID-19 is boosted via enhanced viral replication **(e)**.

in Fig. 1.

2.2. T Lymphocytes in COVID-19

Studies show SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells –in harmony with other components of the immune system– first fight acute viral infection and then protect the body against re-infection. The results of some studies show that the number of T cells in severe and critically ill patients is significantly less than those who have mild/moderate cases of COVID-19. Among the different types of immune cells, it seems that T cells are more vulnerable to SARS-CoV-2, as the number of these cells is reduced to half the reference limit. Moreover, there is a correlation between the age and the number of T cells in COVID-19, as the patients with the age older than 60 tend to have a lower number of T lymphocytes; an event which may explain, at least partially, why older patients are at a higher risk for the SARS-CoV-2 infection [20,21]. Apart from an alteration in T cell count, there are several studies that have investigated the effect of SARS-CoV-2 on T cell activation. A study provides evidence of T cell activation in mild disease, but not severe, as compared to healthy controls [20]. While the superiority of $CD8^+$ T cell activation to $CD4^+$ T cells was evident in some reports [20,22], another study demonstrated that $CD4^+$ T cell activation was more pronounced in patients with the clinically more severe condition [7]. In the following, we discuss separately the $CD4^+$ and $CD8^+$ T cell responses to SARS-CoV-2 infection.

2.2.1. $CD4^+$ T cell responses in COVID-19

Grifoni et al. showed that the stronger SARS-CoV-2-specific $CD4^+$ T cell responses would result in a greater level of antibody in COVID-19 patients. The results of TCR-dependent AIM assays revealed that SARS-CoV-2 spike-specific $CD4^+$ T cells are detectable in all the cases with COVID-19. Their results also showed that COVID-19 infection

mostly stimulated TH1 cell polarization, since they produced a significant amount of IFNγ rather than IL4, IL5, IL13, or IL17a [23]. In agreement, Chen et al. declared that the proportion of IFNγ-producing TH1-like cells was higher in moderate COVID-19 cases compared to those with a severe condition [24]. While the role of TH2 responses in SARS-CoV-2 infection is not fully described, there are several studies proposing a potential role for TH17. In a study conducted by Xu et al, it has been indicated that the concentration of highly pro-inflammatory $CCR6^+$ TH17 cells was increased significantly [25]. The frequency of different types of $CD4^+$ T cells, including naïve T cells, effector memory T cells (EM-1/2/3), central memory T cells (CM), and $CD45RA^+$ effector memory cells as well as circulating TFH (cTFH) cells were examined in COVID-19 cases in an interesting study conducted by Mathew et al. While the population of naïve $CD4^+$ T cells was diminished in COVID-19 cases, the populations of both EM2 and $CD45RA^+$ effector memory cells were increased significantly. Also, the number of activated cTFH cells [$CD38^+$ $ICOS^+$] was higher in recovered donors than in healthy donors, perhaps reflecting residual COVID-19 responses in the former [7]. Accordingly, it has been indicated that the number of memory $CD4^+$ T cells with high levels of IL-7Rα (CD127) was significantly increased in the recovered donors. Indeed, patients with SARS-CoV-2-specific memory T cells have a higher chance of disease recovery, and the risk of COVID-19 is extremely low in individuals with peripheral COVID-19-specific memory T cells [26]. Taken together, $CD4^+$ T cells are directly and indirectly involved in the immune response against COVID-19 infection not only by the coordination of anti-viral responses but also via hampering the ability of SARS-CoV-2 to replicate; however, there are several studies now underway to precisely elucidate whether $CD4^+$ T cells are over-activated or functionally impaired within acute phase of COVID-19.

2.2.2. $CD8^+$ T cell responses in COVID-19

It was at the early stages of the COVID-19 outbreak that the adverse impact of the virus on the number, activation, as well as differentiation status of $CD8^+$ T cells, known as cytotoxic T cells (CTL), became evident in the severe cases. By using an activation-induced marker (AIM) and intracellular cytokine staining (ICS) assays, Grifoni et al. provide evidence suggestive of the prominent participation of $CD8^+$ T cells against SARS-CoV-2 in the recovered COVID-19 patients [23]. Accordingly, it has been declared that $CD8^+$ T cell clonal expansion either in bronchoalveolar lavage fluid [27] or peripheral blood [28] was directly correlated with COVID-19 recovery or the emergence of milder condition; however, the question concerning the fact that it is the reason or result of the recovery is still open to debate. In an interesting study and based on the increased ratio of $CD4^+$ T cells to $CD8^+$ T cells in COVID-19 patients, the authors concluded that probably cytotoxic T cells were migrated to the respiratory tract [29]. Accordingly, the results of a study conducted by Jiang et al. shed light on the fact that there is a negative correlation between the number of $CD8^+$ T cells and their activity during COVID-19 progression. In fact, they claimed that as the virus could probably diminish the population of peripheral lymphocyte subsets, the activity of $CD8^+$ T cells is compensatorily reinforced to bypass the pathogenesis of the virus [20]. In this vein, a previous study suggested the presence of an overaggressive $CD8^+$ T cell response in COVID-19 cases [30]. Also, increased cytotoxicity of CTLs has been proposed in several studies as $CD8^+$ T cells could express high levels of NK cell-related markers [31–33].

It has been indicated that the number of $Ki67^+$ proliferating CTLs or activated $CD8^+$ T cells that express CD38 and HLA-DR may be remarkably increased after vaccination with live-attenuated viruses or during the acute phase of a viral infection; an event which may possibly reflect the contributory role of virus-specific CTLs [34]. Accordingly, several studies are suggesting that the percentage of the aforementioned cells may elevate in most, if not all, of COVID-19 patients [31,35–37]. In addition, recent disclosures provide evidence indicating an increased number of SARS-CoV-2-specific CTLs in recovered COVID-19 cases [38–40]; further highlighting the fact that virus-specific $CD8^+$ T cell responses may be in charge of battle with the SARS-CoV-2 virus. Although there is implicit agreement on the role of $CD8^+$ T cells in the response against SARS-CoV-2, there are still conflicting issues that must be considered. In fact, one of the most important of these is how the results achieved through examination of T cell responses in peripheral blood can be generalized to lung events. Also, different CTL responses according to disease features should be considered while investigating adaptive immunity and its ability to defend from future infection with SARS-CoV-2.

2.2.3. Regulatory T cells in COVID-19

As regulatory T cells (Tregs) have a fundamental role in the maintenance of immune homeostasis, the reduction in the number of this group of lymphocytes during COVID-19 infection may be coupled with the aberrant activation of the immune system and subsequently induction of tissue damage, especially in the lungs, where the infection has the most activity. A remarkable decrease in the population of Tregs has been reported in COVID-19 patients, especially in the severe group [20,41,42]. Although there is little evidence about the mechanism that is responsible for the reduction of circulating Tregs, one of the most possible scenarios is they migrate to the lungs in order to protect the host cells from the deteriorating effects of immune responses. Notably, there is a piece of evidence showing that the Middle East coronavirus possesses the ability to infect T cells [43]; so there is a possibility that the reduction of Tregs in COVID-19 patients may be attributed to the direct impact of the virus on these cells [44]. Decreased transcription of IL-2 has been also detected in the isolated $CD4^+$ T cells from the bronchoalveolar lavage of severe COVID-19 patients. One of the obvious conclusions that can be drawn is that the reduction in IL-2 is associated with Tregs apoptosis, which was further confirmed by the reduced expression of FoxP3 [44]. Additionally, the identification of elevated concentrations of soluble CD25 (sCD25) in severe cases of COVID-19 patients [2] shed light on the possibility that COVID-19-induced inflammation may cleave cell surface CD25 and release it in the form of sCD25 in the bloodstream. Soluble CD25 now has the ability to interact with IL-2 and prevent its association with CD25 expressed on Tregs, thereby trigger apoptosis.

2.2.4. T Cell exhaustion

The decrease in the number of T cells is not the only abnormality during COVID-19 infection, as due to the severe and prolonged infection a group of T cells may acquire the exhausted phenotype that is mainly detectable by the massive expression of markers known as exhausted markers, including PD-1 (programmed cell death 1; CD279) and TIM-3 (T cell immunoglobulin and mucin domain-containing-3; CD3 [21,45,46]. In fact, the increase in the expression of PD-1 and Tim-3 is representative of the progression of the disease from prodromal to symptomatic stages. FACS analysis in severe cases of COVID-19 patients, especially those who were admitted to ICU, revealed a higher expression of PD-1 as compared to healthy counterparts, suggestive of the ability of SARS-CoV-2 in enforcing T cells to express exhaustion markers [21]. Weiskopf et al. suggested Tim-3 overexpression as a hallmark of poor prognosis in COVID-19 patients [30]. Apart from the indicated markers, elevation in the expression levels of NK group 2 member A (NKG2A) on either NK cells or effector T cells was reported in COVID-19 patients. Indeed, expressed NKG2A could halt the cytotoxic effects of these antiviral members of the immune systems and lead to disease progression [47]. Interestingly, Zheng et al. found that decreased expression of NKG2A was coupled with the elevation in the number of cytotoxic lymphocytes (NK cell and T cells) after disease recovery [24]; shedding more light on the competency of exhausting markers as plausible prognostic factors.

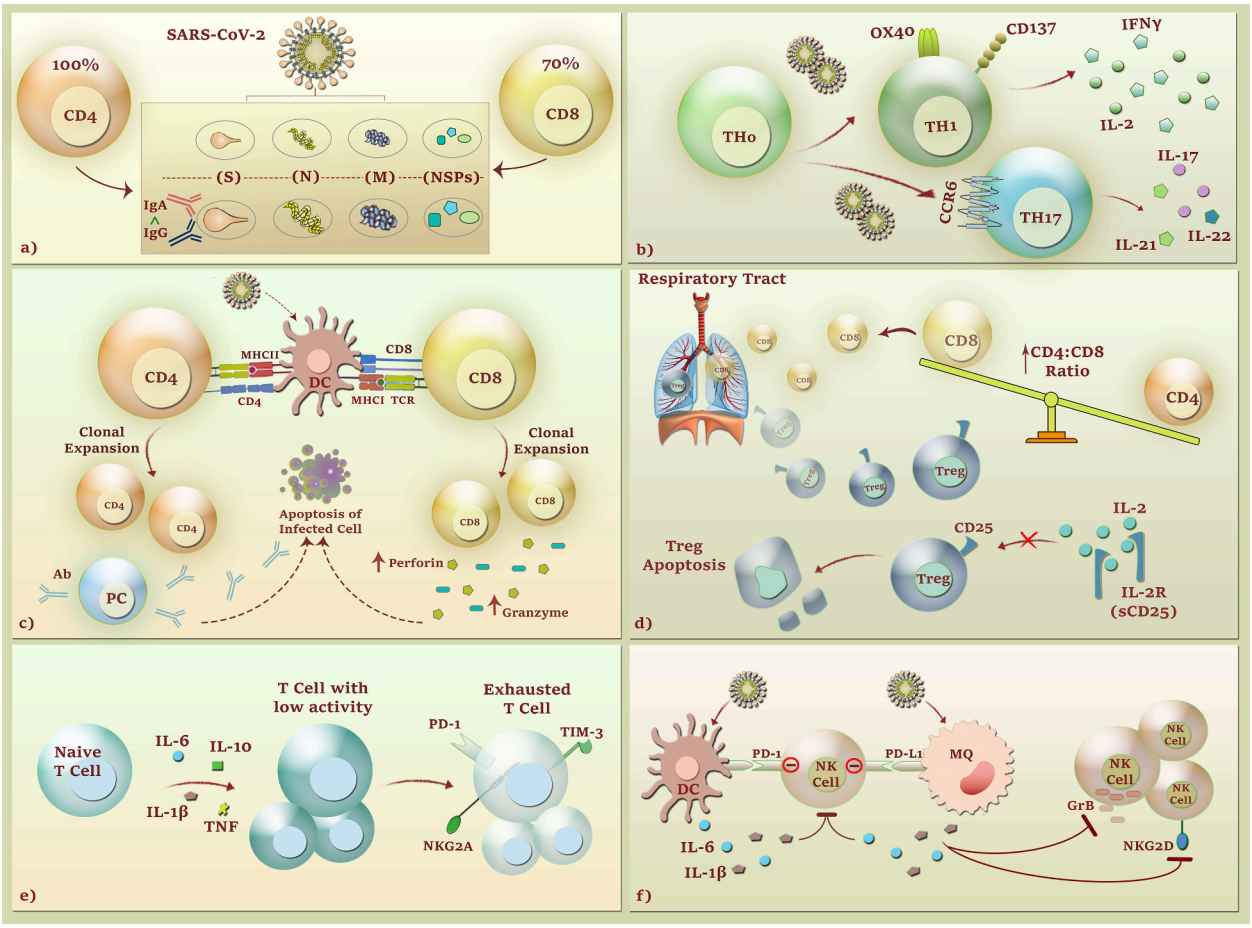

Fig. 2. A glance at the effect of SARS-CoV-2 on T cells and NK cells. Both $CD4^+$ and $CD8^+$ T cells –specific against different parts of the virus including spike (S), membrane (M), nucleocapsid (N), and nonstructural proteins (NSPs)– were recognized in 100% and 70% of convalescing COVID-19 patients' epitope pools, respectively. Spike-specific $CD4^+$ T cell responses were associated with the elevation in the amount of the anti-virus IgG and IgA **(a)**. In addition, SARS-CoV-2 infection frequently stimulated TH1 cell polarization, as they produced a high level of IFNγ. Also, the percentage of TH17 may increase in COVID-19 patients **(b)**. The virus endocytosis and degradation are taken place by the APCs like dendritic cells (DCs) and then the antigens of the virus are presented by MHCs to the T cells. When a $CD8^+$ T cell binds to MHC-I, clonal expansion occurs and infected cells are directly targeted via secretion of cytotoxic proteins, such as perforin and granzymes. In the case of $CD4^+$ T cells involvement, they can activate SARS-CoV-2-specific B cells to clonally proliferate and secrete antibodies to target the SARS-CoV-2 virus **(c)**. The CD4:CD8 ratio is increased in COVID-19 patients which may be explained, at least partly, by the migration of $CD8^+$ T cells to the respiratory tract. Also, the percentage of circulating Tregs is decreased mainly due to apoptosis of these regulatory cells as a result of IL-2 neutralization by the increased level of sCD25 **(d)**. Increased secretion of pro-inflammatory cytokines leads to over-expression of PD-1, Tim-3, and NKG2A, which is suggestive of the ability of SARS-CoV-2 in enforcing exhaustion phenotype in T cells **(e)**. Also, IL6 could hamper the expression of both GrB and NKG2D, an event which in turn leads to decreased cytotoxicity of NK cells **(f)**.

2.3. NK cell responses in COVID-19

Natural killer (NK) cells are one of the most important arms of the innate immune system that their significant responsibility is to defend against viral infections at the initiating stage without the need for specific identification of antigens. Also, by constructing a network with other immune cells like dendritic cells, NK cells are enabled to simultaneously transmit the stimulating signal to the adaptive arm of the immune system [48]. It seems that NK cells are one of the targets of SARS-CoV-2, as several pieces of evidence clearly showed their reduction in COVID-19 patients, especially those who are classified as critically ill [8,45,49]. Despite a piece of evidence, still it is ambiguous whether the reduction in the number of NK cells is due to the ability of the virus to induce apoptotic death or is due to their redistribution into infected sites. In support of the latter, the identification of a great number of NK cells in bronchoalveolar lavage fluid (BALF) samples from COVID-19 patients clearly suggests that NK cells are accumulated in the lungs during viral infection [50]. In another analysis of BALF samples of COVID-19 patients, while the number of quiescent NK cells was reduced, there were no differences in the population of activated NK cells [45].

Apart from the decreased number, there are also several studies reporting SARS-CoV-2-induced effects on the function of NK cells. Notably, while the expression of Granzyme A (GrA) was increased in NK cells in mild and severe types of the disease, their capability to express this enzyme was reduced as the disease progressed into more serious stages [20]. As mention previously, Zheng et al. hypothesized that due to the induction of exhaustion phenotype in NK cells during COVID-19 infection, the innate immune responses might be severely compromised in severe cases of the disease [45]. It has been indicated that local and systemic inflammation, specifically increased secretion of IL6 by the infected dendritic cells and macrophages, could suppress GrB expression and hamper NK cell cytotoxicity in COVID-19 [51]. IL-6 could also halt the expression of NKG2D, one of the leading receptors on the surface of NK cells that are responsible for eliminating infected cells [52]. All in all, the abstract of these finding suggests that while a group of NK cells abandon peripheral blood and migrate to the lungs to defend against the virus, an event which ultimately results in local inflammation and injury, circulating NK cells undergo the exhaustion phenotype that

paralyzes the immune system to prevent virus spread. Fig. 2 represents a schematic to provide a well-conceptualized overview of the roles of T cells and NK cells in SARS-CoV-2 infection.

Taken all together, infection with SARS-CoV-2 may affect B cells, T cells, and NK cells. Concerning B lymphocytes, SARS-CoV-2 decreases the number of both naïve and memory B cells. There is also evidence of prominent loss of germinal centers in the lymph nodes and spleen coupled with the depletion of Bcl-6$^+$ B cells in acute COVID-19 which may explain, at least partly, the low durability of humoral responses. In the case of CD4$^+$ T cell involvement, SARS-CoV-2 infection frequently stimulates polarization of TH_1 cells which are able to activate specific B cells to clonally proliferate and secrete antibodies to target the virus. On the other hand, virus-specific CD8$^+$ T cells can directly target infected cells via secretion of cytotoxic proteins, such as perforin and granzymes. While SARS-CoV-2 can reduce both CD4$^+$ and CD8$^+$ T cells, it mainly affects the latter as the ratio of CD8:CD4 often decreases in COVID-19 patients. Also, this infection may reduce the percentage of circulating Tregs mainly due to apoptosis induction as a result of IL-2 neutralization by the increased level of sCD25. Induction of exhausted T cells and NK cells also should be taken into account as an important way that SARS-CoV-2 may apply to hamper host immunity.

3. Prognostic factors associated with lymphocytes in COVID-19

3.1. Lymphocyte count

Several pieces of evidence suggest that lymphocyte count can be a valuable parameter for risk stratifying of COVID-19 patients into 3 groups of moderate, severe, and critically ill. This idea is originated from the fact that the total number of lymphocytes may be significantly diminished in COVID-19 patients, with the notion that the reduction is more highlighted in the critically ill patients [20]. A significant decrease of T lymphocytes has been also shown to be positively correlated with in-hospital death and severity of illness [53]. Accordingly, Huang et al. demonstrated that patients with increased mortality, acute respiratory distress syndrome, and those who need intensive care have a lower number of lymphocytes as compared to their counterparts with better prognosis [54]. The results of a recent meta-analysis suggested that the estimated pooled mean of lymphocytes in non-severe cases was 0.95 (95% CI, 0.81–1.10), whereas it was 0.70 (95% CI, 0.52–0.87) in severe COVID-19 patients ($P < 0.01$) [55]. The same result was obtained in another study conducted by Bermejo-Martin et al. [56] who introduced lymphopenia as a severe COVID-19 signature. Notably, lymphopenia was common in the critically ill patients with the MERS infection which may be a result of lymphocytes apoptosis [57,58]. Overall, these findings indicated that the number of lymphocytes retains a specific clinical and biological significance in COVID-19 and lymphopenia is seemingly an important hematological abnormality that contributes to mirror the evolution toward an unfavorable outcome.

3.2. TLM (time to lymphocyte model)

Tan et al. have established a new index for categorizing COVID-19 patients according to a time to lymphocyte model (TLM). Based on their observation, the lymphocyte count of the patients could be used to determine the prognosis of the patients; however, the results might be a bit different according to the time of evaluation. At the first time period (TLM-1), which is in 10–12 days after the onset of the symptoms, those whose lymphocyte percentage (LYM%) is higher than 20 would have a moderate form of the disease and conversely, those with LYM% lower than 20 are categorized as high risks. If LYM% is evaluated 17 to 19 days after the manifestation of the symptoms, known as TLM-2, the classification of the patients is quite different. LYM% higher than 20 is suggestive of the recovery of the patients, LYM% between 5 and 20 is showing that the patient still needs supervision, and less than 5 is indicative of the patient with a high risk of mortality who needs intensive care [59].

3.3. NLR (neutrophil to lymphocyte ratio)

The ratio of neutrophil to lymphocyte counts, known as NLR, is a predominant biomarker of systematic inflammation and is widely used in bacterial infections to predict the outcome of the patients, especially those who have pneumonia. Bacterial infections are not the only condition in which calculating NLR would be beneficial, as this index could provide valuable information about the prognosis of patients in different types of diseases ranging from human cancers to other inflammatory diseases such as acute coronary syndrome, intracerebral hemorrhage, polymyositis, and dermatomyositis. Interestingly, it became recently evident that the shift in the count of WBCs towards neutrophils rather than lymphocytes occur in severe cases of COVID-19, suggesting that probably the calculation of NLR could help the physician to appropriately risk stratify the patients [20]. Feng et al. designed an interesting study to investigate if there is a correlation between NLR and prognosis in COVID-19 patients. In their study, they observed a significant increase in NLR during the severe phase [60]. Their results have been confirmed by a study conducted by Eid et al. They indicated high sensitivity of this ratio in patients aged over 50 years and suggested it as a quick and simple tool with excellent efficacy for screening patients who need more attention and careful evaluation due to their high vulnerability to the respiratory deterioration [61]. A double-center study also obtained interesting results as they found that patients with NLR value greater than 6.5 had more complicated clinical outcomes and those with NLR value of 9 or higher mostly faced death [62].

3.4. PLR (platelet to lymphocyte ratio)

The platelet to lymphocyte ratio (PLR) is confirmed to be a good candidate for predicting the outcome of patients with different diseases, including human cancers, acute pancreatitis, and cardiovascular disease. Meng et al. reported the correlation between PLR and disease severity in hepatitis C-infected patients [63]. The same result was reported by Ye et al. who showed that acute heart failure (AHF) patients with the higher PLR experienced a poorer outcome [64]. Notably, it has been indicated that PLR could be an indicator of disease severity in COVID-19 cases and may be helpful in predicting disease prognosis [65]. According to a recent investigation perform by Qu et al., while univariate analysis introduced both age and PLR as prognostic factors for COVID-19 patients, the multivariate analysis failed to find an association between these parameters and the outcome of the patients. They also reported that ΔPLR could provide a valuable schematic about the duration of hospitalization of patients; the higher the value is, the longer patients should stay at the hospital [66]. Lu et al. revealed that once patients improve (on the 14th day), the value of RLP is going to decrease and gradually be close to normal [67]. Taken together, all these results shed light on the fact that PLR may probably serve as one of the key prognostic factors in the COVID-19 infection.

3.5. Existence of atypical lymphocytes

The role of atypical lymphocytes (ALC), which are a group of medium to large size lymphocytes with the condensed chromatin and basophilic cytoplasm, in the prognosis of infectious diseases is still a debatable issue. For example, while Clarice et al. indicated that ALC is a marker of poor prognosis in patients with severe dengue infection (DI) [68], other studies failed to find such a correlation [69]. In a study by Jamal et al., it has been established that the presence of ALC in the peripheral blood of COVID-19 patients is a common event, even in cases with lymphopenia. More interestingly, unlike other viral infections in which the presence of classic Downey II-like cells are very common, SARS-CoV-2 infection is associated with the presence of atypical plasmacytoid lymphocytes in the peripheral blood [70]. Accordingly, Foldes

Table 1
A summary of the prognostic factors associated with lymphocytes in COVID-19.

Patients No.	Age (Y)	Female	Outcome	Ref
Lymphocyte count				
198	X: 50	49%	In this single centre cohort of COVID-19 patients, the most common symptom was fever, and the most common laboratory abnormality was decreased blood T cell counts (45.8% patients on admission). Patients admitted to ICU were older and had a significant reduced T lymphocytes.	[72]
452	M: 58	48%	T lymphopenia, in particular, decrease of $CD4^+$ T cells, were common among patients with COVID-19, and more evident in the severe cases. But no significant change was observed in the number of $CD8^+$ cells and B cells.	[20]
187	M: 62	45%	As the severity of COVID-19 getting worse, the counts of T lymphocyte drop lower. 28 patients died in hospital, the median T and B lymphocyte were significantly lower in the expired cases than other patients. Lower counts (/μL) of T lymphocyte subsets and B cells were associated with higher risks of in-hospital death of CIVID-19.	[73]
1099	M: 47	42%	Lymphopenia was present in 83.2% of the patients on admission, and patients with severe disease had more prominent lymphopenia and leukopenia than those with non-severe disease.	[74]
191	≥18	38%	Baseline lymphocyte count was significantly higher in survivors than non-survivors. In survivors, lymphocyte count was lowest on day 7 after illness onset and improved during hospitalization, whereas severe lymphopenia was observed until death in non-survivors	[75]
135	M: 47	50	Compared to the mild cases, the severe ones had lower lymphocyte counts and higher plasma levels of d-dimer, LDH, and CRP	[76]
138	M: 56	46%	During hospitalization, most patients had marked lymphopenia, and non-survivors developed more severe lymphopenia over time. White blood cell counts and neutrophil counts were higher in non-survivors than those in survivors.	[77]
242	M: 57	50%	Lower lymphocyte percentage was found in severe cases, compared to non-severe cases. Noticeably, the percentages, but not absolute counts of lymphocytes, were lower in severe patients when compared to non-severe patients.	[78]
150	<20–90	NA	There were significant differences in white blood cell counts, absolute values of lymphocytes, CRP, and IL-6 between died and discharged groups.	[79]
52	M: 60	43%	Lymphopenia occurred in more than 80% of critically ill patients. In non-critical, 35% of patients had only mild lymphopenia, suggesting that the severity of lymphopenia reflects the severity of SARS-CoV-2 infection.	[75]
112	NA	NA	Compared with the general group, the lymphocyte count (0.74×10^9/L vs. 0.99×10^9/L, $P = 0.03$) was extremely lower in the critical group.	[80]
NLR				
452	M: 58	48%	A higher number of neutrophils and a lower number of lymphocytes were found in the severe group with COVID-19 compared to the mild group.	[20]
245	X: 54	53%	There was 8% higher risk of in-hospital mortality and the fully adjusted OR for mortality was 1.1 in males for each unit increase in NLR. NLR is an independent risk factor of the in-hospital mortality for COVID-19 patients especially for male.	[81]
1320	M: 52	39%	NLR greater than 6.5 may reflect the progression of the disease towards an unfavorable clinical outcome, with this notion that the ratios higher than 9 may strongly result in death.	[62]
93	M: 46	50%	Elevated age and NLR can be considered independent biomarkers for indicating poor clinical outcomes.	[65]
301	M: 51	50	Having an NLR ⩾2.973 (HR 2.641, 95% CI 1.421–4.908; p = 0.002), age ⩾50 years (HR 2.504, 95% CI 1.202–5.215; p = 0.014) and being male (HR 2.004, 95% CI 1.101–3.647; p = 0.023) were identified as risk factors for progression by multivariate Cox regression analyses.	[82]
74	M: 63	31%	Patients with severe disease were significantly older and had a significantly higher NLR compared with non-severe cases. A higher NLR at hospital admission was associated with a more severe outcome: in particular, a NLR of *greater than 4* was a predictor of admission to the ICU.	[83]
131	M: 64	57%	The NLR of 3.3 was associated with all-cause mortality, with a sensitivity of 100% and a specificity of 84%. NLR of 2.3 might have potential value for helping clinicians to identify patients with severe COVID-19, with a sensitivity of 100% and a specificity of 56.7%.	[2]
225	M: 60	42%	Not only admission NLR correlated with mortality but also the nadir quantity of NLR was higher in the non-survived patients. NLR was higher than 3 in all of the deceased patients, and it seems that elevated NLR could be used as a prognostic value for improved mortality prediction.	[84]
81	X: 50	37%	NLR could be a valuable biomarker to recognize severe COVID-19 patients with moderate-severe ARDS, which facilitated clinicians to give effective respiratory supporting strategies and quickly find out moderate-severe ARDS	[85]

(*continued on next page*)

Table 1 (*continued*)

	Patients No.	Age (Y)	Female	Outcome	Ref
				patients who are at high indication for V-V ECMO.	
	100	X: 52	31%	The NLR was found the most sensitive hematological marker with an AUC of 0.799 for ICU stay at 88% sensitivity and 90% PPV, while a value of 4.16 predicted mortality at a sensitivity of 91% and PPV of 96%. Around 90% of ICU patients along with non-survivors had NLR > 3 and one-half of them had NLR > 9 at admission.	[86]
PLR					
	30	M: 50	46%	The PLR of patients might provide a new indicator in the monitoring of patients with COVID-19: the higher PLR of patients during treatment, the longer time of the hospitalization.	[66]
	93	M: 46	40%	The age and PLR of severe ill patients were significantly higher than those of non-severe patients.	[65]
	131	M: 64	57%	There were no significant differences in PLR for non-survivors, when compared to survivors. The PLR has no observed value for distinguishing the severity and predicting the death of patients with COVID-19.	[2]
	225	M: 60	42%	PLR correlated with mortality and was significantly higher in non-survivors. Elevated PLR and lower Hb at the time of admission associated with mortality and also ICU admission.	[84]
	100	X: 52	31%	PLR was elevated more in ICU (P = 0.004), and deceased patients.	[86]
Existence of atypical lymphocytes					
	33	NA	NA	Atypical lymphocytic is common in SARS-CoV-2 patients' peripheral blood. Specifically, atypical plasmacytoid lymphocytes are highly associated with SARS-CoV-2, which is unusual among viral infections. The classic Downey II-like cells, which are generally common in viral infections, are less frequently found in SARS-CoV-2 infection.	[70]
	1	59	0	Lymphoplasmacytoid lymphocytes with an eccentric nucleus, deeply basophilic cytoplasm and a prominent paranuclear hof were observed.	[71]

LDH: Lactate Dehydrogenase; **CRP:** C-reactive protein; **NLR**: Neutrophil-to-lymphocyte ratio; **HR**: Hazard ratio; **V-V ECMO:** Veno-venous extracorporeal membrane oxygenation; **AUC:** Area under the curve; **PPV:** Positive Predictive Value; **PLR:** Platelet-to-lymphocyte ratio; **NA**: Not available; **X:** Mean; **M:** Median; **Y:** Year.

et al. reported that blood film of COVID-19 showed Mott cells, which are a group of ALCs with noticeable cytoplasmic inclusions [71]. Taken together, the presence of atypical lymphocytes has been shown in COVID-19 patients, but the association between these lymphocytes and the severity of the disease is still unclear and further studies are needed. To provide a well-conceptualized overview of the prognostic factors associated with lymphocytes in COVID-19, we summarized all these data in Table 1.

4. Pathophysiology for lymphopenia in COVID-19

4.1. Direct infection of lymphatic organs

One of the main mechanisms that causes lymphopenia in COVID-19 patients could be due to the direct attack of SARS-CoV-2 on lymphatic organs, such as the thymus and spleen [87]. Ding et al. have previously reported that the SARS virus could destroy the germinal centers, especially in the pulmonary hilar lymph nodes [88,89]. A mounting body of evidence showed that SARS infection is associated with the atrophy of splenic white pulp and disappearance of the germinal centers [89–92]. The same results were obtained by Falasca et al., who reported that the splenic white pulps of COVID-19 patients showed lymphoid hypoplasia with congested red pulp [93]. Notably, direct destruction of some lymphatic organs such as lymph nodes and spleen with COVID-19 has been reported by Feng et al. Their histopathological examination showed that the spleens were congested, hemorrhagic, and the spleen corpuscles were atrophic with increased fibrous tissue hyperplasia in the splenic sinus [94]. They also proposed a model in which SARS-CoV-2 disseminates to the secondary lymphoid organs, leading to Fas up-regulation and IL-6 secretion, which in turn, trigger activation-induced cell death in lymphocytes resulting in lymphopenia. Interestingly, the authors detected that ACE2-expressing $CD68^{+}CD169^{+}$ macrophages were in the splenic marginal zone and marginal sinuses of lymph nodes. In addition, the expression of IL-6 was elevated in SARS-CoV-2 infected macrophages which have nucleoprotein antigen. Of particular interest, the results of the tunnel test indicated that apoptosis and disorganization in the secondary lymphoid organs of COVID-19 patients were very common [94]. In another study, Liu et al. reported that the postmortem spleens were generally contracted with shrinking capsules, mixed thrombi, hemorrhage, and anemic infarction. Moreover, COVID-19 spleens showed a prominent absence of lymphoid follicles as compared to control spleens from patients with abdominal trauma necessitating splenectomy [95]; further highlighting the deteriorating effect of SARS-CoV-2 on the spleen. Elsoukkary et al. also indicated that the subcapsular and intraparenchymal sinuses of the spleen and/or lymph nodes were enlarged and often contained a variable number of larger transformed cells [96]. All in all, these results further highlighted the probable ability of COVID-19 to induce direct damage to lymphoid tissues and lymphopenia under its influence; however, this hypothesis requires to be validated by further comprehensive pathological studies.

4.2. Direct infection of bone marrow

The evidence supported that the influence of SARS-CoV-2 on lymphocyte precursors could also be traced back to bone marrow (BM), where hematopoietic stem cells (HSCs) developed into common lymphoid progenitors (CLP). This idea has been developed from the recent disclosure which indicated the high expression of both CXCL10 and CCL2 in COVID-19 patients, especially in severe cases who suffer from lymphopenia [97–99]. The suppressive impacts of these chemokines on the survival of HSCs have been well-established in previous reports and it could be assumed that perhaps the overexpression of CXCL10 and CCL2 might be involved in the SARS-CoV-2-mediated lymphopenia [100]. Microscopic analyses of BM in COVID-19 decedents also showed several abnormalities, including increased myeloid/erythroid (M/E) ratio, a relative increase in overall cellularity, and in particular, the presence of scattered macrophages that engulfed erythroid cells which is consistent with the diagnosis of hemophagocytic histiocytosis [101]. The same result was obtained in a study conducted by Falasca et al., who reported the presence of numerous macrophages with the features of hemophagocytosis. Notably, the results of BM examination showed a prominent white pulp lymphoid hypoplasia coupled with conspicuous hyperplasia in the megakaryocyte lineage [93]. Another point that could confirm the destructive effects of SARS-CoV-2 on BM and its residential cells is the high expression of the

ACE2 receptor in this organ. ACE2 receptor, as explained earlier, is the main receptor that participates in SARS-CoV-2 entrance into the target cells. Although the expression of this receptor seems to be low in BM, it is reasonable to assume that the devastating effect of SARS-CoV-2 on lymphocytes might, at least partly, be due to BM suppression [102]. However, further investigation in this field is required to validate this mechanism.

4.3. Cytokine storm

It has been suggested that the cytokine storm is probably the main player of COVID-19-induced lymphopenia. While the concertation of the pro-inflammatory cytokines is reduced to the normal level in recovered patients, basic researches confirmed the increased serum levels of pro-inflammatory cytokines and showed that up-regulated TNF-α and IL-6 may take a part in the induction of lymphopenia through inducing apoptosis in T cells [103]. This disclosure was also confirmed by the results of autopsy studies on lymphoid tissues of COVID-19 patients, which showed massive lymphocyte death due to the high level of IL-6 and Fas-FasL interactions [104,105]. Dia et al. also demonstrated the converse correlation between the number of lymphocytes and the serum levels of TNF-α, IL-6. Reciprocally, changes alteration in the level of the lymphocyte subgroups may also lead to an immune dysregulation with the induction of thumping cytokine and chemokine response, all of which may enhance cytokine storm leading to multi-organ dysfunction [21]. Although the lungs are the primary target sites affected by the elevated levels of these cytokines, the injury of non-pulmonary tissues is also common, and damage to the lymphoid organs such as the spleen and thymus shall not be considered an exception to this rule. Taken together, increased secretion of inflammatory cytokines may exacerbate lymphopenia, either through a direct or indirect manner, especially in severe cases of COVID-19. While previous studies reported that the excessive production of inflammatory cytokines may be in charge of COVID-19-induced lymphopenia, on the other hand, there may be a reciprocal connection suggesting that impaired lymphocyte activity may result in overproduction of pro-inflammatory cytokines in a compensatory manner. Indeed, it should be noted that the inability of the adaptive immune system in eradicating the viruses may result in the hyperactivation of the immune system, which in turn, causes the excessive release of inflammatory mediators to compensate either lymphopenia or lymphocyte dysfunction; an event that consequently may lead to the cytokine storm syndrome [106]. However, the precise mechanism of such an issue should be clarified.

4.4. The emergence of metabolic disorders

It has been shown that some metabolic products elevate the risk of poor disease outcomes and mortality in COVID-19, mainly through deregulation of the host immune system. In an interesting study, Fischer. et al. declared that exposure to external lactic acid resulted in the decreased proliferative capacity of human cytotoxic T lymphocytes via blockade of lactate efflux and thereby disturbance of T-cell metabolism [107]. Accordingly, Tan et al. reported the elevation in the lactic acid levels in severe COVID-19 patients and suggested it can diminish the proliferative capacity of lymphocytes [59]. The data extracted from a study conducted by Henry et al. indicated the correlation between elevated lactate dehydrogenase (LDH) and the development of the severe form of the disease as well as the increased risk of mortality in COVID-19 cases [108]. Additionally, Lu et al. indicated that there is a significant difference between the levels of lactic acid and LDH in patients who suffer from severe COVID-19 and those who have a mild form of the disease [109]. In a similar study, the serum level of LDH has been proposed as a biomarker for risk stratification of the patients and also for monitoring the response rate to the treatment [110]. Cure et al. reported that Dapagliflozin –a sodium-glucose cotransporter-2 (SGLT2)– is enabled to postpone the severe course of COVID-19 by preventing the acidification of cytoplasm and diminishing the proportion of viral particles [111]. The same result was obtained in a case report study conducted by Chhetria et al. [112]. Lactic acid acidosis may also increase the risk of COVID-19 complications for patients through inhibition of lymphocyte proliferation, and subsequent lymphopenia [113].

4.5. Expression alteration of genes that are involved in lymphocyte proliferation/apoptosis

SARS-CoV-2 can reduce the expression of certain genes that affect lymphocytes, thereby affecting the proliferation and activity of these cells. Xiong et al. indicated that several genes that are involved in the apoptosis pathways are upregulated in mononuclear cells of peripheral blood from COVID-19 patients; suggesting that lymphopenia may occur as a result of SARS-CoV-2-mediated apoptosis [114]. In this vein, the results of a previous study demonstrated that the infection of Vero E6 cells (monkey kidney cells) with SARS-CoV induced death in the infected cells. As the phosphorylation level of p38 MAPK was increased 18 h post-infection in virus-infected cells, they suggested that the activation of this pathway may participate in SARS-CoV-induced cell death [115]. In addition, a report suggested that the expression levels of MAP2K7 and SOS1 were decreased in T cells of severe COVID-19 [105], which is in agreement with the results reported by Ouyang et al. Interestingly, while the expression of MAP2K7 and SOS1 were down-regulated during the disease progression, Ouyang et al. showed that the expression pattern of these genes was reversed and returned to normal levels upon recovery [116]; further highlighting the fact that the alteration of the aforementioned genes may be considered, at least partly, as a mechanism underlying SARS-CoV-2-induced lymphopenia.

4.6. Epigenetic alteration and dysfunction of lymphocytes

One of the questions that occupied the mind of researchers from the emergence of COVID-19 infection was why some patients ended with the severe condition, while others may have mild respiratory disease. To answer this heterogeneity, many researchers have narrowed their view on epigenetic alteration as it is an age-related phenomenon [38,117–119]. The validation of this idea became even stronger when the evidence reported the presence of the methylation site on the CpG Island (cg08559914) located near the promotor of the ACE2 receptor, which controls the permeability of cells to SARS-CoV-2 [120]. Another support that suggested the importance of epigenetics in COVID-19 is the association between sex and the severity of the disease. It has been indicated that women with different age categories have a better outcome than men probably due to the sexual dimorphism in the immune system. These results became even more interesting as previous studies suggested the higher frequency of lymphopenia in male patients compared to female counterparts [79,121]. Given these and based on the role of epigenetic alterations in regulation of lymphocyte functions in men and women [122,123], intense attention has been attracted to the role of this phenomenon in the vulnerability of lymphocytes to SARS-CoV-2. It did not take long that the correlation between age-associated DNA methylations and COVID-19-induced lymphopenia has been described. Wang et al. noticed that there is an association between the expressions of a histone methyltransferase G9a and lymphopenia incidence in COVID-19 patients. Their results suggested that those patients with overexpressed G9a had server conditions due to the impairment in T cell functions and excessive inflammatory responses [124]. Kouidou et al. also cited the correlation between ten-eleven translocase 2 (TET2) expression in COVID-19 patients and disease mortality due to the immune system failure, as TET2 is a DNA methyltransferase that widely regulates immune responses [125]. In another study, the results of genome-wide DNA methylation profiles revealed the presence of DNA methylation dysregulation in PBMCs harvested from COVID-19 patients. Their results showed the incidence of the hypermethylation pattern in the promotor of genes encoding different types of

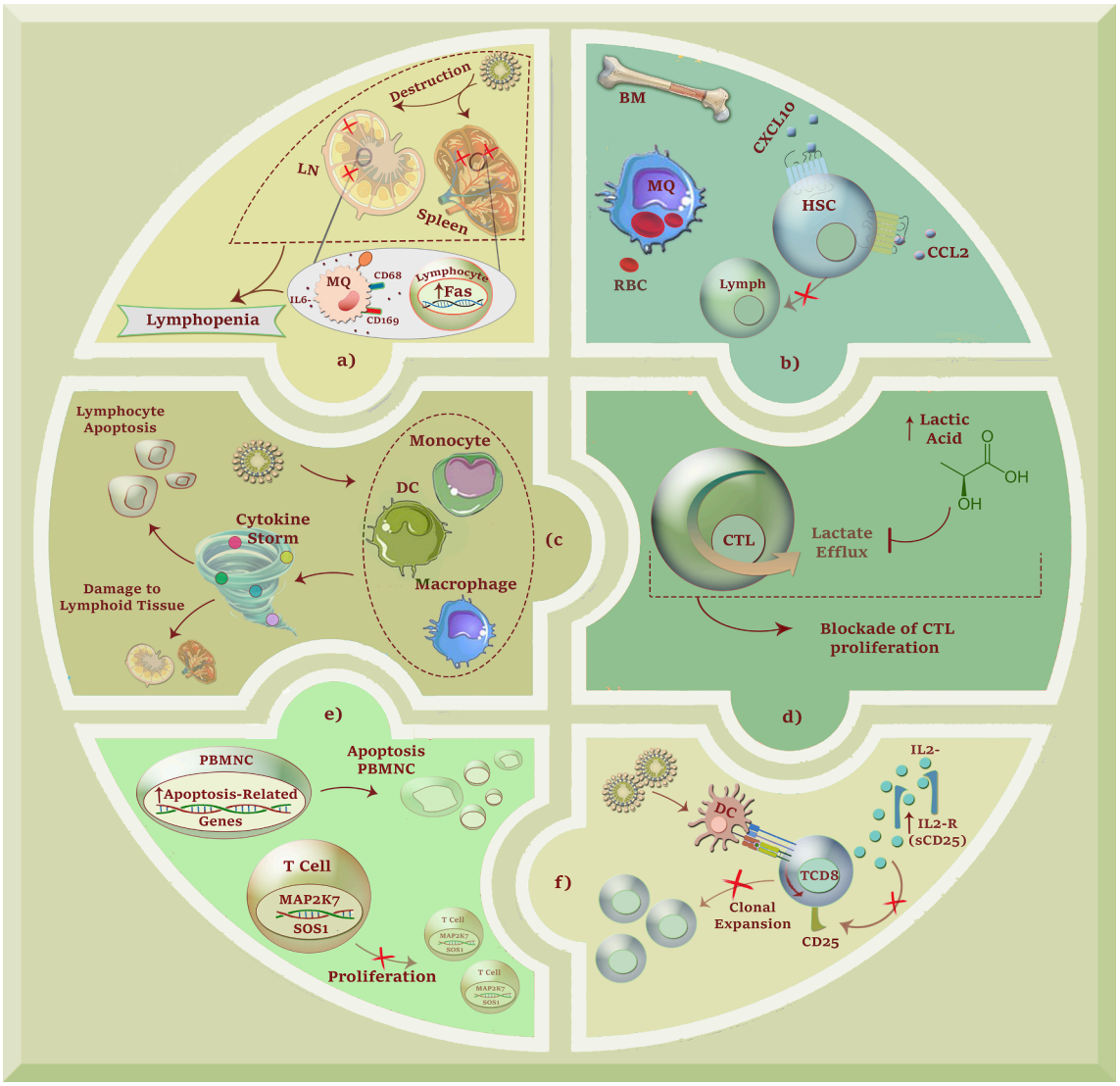

Fig. 3. Pathophysiology of lymphopenia in COVID-19. SARS-CoV-2 is also capable of inducing lymphopenia via direct destruction of lymphoid tissues, overexpression of Fas, and stimulation of IL-6-producing macrophage **(a)**. The overexpression of CXCL10 and CCL2, which have well-known suppressive impacts on the survival of HSCs, may cause reduced lymphopoiesis and might be involved in the SARS-CoV-2-mediated lymphopenia. The presence of scattered macrophages that engulfed erythroid cells is also a conspicuous finding in BM of COVID-19 patients **(b)**. The increased serum levels of pro-inflammatory cytokines such as TNF-α and IL-6 may take part in induction of lymphopenia, either via a direct or indirect manner **(c)**. Lactic acid acidosis may increase the risk of COVID-19 complications through blockade of lymphocyte proliferation and subsequent lymphopenia **(d)**. Not only the transcription of apoptosis-related genes is increased in peripheral blood mononuclear cells (PBMNCs) but also the expression levels of proliferation-related genes (MAP2K7 and SOS1) are decreased, leading to reduced T cell proliferation **(e)**. Extra-released sCD25 binds to IL-2 and impedes its interaction with T lymphocytes, an event that adversely affects T cell clonal expansion **(f)**.

INFs, as well as the hypo-methylation pattern of inflammatory-related genes [126]. Taken together, these findings not only shed light on one of the probable mechanisms through which SARS-CoV-2 may develop lymphopenia in patients but also suggested that perhaps the study of methylation pattern of some immunoregulatory genes in COVID-19 could provide a new insight in stratifying the patients and predict their outcome [127].

4.7. Immune activation and increased expression of CD_{25}

When it comes to IL-2, many studies have tightened this cytokine to the proliferation, differentiation, and function of different types of T lymphocytes ranging from $CD4^+$ T regs and helper T cells to $CD8^+$ cytotoxic effectors [128]. Upon immune stimulation, CD25 (IL-2Ra) is expressed on activated T cells and its soluble form (sCD25) releases into the bloodstream to prevent the unnecessary activation of T cells. In many inflammatory-based diseases, extra-released sCD25 binds to IL-2 and prevents its interaction with T lymphocytes, an event which in turn negatively regulates T cells, and may induce lymphopenia [44,54]. In a study done by Wang et al., they concluded that the level of sCD25 was gradually increased with increased severity of illness but had no significant difference among the mild, severe, and extremely severe groups [41]. Hou et al. also found a negative association between sCD25 and lymphocyte number. Interestingly, their finding was confirmed during disease recovery: while the concentrations of sCD25 were decreased, the number of lymphocytes was increased in recovered patients [129]. Taken together, these results shed light on the fact that increased levels of sCD25, especially in COVID-19 patients with severe illness, may cause lymphopenia by interfering with IL-2 signaling. Fig. 3 was designed to provide a summary of the plausible mechanisms involved in COVID-19-induced lymphopenia.

5. Treatment of COVID-19: Inhibiting inflammation-mediated lymphopenia and enhancing lymphocytes

Unfortunately, existing antiviral therapies such as glucocorticoids and immunoglobulin therapy cannot significantly increase the chances of survival of patients with severe COVID-19. One successful strategy is using convalescent plasma from patients recovered from a viral infection without severe adverse events [130,131]. On the other hand and given the importance of cytokine storm and inflammation-mediated lymphopenia in the pathogenesis of COVID-19, especially in severe cases, there are endorsed attempts to design new therapeutic strategies that are intended to control these events.

5.1. Blockade of pro-inflammatory cytokines

Once SARS-CoV-2 binds to TLRs, a cascade of events is stimulated within the cells resulting in the production of pro-inflammatory cytokines, such as IL-1β and IL-6. The results of several studies revealed that over-secretion of these cytokines are responsible for the dysfunction of the lungs and the emergence of systemic clinical symptoms. Interestingly, when Zhao et al. evaluated the therapeutic potential of Tocilizumab, a specific monoclonal antibody against IL-6, they found that the agent was successful in reducing the lung lesion opacity of severe cases of COVID-19 patients. Notably, administration of Tocilizumab could also increase the number of peripheral lymphocytes; further highlighting the importance of inflammation-mediated lymphopenia in COVID-19 [3]. Sarilumab, an IL6 blocker, also showed significant effects in severe cases of COVID-19 [132]. Apart from IL6 blockage, it seems that IL1 family suppression could bring advantages for COVID-19 patients. The blockage of IL1β by using IL37, an inhibitor of MHC-II, may bring a ray of hope for COVID-19 patients. IL38 is another inhibitory cytokine that has the ability to inhibit IL1β and other members of the IL1 family caused by SARS-CoV-2 [133]. Taken together, these results suggested that inhibition of inflammatory cytokine may help us to control inflammation-induced complications, such as lymphopenia in COVID-19.

TNF-α is notorious in inflammatory diseases due to its ability to induce apoptotic cell death in aged T lymphocytes by interacting with its receptor named TNFR [134]. Since TNF-α accounts for lymphopenia in viral infections, it is not surprising that anti-TNF-based therapies have found their ways into the clinic to treat a wide range of viral infections, such as COVID-19. Accordingly, it became evident that anti-TNF treatment could provide a significant outcome in animal models that mimic lung viral infection in humans [54]. Among the long list of drugs queued as TNF inhibitors, only a few such as infliximab or adalimumab received the approval to be used for reducing the inflammatory responses in COVID-19 [134]. Overall, it seems that anti-TNF treatment may be beneficial if they are administrated at the early stage of the disease.

5.2. JAK inhibitors

The JAK/STAT pathway is one of the common signaling axes that is recruiting by both SARS-CoV-2 host cells and immune cells to transmit their desired messages to the nucleus [135]. Given this, it seems if this pathway is inhibited, an order of unnecessary cytokine production would be interrupted. The superiority of JAK inhibitors to other immunomodulatory agents has been proposed to be attributed to their ability to exert dual anti-inflammatory and anti-viral properties. The favorable pharmaceutical characteristics such as oral administration and short half-life are also other advantages of these inhibitors [136]. One of the JAK inhibitors that received its approval from the FDA is Fedratinib; an agent with a significant ability to suppress inflammation by reducing the secretion of IL17 and IL22 from murine Th17 cells. Since COVID-19-mediated inflammation is characterized by Th17 cytokine signature such as what is happened in MERS-CoV cases, so administration of Fedratinib may be beneficial in the treatment of COVID-19 via suppression of Th17-associated cytokine storm [137]. In addition to Fedratinib, Baricitinib and ruxolitinib are other potent and selective JAK inhibitors. Stebbing et al. introduced Baricitinib as a promising agent in the treatment of COVID-19 due to its safety profile and high efficacy. Notably, the results of this study showed that abrogation of the JAK axis could block the entry of viruses into host cells and prevent the propagation of inflammatory responses [138]. The combinational strategies consisting of Baricitinib and conventional anti-viral agents such as lopinavir, ritonavir, or remdesivir may also be fruitful for patients infected with SARS-CoV-2 [138]. Accordingly, the administration of Ruxolitinib was successful to prevent multiple organ failure in COVID-19 patients with hyper-inflammation [139]; further highlighting the importance of JAK inhibition in the management of patients with COVID-19, especially those with systemic involvement.

5.3. Complement inhibitors

Having established that complement stimulation may have a role in the pathogenesis of COVID-19, it is postulated that if complement inhibitors are used at the initiating stage of the disease, they would bring fortune for patients, especially those who need more intensive treatments [24]. Mastaglio et al. claimed that C3 inhibition using AMY-101 could attenuate the tissue's destructive inflammatory responses and serve as a safe therapeutic option for COVID-19 patients with systemic hyper-inflammation [140]. Other critical members of the complement cascade that may also have a role in COVID-19–associated tissue inflammatory injury are C5a and C5b. The efficacy of anti-C5aR monoclonal antibody, avdoralimab, in severe cases of COVID-19 patients has been tested in a double-blind randomized study [141]. Moreover, Stahel et al. shed light on the advantageous effect of targeting complement components in COVID-19 patients and suggested that this therapeutic approach could prolong the survival of younger patients that had symptoms of hyper-inflammation, thromboembolic complications, and cardiac arrest [142]. Although therapeutic approaches intervening in the activation of complement seem to be effective, it should not be forgotten to be cautious in the administration of such agents to avoid any unfavorable harmful side effects.

5.4. Immunomodulators

The idea behind the application of immunomodulators in the therapeutic approaches of COVID-19 patients is stemmed from the success of pegylated IFNα-2a and 2b immunomodulators in the treatment of hepatitis B and C infections [143]. Through provoking anti-viral innate immune responses against SARS-CoV-2, immunomodulators seem to be successful in eliminating the number of pathogens in patients. A clinical trial is currently underway to examine the effect of combination therapy of ribavirin and pegylated interferon in patients with COVID-19 (ChiCTR2000029387). Other immunomodulators such as Pseudomonas aeruginosa and thymosin also showed befitting results in the treatment of COVID-19 patients; however, their efficacy and safety should be evaluated through more precise investigations [31].

5.5. MSC therapy

The beneficial impact of mesenchymal stem cells (MSCs) for COVID-19 patients is not only due to its regenerative effects in impairing tissue injuries but also is for its influential anti-inflammatory and immunemodulatory properties [144]. Z. Leng et al. conducted a study on seven severe COVID-19 patients who were subjected to 1×10^6 MSCs/kg. After infusion of MSCs, while there was a significant elevation in the number of peripheral lymphocytes, the serum levels of TNF-α and CRP were dropped down remarkably [76]. Choudhery et al. also reported the success of MSC transplantation in diminishing the level of TNF-α as well as elevating the serum level of IL-10 in critical cases of COVID-19 [145]. Incidentally, Rajarshia et al. [146] and Golchin et al. [147] found that

Table 2
A list of clinical trials investigating the efficacies of therapeutic approaches in COVID-19.

Drug	Mechanism	Population/Severity	No.	Phase	Status	Aim and outcome	Identifier
Targeting specific inflammatory molecules & pathways							
Canakinumab	IL-1R antagonist	COVID-19 patients.	451	Phase 3	Not yet recruiting	A multicenter, randomized, double-blind, placebo-controlled study to assess the efficacy and safety of canakinumab-plus-SOC compared with placebo-plus-SOC.	NCT04362813
Anakinra	IL-1R antagonist	COVID-19 patients.	180	Phase 2/3	Recruiting	A randomized, parallel group, 2-arm study, investigating the efficacy and safety of anakinra added to standard treatment, compared to standard treatment alone.	NCT04443881
Sarilumab	mAb against IL-6	COVID-19 patients with moderate & severe pneumonia.	239	Phase 2/3	Not yet recruiting	A randomized controlled trial to determine the therapeutic effect and tolerance of Sarilumab in COVID-19 patients.	NCT04324073
Tocilizumab	mAb against IL-6	COVID-19 patients with systemic inflammation.	243	Phase 3	Completed	A randomized controlled trial to evaluate the effect of Tocilizumab on multi-organ dysfunction among hospitalized COVID-19 patients.	NCT04356937
Situximab	IL-6 neutralization	COVID-19 patients with acute respiratory failure & systemic CRS.	342	Phase 3	Not yet recruiting	To evaluate safety and effectiveness of individually or simultaneously blocking of IL-6 and IL-1 versus standard of care on blood oxygenation and systemic CRS.	NCT04330638
Infliximab	TNF α blocker	COVID-19 patients with severe or critical symptoms.	17	Phase 2	Recruiting	A single center trial to assess the efficacy of infliximab or infliximab-abda in hospitalized COVID-19 patients.	NCT04425538
Baricitinib	Jak inhibition	COVID-19 patients.	1400	Phase 3	Recruiting	To evaluate the efficacy of baricitinib in hospitalized COVID-19.	NCT04421027
Emapalumab	INF II blocker	COVID-19 patients.	16	Phase 2	Terminated	To reduce the number of patients requiring mechanical ventilation, and to address the most urgent need to preserve the access to ICU support to the lowest possible number of patients.	NCT04324021
Inhibition of complement activation							
Eculizumab	C5 Inhibitor	ICU-admitted COVID-19 patients with ARDS.	N/A	N/A	Available	To evaluate if mortality can be halted while the patient has time to recover from the virus.	NCT04288713
AMY-101	C3 Inhibitor	COVID-19 patients with ARDS.	144	Phase 2	Not yet recruiting	To assess both the efficacy and safety, as well as pharmacokinetics and pharmacodynamics of C3 inhibition in COVID-19.	NCT04395456
Avdoralimab	C5aR Antibody	COVID-19 patients with severe pneumonia and ARDS.	168	Phase 2	Recruiting	To reduce the need for and duration of mechanical ventilation in patients with COVID-19 pneumonia ARDS.	NCT04371367
Immunomodulators							
HCQ	increases lysosomal pH in APCs	COVID-19 patients.	58	Phase 2	Terminated	A randomized, blinded trial to confirm or refute the efficacy of HCQ in early treatment of COVID-19 to reduce viral load.	NCT04353271
p-IFN lambda	Anti-viral activity	COVID-19 patients with mild to moderate symptoms.	40	Phase 2	Recruiting	To assess safety and tolerability of Lambda by adverse event monitoring, and vital signs assessment.	NCT04534673
MSC therapy							
MSCs	Immunomodulation	COVID-19 patients with moderate and severe symptoms	20	Phase 2	Recruiting	To assess the efficacy of MSCs as an add-on therapy to standard supportive treatment for COVID-19 patients.	NCT04444271
WJ-MSCs	Immunomodulation	Symptomatic COVID-19.	5	Phase 1	Recruiting	To investigate the potential use of WJ-MSCs for treatment of patient diagnosed with COVID-9.	NCT04313322
NestaCell®	Immunomodulation	COVID-19 patients with severe symptoms.	90	Phase 2	Not yet recruiting	To assess the efficacy of NestCell® as an add-on therapy to standard treatment to treat patients with COVID-19 pneumonia.	NCT04315987
Modulation of lymphocyte exhaustion							
Nivolumab	PD-1 blockade	COVID-19 patients with severe symptoms.	92	Phase 2	Not yet recruiting	To evaluate the efficacy of nivolumab in combination with standard treatments.	NCT04343144
Pembrolizumab	PD-1 blockade	COVID-19 patients.	24	Phase 2	Recruiting	To assess the efficacy of continued standard care together with tocilizumab plus pembrolizumab.	NCT04335305
NK cell-based therapy							
NK cells	NK Cells cytotoxicity	COVID-19 patients with moderate symptoms.	10	Phase 1/2	Not yet recruiting	To evaluate the safety and immunogenicity of allogeneic NK cells from PBMCs of healthy donors in patients with COVID-19.	NCT04344548
CYNK-001	NK cells cytotoxicity	COVID-19 patients with moderate symptoms.	14 72	Phase 1 Phase 2	Recruiting Recruiting	To evaluate the safety and efficacy of multiple doses of CYNK-001. A randomized, open-label design of multiple doses of CYNK-001 compared to the control.	NCT04365101
Novocellbio	Memory T and NK cells cytotoxicity	COVID-19 patients with worse prognosis.	58	Phase 1/2	Recruiting	A randomized escalating-dose trial to determine safety, alloreactivity, and efficacy of	NCT04578210

(*continued on next page*)

Table 2 (*continued*)

Drug	Mechanism	Population/Severity	No.	Phase	Status	Aim and outcome	Identifier
						adoptive cell therapy of NK cells or memory T cells.	

NK: Natural Killer; **PBMCs:** Peripheral blood mononuclear cells; **CYNK-001:** CYNKCOVID; **ARDS:** Acute respiratory distress syndrome; **PD-1:** Programmed cell death-1; **SOC:** Standard-of-care; **CRS**: cytokine release syndrome; **IL-1:** Interleukin-1; **HCQ:** Hydroxychloroquine; **p-IFN:** Pegylated Interferon Lambda; **WJ-MSCs:** Wharton's Jelly-Mesenchymal Stem Cells; **APC:** Antigen-presenting cell.

MSCs may be possibly one of the most ideal therapeutics, or considered as a combinational treatment for COVID-19 patients. It has been also reported that allogeneic bone marrow MSC-derived exosomes are enabled to reduce cytokine storm, restore oxygenation, and reconstruct the immune responses in the favor of the COVID-19 patients' survival [145]. All in all, the evidence supports the effectiveness of MSC products in the treatment of COVID-19 patients, as they may reduce pro-inflammatory cytokines and improve lymphopenia-related complications.

5.6. Modulation of lymphocyte exhaustion

Induction of lymphocyte exhaustion holds a respectable share in the pathogenesis of viral diseases and COVID-19 is not an exception. Given this, strategies that block PD-1/PD-1L and TIM3 appear to be effective in preventing lymphopenia or lymphocyte dysfunction in viral infections. So far, several monoclonal antibodies (mAbs) against PD-1 or NKG2A are designed to restore the cytotoxic phenotype to T and NK cells and facilitate the elimination of the virus [47,148]. Given the results of a study showing that increased absolute lymphocyte count and decreased absolute neutrophil count levels during nivolumab therapy correlated with a better response in melanoma [149], it is reasonable to assume that anti-PD-1 antibodies such as nivolumab or pembrolizumab may act as promising candidates for the treatment of lymphopenia in patients with COVID-19.

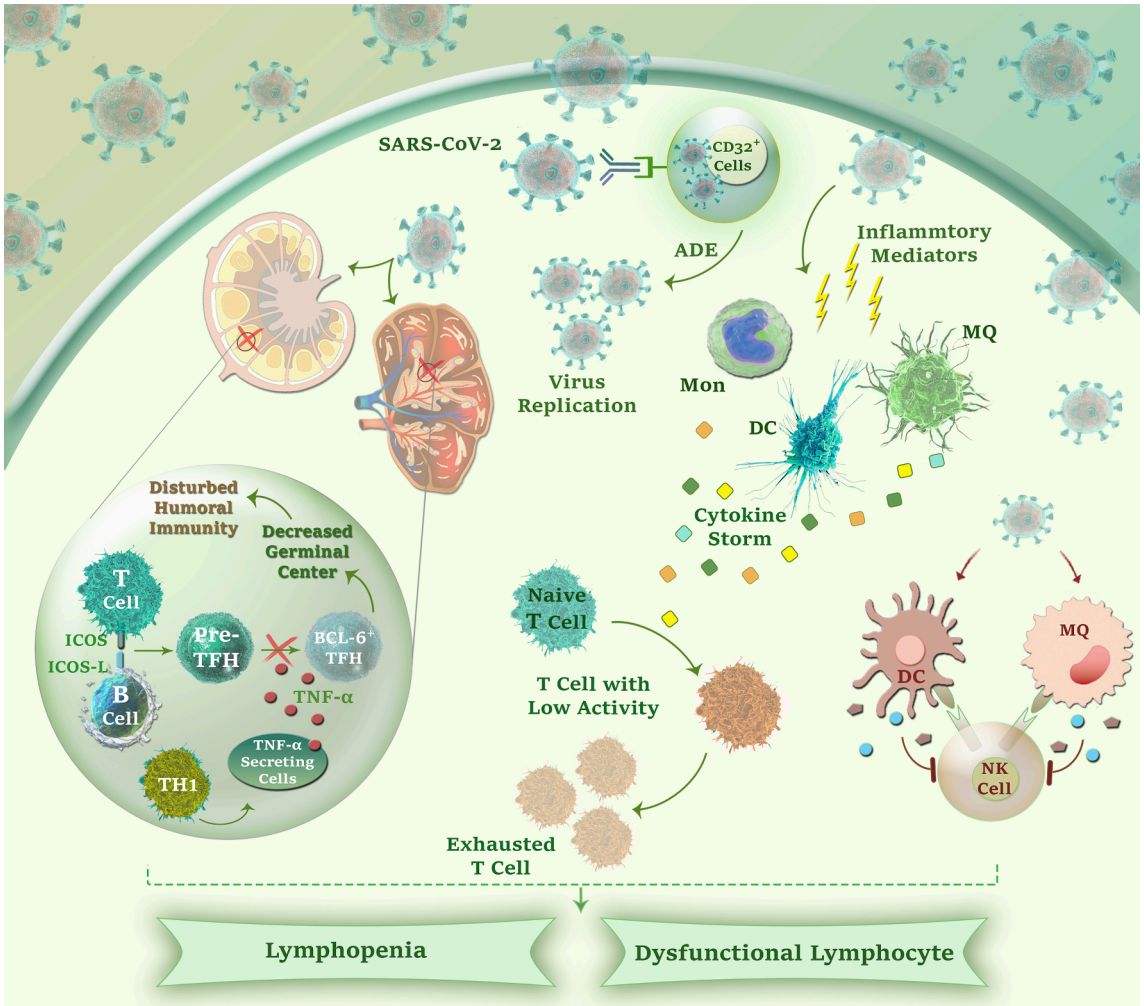

Fig. 4. The brief story behind the inhibitory effects of SARS-CoV-2 on lymphocytes. By entering SARS-CoV-2 into the body, a group of events takes place to have destructive effects on lymphocytes. Elevation in TH1 cells and abnormal aggregation of extra-follicular TNF-α could hamper differentiation of Bcl-6^{+} T follicular helper cells (TFH), an event which in turn results in decreased formation of germinal centers and disturbed humoral immune responses. On the other hand, unrestrained production of inflammatory cytokines is triggered during COVID-19 and leads to aberrant systemic inflammatory responses, which in turn, may induce exhausted phenotype in T cells. SARS-CoV-2 also affects NK cells and makes them inefficient. All the above-mentioned events go hand in hand to exacerbate COVID-19 via induction of lymphopenia and dysfunctional lymphocytes.

5.7. NK cell-based therapy

The lower number of circulating NK cells in SARS-CoV-2 infected patients has fired up an idea of application of the NK cell-based therapies as a weapon to combat the disease through boosting the host immune system's function [150]. Recently, the FDA permitted Cellularity to evaluate the therapeutic value of a universal NK cell therapy CYNK-001 named NKG2D-ACE2 CAR-NK cell therapy in COVID-19 patients [151]. Not only this approach was doing well in eliminating virus-infected cells also it could prevent the ability of SARS-CoV-2 in infecting the susceptible cells, such as alveolar epithelial cells. In the middle of 2020, autologous NK cell treatment agent "Novo-NK" has achieved a breakthrough in the treatment of COVID-19 patients, as it could rapidly remove a considerable proportion of viruses [152]. Odabasi et al. revealed that suppression of NKG2A receptors could be used as an approach to reinforce the antiviral activity of cytotoxic T cells and NK cells, resulting in the elevation of absolute lymphocyte count. They also suggested that the synergistic effect of NKG2A inhibitor (as an immune system booster) with IL-6R antibody (as an anti-inflammatory agent) could bring a ray of hope for the severe cases of COVID-19 patients [153]. Although it is early to hazard a conclusion, it seems that the combination of NK cell-based therapy and standard therapy may provide outstanding results in COVID-19. A list of clinical trials summarizing all these approaches was presented in Table 2.

6. Conclusion

With the massive numbers of studies that scrutinize the biology of SARS-CoV-2, now, the basic knowledge about the virus is to the degree that several therapeutic approaches have been designated for the disease; however, still there are several questions that need to be answered. By putting the current knowledge together, we can step toward diagnosis, prognosis, and treatment of the COVID-19 infection at an early stage. By direct damage to lymphatic organs and also by increasing the release of sCD25, it seems that not only SARS-CoV-2 diminishes the number of host's lymphocytes but also induces exhaustion phenotype in effector T cells and NK cells; an event which in turn paralyzes the immune system and may result in overproduction of pro-inflammatory cytokines in a compensatory manner. Reciprocally, the emergence of cytokine storm may exacerbate both lymphopenia and induction of exhausted lymphocytes (Fig. 4). Given this, it is reasonable to assume that any treatment strategy that targets lymphopenia −either directly or indirectly− would bring advantages for COVID-19 patients. So far, many of these approaches, including inhibition of inflammation and enhancing lymphocytes have been tested on COVID-19 patients; however, to ensure the efficacy and the safety profile of these treatments, more clinical investigations are required.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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