Cytopenia in T Cell Subsets: A Predictor of Severe COVID-19

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Abstract:

Introduction: *The outbreak of Coronavirus Disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has posed great threat to human health. T cells play a critical role in antiviral immunity but their numbers and functional state in COVID-19 patients remain largely unclear. The present study was carried out at Armed Forces Institute of Pathology (AFIP), Dhaka to assess the degree of cytopenia of T cell subsets in COVID 19 and its association with severity of the disease. The aim of this study is to assess the degree of reduction of T cell subsets in both non severe and severe COVID 19 patients.*

Methods: *Total 100 patients having positive result of RT-PCR for SARS-CoV-2 and lymphopenia were recruited for this study. Patients were grouped as ICU and non- ICU according to the severity of clinical conditions, consisting of 50 patients in each group. Data of T cell subsets were obtained by flow cytometric analysis of peripheral blood using monoclonal antibodies.*

Results: *In this study, the absolute value of $CD3^+$ T cells was below the normal range in 47 (94%) ICU patients. Compared to the non-ICU group, the median absolute value of $CD3^+$ T cells was significantly lowered (P=0.019) in the ICU group. The absolute value of $CD4^+$ T cells was also below the normal range in 91patients (91%). All the patients in the ICU group showed low $CD4^+$ T cell counts. Moreover, a significantly lower median absolute value of $CD4^+$ T cells was observed in the ICU group compared to the non-ICU group (P = 0.004). The absolute value of $CD8^+$ T cells was below the normal range in 64 patients (64%). Similar to $CD4^+$ T cells, compared to the non-ICU group, the median absolute value of $CD8^+$ T cells was significantly lower in the ICU group (P = 0.028).*

Conclusion: *Significant reduction of T cell subsets occurs in severe COVID-19. Flow cytometric analysis of T cell subsets in COVID 19 patients with absolute lymphopenia can guide the physician to predict the severe outcome of the disease.*

Keywords: *COVID 19, CD: Cluster Differentiation, ICU: Intensive Care Unit, Lymphopenia, RT-PCR: Reverse Transcriptase Polymerase Chain Reaction.*

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Introduction

A novel strain of Coronavirus SARS-CoV-2 was first detected in December 2019 in Wuhan, a city in China's Hubei province with a population of 11 million, after an outbreak of pneumonia without an obvious cause. The virus has now spread to over 200 countries and territories across the globe, and was characterized as a pandemic by the World Health Organization (WHO) on 11 March 2020. As of 3 November 2020, there were 46,591,622 laboratory-confirmed cases of coronavirus disease 2019 (COVID-19) infection globally, with 1,201,200 reported deaths[1].

As the number of COVID-19 patients is dramatically increasing worldwide and treatment in intensive care units (ICU) has become a great challenge. The possibility of estimating the evolutionary trend and the final outcome of the infection at an early stage of the disease

with an early and effective therapy for those who may progress into a more critical condition could reduce the mortality rate. Ascertaining trustworthy indicators of disease gravity and, above all, reliable markers of a possible negative progression is therefore essential. Most critically ill and deceased patients did not show serious clinical symptoms in the early stages of the infection. Most patients only displayed cough, mild fever, or muscle ache. Clinical conditions of these subjects worsened unexpectedly in the later stages of the disease. Acute respiratory distress syndrome (ARDS) and multiple-organ failure occurred precipitously, resulting in death in a short time [2]. It has been speculated that when the body is unable to perform an adequate adaptive T cell mediated immune response against the infection, an innate relentless inflammation can then cause a cytokine storm with ARDS and organ failure. Decreased T cell population may contribute to severe COVID 19 due to exaggerated cytokine release resulting from an uninhibited innate immune activation [3, 4]. Hence this study was done to assess the degree of reduction of T cell subsets and association with severe COVID-19.

Methods

This cross sectional analysis was conducted at Armed Forces Institute of Pathology, Dhaka. Purposive sampling technique was used among 100 patients who got admitted in Corona-ICU and Corona ward with confirm COVID-19 from 5th August 2020 to 5th November 2020. Patients were grouped as ICU (Severe and critical patients) and non- ICU (mild and moderate patients) according to the severity of clinical conditions, consisting of 50 patients in each group. Variables likes; age, sex, Absolute lymphocyte count, CD3+, CD4+ &CD8+ T cell count were analyzed. Privacy and anonymity of the participants were maintained. Approval of the study protocol, procedures and ethical clearance were obtained from the Institutional Ethical Review Board.

Operational definition of clinical spectrum (*SARS-CoV-2)[5,6]*:

Based on the New Coronavirus Pneumonia Prevention and Control Program from the National Health Commission of China, and National Guidelines on Clinical Management of Coronavirus Disease 2019 (COVID-19), Bangladesh, version-6, patients with SARS CoV-2 infection were divided into asymptomatic carriers, mild patients, moderate patients, severe patients and critically severe patients. According to the guideline, asymptomatic carriers were not classified as confirmed cases. In our study, asymptomatic carriers were not included. In this study the criteria for different clinical spectrum of cases with SARS-CoV-2 infections considered are as follows:

Types	Characteristics
Asymptomatic carriers[1]	Laboratory confirmed SARS-CoV-2 infection without symptoms and imaging findings.
Mild	Mild clinical symptoms without imaging findings of pneumonia
Moderate	Fever or respiratory symptoms with imaging findings of pneumonia.
Severe	Meet any of the followings: 1. Respiratory distress with respiratory frequency e"30 breaths/min. 2. Pulse oximeter oxygen saturation (SpO_2) d" 93% in resting state. 3. $PaO_2/FiO_2 \leq 300$mmHg (1mmHg = 0.133kPa) 4. Showing rapid progression (>50%) on CT imaging within 24-48h).
Critical severe	Meet any of the followings: 1. Respiratory failure in need of mechanical ventilation. 2. Shock 3. With other organ dysfunction

[1]Asymtomatic carriers were not classified as confirmed cases of COVID-19.

Selection criteria

- Inclusion criteria
  - RT-qPCR for SARS-COV-2: Positive
  - >18 years
  - ICU patients with criteria of severe and critical severe COVID-19 patients.
  - Non-ICU patients with criteria of mild and moderate COVID-19 patients.
  - Absolute lymphocyte count <1500/µl
- Exclusion criteria
  - RT-qPCR for SARS-COV-2: Presumptive Positive (Single gene detected)
  - Pregnant women.
  - Patients with other diagnosed cytokine release syndrome and any malignancy.

***Sample collection**:* 02 ml venous blood sample was taken in a purple top vacutainer containing EDTA from each patient for complete blood count & flow cytometry.

Laboratory assay: Absolute lymphocyte count was obtained from complete blood count using automated hematology analyzer (Sysmex XN 1000) and cross checked by peripheral blood film examination. Samples with lymphopenia(<1500/µl) were processed within 2 hours of collection by stain-lyse-wash method for flow cytometry to analyze the lymphocyte subsets. 50µl blood &10µl monoclonal antibodies consisting of anti-CD3 PC5.5-A, anti-CD8 ECD-A and anti-CD4 PE-A were mixed in a test tube. This cell suspension was incubated at room temperature in the dark for 15 minutes. Red blood cells were removed using 500µl of lysis buffer and again incubated at room temperature in the dark for 10 min. Cells were washed with phosphate buffer saline and 50µl flow count was added. Finally, the cells were analyzed using Beckman coulter flow cytometer (model: DxFLEX). The count of CD3+, CD4+, and CD8+ T lymphocytes were obtained. Samples from 10 healthy control subjects were taken to check the validity of investigation.

Statistical analyses: Statistical analysis was performed using SPSS 24.0. Median values were used to present the analysis of data. The enumeration data were analyzed using Mann-Whitney U test. P value <0.05 were considered statistically significant.

Results

Total 100 patients who were RT-qPCR for SARS-CoV-2 positive and having lymphopenia were recruited for this study. Patients were grouped as ICU and non- ICU group according to the severity of clinical conditions. Each group consists of 50 patients.

Table I

Age statistics of study population (N=100)

	Age(years)	
	Non-ICU group (N=50)	ICU group (N=50)
Mean	50.34	58.2
Minimum	19	22
Maximum	78	77

Table I shows mean age of non-ICU group and ICU group is 50.34 years & 58.2 years respectively.

Table II

Frequency distribution of age group (n=100).

Age group(years)	Frequency	Percentage (%)
18-40	22	22
41-60	35	35
>60	43	43
Total	100	100

Table II shows most of the COVID-19 patients belong to >60 years age group (43%).

Table III

Gender distribution of patients (n=100).

	Sex of the patients	
	Male n (%)	Female n (%)
Non-ICU group	37/50(74)	13/50(26)
ICU group	39/50(78)	11/50(22)

Table III shows most of the patients admitted in ICU with severe COVID-19 are male (78%).

Table IV

Frequency distribution of lymphocytes and T cell subsets in COVID 19 patients (n=100).

	Count/µl	All patients (N=100)	Non-ICU group (N=50)	ICU group (N=50)
Absolute Lymphocyte count	<1000	62/100(62%)	13/50(26%)	49/50(98%)
	≥1000-1500	38/100(38%)	37/50(74%)	01/50(02%)
CD3 count	<600	57/100(57%)	10/50(20%)	47/50(94%)
	≥600	43/100(43%)	40/50(80%)	03/50(06%)
CD4 count	<400	91/100(91%)	41/50(80%)	50/50(100%)
	≥400	09/100(9%)	09/50(18%)	00
CD8 count	<200	64/100(64%)	20/50(40%)	44/50(88%)
	≥200	36/100(36%)	30/50(60%)	06/50(12%)

We observed the distribution of T lymphocytes and their subsets in patients infected with COVID-19 and compared between the non-severe and severe group. As shown in the Table III, the absolute lymphocyte count was greatly reduced in 62(62%) patients. Compared to the non-ICU group (26%), most of the ICU patients (98%) developed remarkable absolute lymphopenia. In our study, the absolute value of $CD3^+$ T cells was below the normal range in 47 (94%) ICU patients. Whereas all the patients in the ICU group showed low $CD4^+$ T cell counts. Similarly, the absolute value of $CD8^+$ T cells was below the normal range in 20(40%) non-ICU patients and in 44(88%) patients in the ICU group.

Table V:

The comparison of T lymphocyte subsets of patients infected with COVID-19 between Non- ICU and ICU group (n=100).

Lymphocyte subsets		All patients (n=100) Median	Non-ICU group (n=50) Median	ICU group (n=50) Median	P-value
Absolute lymphocyte	<1000	735.5	924	696	<0.001
count/µl	≥1000-1500	1194.5	1224	1024	0.013
CD3 count/µl	<600	450	559.5	410	0.019
	≥600	719	730	658	0.23
CD4 count/µl	<400	182	252	137	0.004
	≥400	474	474	0	—
CD8 count/µl	<200	130	159	116	0.028
	≥200	251	245.5	247	0.07
CD4/CD8 ratio	<0.7	0.58	0	0.58	-
	0.7-3.0	1.28	1.27	1.18	0.006
	>3.0	3.97	3.86	3.97	0.14

Note: Data contains median value of all patients, non-ICU and ICU patients. P values comparing non-ICU and ICU group are obtained from Mann-Whitney U test. P<0.05 was defined as statistically significant.

Table V revealed that the median absolute value of $CD3^+$ T cells was significantly lowered (P 0.019) in the ICU group compared to non-ICU group. Moreover, significantly lower median absolute value of $CD4^+$ T cells (P= 0.004) and $CD8^+$ T cells (P=0.028) were observed in the ICU group compared to the non-ICU group.

Discussion

$CD3^+$ T cells are mainly composed of $CD4^+$ T cells and $CD8^+$ T cells. $CD4^+$ helper T cells have a crucial role in adaptive immune responses. Upon antigen presentation, naïve $CD4^+$ T cells can differentiate into distinct subsets. Among them, Th1 cells, which are induced by IL-12 and produce large quantities of IFN-ã, are involved in enhancing the clearance of certain intracellular pathogens, including viruses[7]. Besides, $CD8^+$ T cells restricted by class I major histocompatibility complex molecules are important in establishing immunity to influenza virus because they recognize internal viral proteins that are conserved between multiple viral strains. Both $CD4^+$T cells and $CD8^+$ T cells are critical in defending against influenza viruses[8]. Since the sequence of the new coronavirus is highly homologous to SARS and MERS (Middle East Respiratory Syndrome) related corona virus, both of which are severe respiratory viruses, probably that $CD4^+$T cells and $CD8^+$ T cells are also responsible for controlling the new coronavirus[9]. Here, we have shown that the reduction of those population was associated with the severity of patients with the COVID-19.

From our cross-sectional analysis of 100 patients having lymphopenia, 57%, 91% and 64% patients had remarkably low total T cell count, $CD4^+$ and $CD8^+$ T cell count respectively. Among milder disease patients in the Non-ICU group, the median value of total T cell, $CD4^+$ and $CD8^+$ T cell count were 559.5, 252 and 159 respectively; the median value decreased to 410,137 and116 respectively in the ICU group (Table IV). The count of total T cell, $CD4^+$, and $CD8^+$ T cell were significantly lower in ICU patients than Non-ICU cases. Elderly patients are mostly affected. 43% patients belong to >60 years age group. Males are more affected than females.76% of the study population are male.

Liu et al.[10] showed similar result in their study. They found a strong correlation between the severity of COVID-19 and the $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocytes. They have shown that the population of $CD3^+$, $CD4^+$ and $CD8^+$ Lymphocyte subsets was decreased when patients went from severe to critical whereas the population of T cells were comparable between moderate and severe patients.

Diao et al.[11] showed in their study that T cells not only reduces in severe COVID 19, but also these cells are functionally exhausted by increasing expression of some immune inhibitory factors including PD-1 & Tim-3 on cell surface. They found increased percentage of PD-1+ CD8+ T cells and Tim-3+ CD4+ T cells in ICU period in comparison to prodromal and symptomatic stages. Our study would have been more informative if these functional markers were included.

Fan Wang et al.[12] measured lymphocyte subsets in 60 hospitalized COVID-19 patients before and after treatment, and their association with clinical characteristics and treatment efficacy was analyzed. Like our study results, total lymphocytes, CD4+ T cells, CD8+ T cells decreased in COVID-19 patients and severe cases had a lower level than mild cases. After treatment, 37 patients (67%) showed clinical response, with an increase in CD8+ T cells and B cells. No significant change in any subset was detected in nonresponsive cases. Post treatment T cell response was not analyzed in our study. We have few limitations in our study like; Age limit of the population was >18 years. So, the results do not project about severity in all ages of COVID-19 patients. This was a single center small group study. Dynamic changes in peripheral blood lymphocyte subsets in patients with COVID-19 was not studied. So the results could be better if we overcome these limitations.

Conclusion

Significant reduction of T cell subsets occurs in severe COVID-19. Flow cytometric analysis of T cell subsets in COVID 19 patients with absolute lymphopenia can guide the physician to predict the severe outcome of the disease. Further large-scale study is needed in this regard. Dynamic changes and functional status of the T cell subsets should be explored for better implications.

Conflict of interest: none.

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