Alterations in lymphocyte subsets and monocytes in patients diagnosed with SARS-CoV-2 pneumonia: a mini review of the literature

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Abstract. – OBJECTIVE: Complete blood count parameters are frequently altered in COVID-19 patients. Leucopenia and lymphopenia are the most common findings. This is not specific to COVID-19 as similar alterations are found in various other viral infections. This work is intended to summarize the evidence regarding white blood cell and lymphocyte subset alterations in COVID-19 and their clinical implications.

MATERIALS AND METHODS: A PubMed search was conducted to identify relevant original studies. Articles not available in English or referring exclusively to pediatric patients were excluded. The study was designed as a narrative review from its inception.

RESULTS: Complete white blood cell number and lymphocytes may be reduced in COVID-19 patients. Circulating CD4+ cells (helper T lymphocytes), CD8+ cells (cytotoxic T lymphocytes), regulatory T cells and natural killer (NK) cells may be reduced, with a greater reduction observed in critically ill patients. CD4+ and regulatory cell deficiencies may contribute to the cytokine storm and subsequent tissue damage observed in severe COVID-19 infection. NK and CD8+ cell deficiency might delay infection clearance. These aberrations of cellular immunity may contribute significantly to the pathogenesis of the disease. Alterations observed in monocyte function can also be implicated as they are effector cells responsible for tissue damage and remodeling. B cell dysfunction and maturation abnormalities have also been reported, suggesting that the virus also impairs humoral immunity.

CONCLUSIONS: Lymphocyte subset abnormalities may be useful prognostic biomarkers for COVID-19, with circulating CD8+ cell count being the most promising as a predictor of severe disease requiring mechanical ventilation and mortality.

Key Words:
Coronavirus, COVID-19, CD4+ T cells, CD8+ T cells, Interleukin, Lymphocytes subsets, Macrophage, Monocytes.

Introduction

SARS-CoV-2 infection (COVID-19) spread worldwide rapidly and became a pandemic with over 3 million individuals infected, over 300000 new cases per day, and over one million deaths all over the world[1].

Lymphocytes and the subsets of CD4+ T cells, CD8+ T cells, B cells, monocytes, and natural killer (NK) cells play an important role in the immune system function[1]. After viral infection, alterations in absolute lymphocyte numbers and subsets vary and a different pattern is observed based on the pathogen involved. This indicates a potential association between lymphocyte subset alteration and the pathogenetic mechanisms in viral infections[2].

Changes in adaptive immune cells in COVID-19 patients are a matter of considerable clinical and research interest. Lymphopenia is commonly observed at the disease onset. Furthermore, absolute lymphocyte count (ALC) was reported to be correlated with disease severity. A number of changes in B cells, CD4+ T cell and CD8+ T cell number and functions have been reported. These

include CD4+ and CD8+ T cell lymphopenia, presence of activated T cells, increased or reduced T cells, dysregulated humoral immune responses, and impaired cellular immunity responses[2].

The aim of this review is to clarify the impact of COVID-19 infection on the human immune system by summarizing the changes in peripheral blood lymphocyte subsets in adult patients with COVID-19.

Materials and Methods

Literature Search

For the present narrative review, we conducted a PubMed search from March 2020 to April 2020 using the terms "Alterations in Monocytes, T cells subsets, B cells and Natural killer cells (NK) in patients diagnosed with COVID-19 pneumonia" as "Title/Abstract". Only articles referring to humans were enrolled in this review. In this review all published studies dealing with alterations in monocytes, NK cells, and T cells subsets related to COVID-19 pneumonia in hospitalized adult patients were included. We excluded articles including only paediatric patients and articles not available in English.

Alterations in Lymphocyte Subsets

The most effective response of the human organism against multiple viral infections is the activation of the cellular immune response, especially T cell activation[3]. Lymphocytes in the peripheral blood including T cells, B cells, and natural killer (NK) cells are involved in the humoral and cellular immunity against viral infections. Especially T cell subsets have been reported to be profoundly affected in severe cases of SARS-CoV-2 infection. In two studies[4,5] it was reported that the incidence of lymphopenia in SARS-CoV-2 infection was 84%, with CD8+ T cells being decreased in 87% of patients, B cells decreased in 76%, NK cells decreased in 55% and CD4+ T cells in the entirety of the sample. The studies reporting on the lymphocyte subsets alterations are shown in Table I.

CD8+ T Cell Subsets

Among COVID-19 patients, Intensive Care Unit (ICU) patients had significantly lower total lymphocyte, CD4+ T cell, CD8+T cell, and B cell counts[6]. CD8+ T cells are specifically efficient in clearing virus-infected cells. After activation from CD4+ T cells, CD8+ T cells can induce the activation and differentiation of cognate B cells and subsequently promote the production of virus-specific antibodies. In turn, neutralizing antibodies are able to mediate antibody-dependent cell-mediated cytotoxicity to kill virus infected cells and block the entrance of extracellular virions. A study[6] on the related coronavirus SARS found that low CD4 and CD8 counts on admission were associated with adverse outcomes. A possible explanation is that CD8+ cytotoxic T cells (CTLs) can eliminate viruses from the host body by secreting a number of molecules, including perforin, granzyme, and interferons (IFNs). CD4 helper T (Th) cells also help to eliminate viral infection by regulating the function of cytotoxic T cells and B cells. Hence, the reduction in the number and the possible disturbance in their function affects the response to SARS-CoV-2 contributing to the increased severity and mortality of the disease[7].

CD4+T Cell Subsets

CD4+ T cells regulate the response to acute and chronic viral infections as they coordinate the immune system primarily by secreting cytokines. CD4+ T cells activate multiple cells of the innate immune system, such as B cells, cytotoxic CD8+ T cells, and non-immune cells. CD4+ T cells also play a key role in the establishment of long-term cellular and humoral antigen-specific immunity, which is the basis of life-long protection from many viral infections and can also be conferred by vaccines[7]. COVID-19 patients exhibit a reduction in absolute numbers of CD4+ lymphocytes. These lymphocytes also express a different molecular profile of markers related to activation and exhaustion/senescence, along with altered expression of master regulator genes and chemokine receptors. Furthermore, it was shown that COVID-19 patients with severe disease had lower counts of CD4+ T cells compared to those with milder symptoms[8]. In addition, CD4+ T cells in patients diagnosed with COVID 19 are characterized by altered cellular proliferation but not mitochondrial functionality, as measured by mitochondrial oxygen consumption and extracellular acidification rate (ECAR)[9]. Moreover, Yang et al[9] reported that as the disease progresses, CD4+ and CD8+ T cells significantly decline in critically and severely ill patients. Therefore, CD4+ and CD8+ cell counts may be useful easily measured, predictive biomarker in COVID-19.

T-Helper (Th) Cell Subset

The Th cells are divided into the subgroups Th1, Th2, Th9, Th17, and T follicular helper cells,

each one having a specific function against infections[10]. The Th1/Th2 balance in COVID-19 has been associated with the final outcome of the disease. When the viral infection is established, an appropriate immune response by Th1 is able to clear it. However, if this immune response is not well organized, an exacerbated reaction leads to a cytokine storm, triggering Th2 cells, thus associated with poor prognosis[10,11]. Based on these results, Th cell activation seems to play an important role in determining COVID-19 severity, although the exact mechanism is still not totally understood. Javier Gil-Etayo et al[12] reported a significant reduction of Th1 and Th17 cell numbers in COVID-19 patients compared with the control group, with higher than expected numbers of activated Th2 cells. A higher number of senescent Th2 cells was found in the patients who died compared to those who survived. COVID-19 patients had an overreactive Th2 response against the virus. Senescent Th2 cell percentage was an independent risk factor for death when used along with the total lymphocyte count[12].

Regulatory T Cells (Treg)

It has been reported that Regulatory T cells (Treg) are also altered in patients with COVID-19[13]. It is well known that the Treg population inhibits innate and adaptive immune responses. Reduced Treg levels have been observed in severe

Table I. Summary of published studies on alterations in lymphocytes subsets in COVID-19.

Study/Year	Country	Main Findings
Chan et al, 2020[5]	Singapore	Statistically significant differences in lymphocyte subsets between ICU and non-ICU. Peripheral lymphocyte subset alterations are associated with the clinical severity of SARS-CoV-2 infection.
Wang et al, 2020[2]	Wuhan	COVID-19 patients have reduced absolute numbers of CD4+lymphocyte. Those with severe disease had a lower level of CD4+ T cells than mild cases.
Yang et al, 2020[9]	China	As the disease progresses, CD4+ and CD8+ T cell numbers decline in critically and severely ill patients. A higher percentage of Th2 cells was senescent in patients who died compared to survivors.
Gil-Etayo et al, 2021[12]	Spain	A significant reduction of Th1% and Th17% cells was observed, with a higher percentage of activated Th2 cells in the COVID-19 patients.
Wildner et al, 2020[16]	Hamburg	Significant increase in atypical memory B cells and plasmablasts in the peripheral B cell compartment of COVID-19 patients, that was correlated with immune activation and disease severity.
Kuri-Cervantes et al, 2020[17]	--------	Immune system dysfunction is a significant contributor to COVID-19 pathogenesis. An increased neutrophil to lymphocyte ratio is predictive of severe disease and adverse outcomes.
van Eeden et al, 2020[18]	--------	Patients infected with SARS-CoV-2 have significantly decreased numbers of NK cells.
Liu et al, 2020[19]	China	NK cells are significantly reduced in the critically ill COVID-19 group.
Lombardi et al, 2020[20]	Italy	Monocytes display morphological and phenotypical alterations in COVID-19 patients.
Meidaninikje et al, 2021[21]	--------	The size of monocytes was larger than normal with a greater proportion of monocytes exhibiting an inflammatory phenotype in COVID-19 patients.
Paliogiannis et al, 2020[22]	Italy	Blood monocyte count in COVID-19 patients was significantly lower compared to controls.
Giamarellos-Bourboulis et al, 2020[24]	Greece	The phenotype of circulating monocytes in severe COVID-19 cases mostly consists of CD14+ and CD16+ inflammatory monocytes, a subpopulation which exerts inflammatory activity.

COVID-19 patients' peripheral blood. Taking into account their normal function in suppressing the immune system, the reduction of their absolute count could be a possible mechanism contributing to the overactive immune responses in COVID-19 subjects. One can suspect that this could explain in a way the development of adult respiratory distress syndrome (ARDS) in severe COVID-19 patients. Additionally, the reduction of peripheral Treg cells in COVID-19 cases can change the balance between regulatory and effector parts of the immune system. This imbalance may lead to massive expansion and activation of neutrophils, macrophages, dendritic cells, mast cells, and Th17 cells, resulting in uncontrolled inflammatory responses mediated by the cells of innate immunity. This impaired balance may also contribute to tissue destruction[14]. Although it is too early to extract firm conclusions, changes in the Treg number in COVID-19 patients suggest that decreased Tregs in the peripheral blood are associated with more severe disease.

B Cells

B cells have not yet received enough attention during the COVID-19 course. The severity of COVID-19 is accompanied by changes in the B cell subpopulations, either immature or terminally differentiated[15]. A significant increase in atypical memory B cells and plasmablasts in the peripheral blood of patients diagnosed with COVID-19 infection has already been reported[16]. This alteration was found to be correlated with immune activation and disease severity[16,17]. A potentially harmful role of B cells in the acute immune response to SARS-CoV-2 infection was suspected; patients with primary antibody deficiencies who completely lack B cells showed a milder course of SARS-CoV-2 infection[17].

Natural Killer (NK) Cells

Natural Killer (NK) cells play an important role in the immune response against viral infections. NK cells function by killing infected cells through the production of cytokines, particularly interferon gamma (IFN-γ), the secretion of cytolytic granule-containing enzymes such as perforin and granzyme, and the use of death receptor-mediated cell apoptosis. Multiple studies on NK cell count changes have been conducted. The principal finding is that patients infected with SARS-CoV-2 have significantly decreased numbers of NK cells[18]. It has also been reported that these cells exhibited a functionally exhausted phenotype, increased expression of the NK inhibitory marker NKG2A and T cell exhaustion markers PD-1 and Tim-3[18]. Liu et al[19] reported that NK cells were statistically significantly reduced
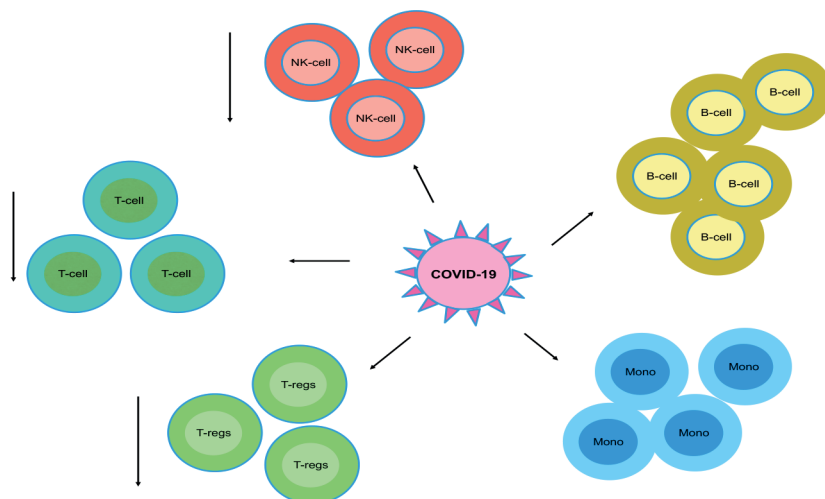

Figure 1. Effects of COVID-19 on cell populations. COVID-19 patients exhibit a reduction in absolute numbers of CD4+ lymphocytes. Among COVID-19 patients, Intensive Care Unit (ICU) patients had significantly lower total lymphocyte, CD4+ T cell, CD8+T cell, and B cell counts. A significant reduction of Th1 and Th17 cell numbers has been reported in COVID-19 patients. Also, reduced Treg levels have been observed in severe COVID-19 patients' peripheral blood. A significant increase in atypical memory B cells and plasmablasts in the peripheral blood of patients diagnosed with COVID-19 infection has already been reported. Patients infected with SARS-CoV-2 have significantly decreased numbers of NK cells. The alteration in the number and size of blood monocytes during the SARS-CoV-2 infection depends on the stage of the disease and varies.

in critically ill patients compared to patients with moderate and severe illness. The decreased NK cell cytolytic function observed in SARS-CoV-2 may be associated with the reduced levels of IFNs and TNF-α induced through NKG2A expression. In addition, the finding that NK cells are significantly reduced in ICU patients compared to non-ICU patients indicates that the NK cell count could be used as a predictor of clinical severity[19].

Monocytes

Peripheral blood monocytes contribute to the immune response against pathogens such as viruses. It has been reported that during the SARS-CoV-2 infection, some of their major functions are disrupted including cytokine production and chemotaxis. Lombardi et al[20] reported that monocytes, although not modified in cell number and distribution, displayed morphological and phenotypical alterations. Flow cytometry analyses of blood samples of COVID-19 patients demonstrated that the absolute number of monocytes did not change, while the size of monocytes was larger than normal[21]. On the other hand, another group indicated that the blood monocytes count in COVID-19 patients was significantly decreased[22]. The alteration in the number and size of blood monocytes during the SARS-CoV-2 infection depends on the stage of the disease and varies across studies already published. Diminished monocyte count has been reported in COVID-19 patients. The phenotype of circulating monocytes in severe cases requiring treatment in the ICU mostly includes the CD14+ and CD16+ inflammatory monocyte subpopulation. This subpopulation increases inflammatory activity through production of IL-6 and contributes to tissue damage directly[21,23]. Notably, Lombardi et al[20] detected reduced expression of HLA-DR, either as a percentage or fluorescence intensity, on patients' monocytes with normal scatter properties. The same observation was made by Kuri-Cervantes et al[17] and by Giamarellos-Bourboulis et al[24]. Overall, alteration in monocyte count, phenotype, and function play a significant role in the SARS-CoV-2 pathogenesis.

Conclusions

Alterations in the peripheral blood lymphocyte populations are found in patients with COVID-19 infection (Figure 1). Different lymphocyte counts might be a useful predictive biomarker of disease severity and response to treatment in this complex disease with circulating CD8+ cell count being the most promising so far.

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