Study of Absolute Lymphocyte Count as a Marker of COVID 19 Disease Severity in Tertiary Care Centre, Suryapet

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ABSTRACT

BACKGROUND

The World Health Organization (WHO) has declared Coronavirus disease 2019 (COVID - 19) as a global public health pandemic. Clinical and laboratory biomarkers to predict the severity of corona virus 2019 are essential in this pandemic. Lymphocyte count has been a marker of interest in order to investigate the association of lymphocyte count and severity of COVID-19. We would like to analyse the relationship between absolute lymphocyte count (ALC) & COVID-19 disease severity.

METHODS

We performed a retrospective study on patients admitted to Government general hospital, Suryapet for COVID-19 illness from September 1st 2020 to September 16th 2020. Age, gender and complete blood count of patients admitted in the hospital was collected. Haemoglobin, total leucocyte count (TLC), absolute neutrophilic count (ANC), absolute lymphocyte count (ALC) and platelet counts were compared between ICU and Non-ICU groups and comparison of absolute lymphocyte counts in each group - ICU alive, ICU death and non-ICU groups was carried out.

RESULTS

134 patients who were admitted in the hospital were analysed. Mean age and gender were compared between ICU and Non-ICU groups. We compared ALC between ICU alive, ICU death and non-ICU groups. Mean ALC in ICU death group was 0.81, in ICU alive group 1.04 and in non-ICU as 1.75. We found that patients with disease severity have lower absolute lymphocyte counts. In addition to this we also found that there was neutrophilia and lower haemoglobin levels in ICU patients.

CONCLUSIONS

We conclude that lymphopenia, defined as absolute lymphocyte count less than 1.1 x 10^9/L may be useful in predicting the severity of COVID-19 illness.

KEYWORDS

COVID-19, Absolute Lymphocyte Count (ALC), Lymphopenia, SARS COV2

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BACKGROUND

Coronavirus disease 2019 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS – COV - 2), a positive strand RNA virus belonging to the family Coronaviridae with about 80 % genomic similarities with SARS - CoV.[1-3] It was first identified in Wuhan, China in December 2019 and has gradually spread all over the world affecting approximately 32.7 million until 27th September 2020. While most patients with COVID-19 have mild influenza like illness and may be asymptomatic, a minority of patients will develop severe pneumonia, acute respiratory distress syndrome (ARDS), multi-organ failure (MOF), and death.[4] Lymphopenia, defined as absolute lymphocyte count less than 1.1 x 10^9/L has been associated with severe COVID-19 illness.[5-7]

Objectives

Prognostic markers are essential in this pandemic to understand the clinical course of disease severity for patients admitted in the hospital. Lymphocyte count which is readily available even in remote areas has been a marker of interest in order to investigate the association of lymphocyte count and severity of COVID-19. In the present study we would like to evaluate the effect of lymphocytopenia with the severity and outcome of COVID-19 illness.

METHODS

This is a retrospective study conducted in Government medical college/Government General hospital, Suryapet from September 1st 2020 to September 16th 2020. All the patients who were diagnosed with COVID-19 and admitted in the hospital were evaluated. Data collected include age, gender and complete blood picture. Information regarding patient outcomes was analysed including ICU, Non-ICU admission and mortality rates. A comparison of lymphocyte count in each group – Non-ICU, ICU admission (alive and death) was carried out.

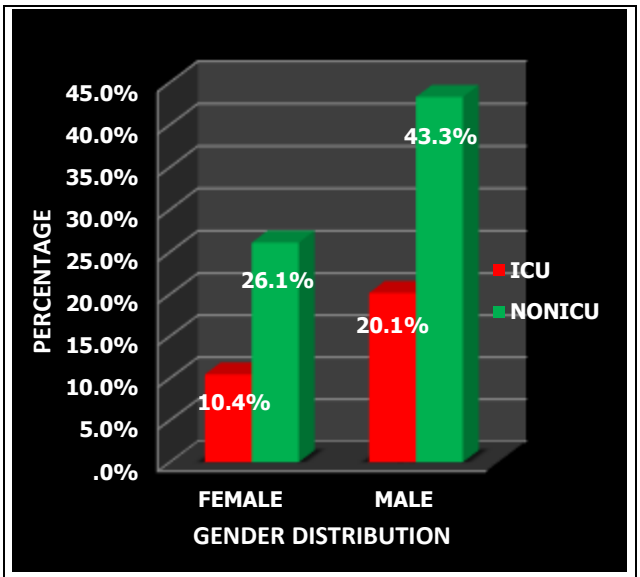
Statistical Analysis

Analysis was done by IBM SPSS software trial/student version 21. Results were expressed in numbers, frequencies, means and standard deviations. Appropriate tests (chi-square, one-way analysis of variance (ANOVA) test, (independent t test) were applied wherever necessary. A P - value less than 0.05 was considered significant.

RESULTS

During the course of the study, a total of 134 patients who were diagnosed with COVID-19 and admitted in the hospital were analysed. Out of 134 patients, 93 were admitted in non-ICU ward and 41 were admitted in ICU ward. According to age, mean age of patients who were admitted in ICU was 58.9 years and mean age of patients who were admitted in non-ICU was 49.9 years. Difference between the means are significant (P = 0.0002).

According to Gender (Graph 1), Out of 134 patients, 85 were males and 49 were females. In ICU admission, 14 (10.4 %) were females and 27 (20.1 %) were males. In non-ICU ward, 35 (26.1 %) were females and 58 (43.3 %) were males. Difference between these groups is not significant (P = 0.6).



Graph 1. Gender Distribution in ICU and Non-ICU Groups

Complete blood picture results were analysed for all the patients admitted in the hospital. Haemoglobin (HB), total leucocyte count, absolute neutrophil count, absolute lymphocyte count and platelet counts were compared between ICU and Non-ICU groups. Mean haemoglobin in ICU group is lower (12.2) when compared to non-ICU group (13.1) but the difference between the means is significant (P = 0.03). Mean total leucocyte count in non-ICU group is lower (9.9) than in ICU group (10.7), but the difference between the mean is not statistically significant (P = 0.4).

Mean absolute neutrophil count was higher in ICU group (9.1) when compared with non-ICU group (7.3) and the difference between means is statistically significant (P = 0.03). Mean absolute lymphocyte count was lower (1.0) in ICU group than in non-ICU group (1.8), but the difference between the means is statistically significant (P = < 0.0001). Difference between the mean platelet counts in ICU group and non-ICU groups is not statistically significant (0.8).

Variables	Place of Admission				Independent t Test P Value
	ICU		Non-ICU		
	Mean	S.D.	Mean	S.D.	
HB	12.2	2.6	13.1	2.0	0.03
TLC	10.7	5.5	9.9	4.7	0.4
ANC	9.1	5.0	7.3	4.3	0.03
ALC	1.0	0.6	1.8	0.7	<0.0001
Platelet Count	280.7	181.0	276.5	131.3	0.8

Table 1. Basic Haematological Parameters of COVID-19 Patients Admitted in the Hospital

HB - Haemoglobin, TLC - Total Leucocyte Count, ANC - Absolute Neutrophil Count, ALC - Absolute Lymphocyte Count, ICU - Intensive Care Unit.

One Way ANOVA								
ALC	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
ICU Alive	32	1.037	0.6333	0.112	0.809	1.266	0.3	3.7
ICU Death	9	0.811	0.2261	0.0754	0.637	0.985	0.6	1.3
Non-ICU	93	1.753	0.7305	0.0757	1.602	1.903	0.6	4.1
Total	**134**	**1.519**	**0.7704**	**0.0666**	**1.387**	**1.65**	**0.3**	**4.1**

Table 2. Mean Absolute Lymphocyte Count (ALC) in ICU (Alive, Death) and Non-ICU Groups

Table 2 shows comparison of absolute lymphocyte count between the three groups (ICU alive, ICU death & non-ICU), mean absolute lymphocyte count was lowest in ICU death group i.e. 0.81 followed by ICU alive group which is 1.03. In Non-ICU group, mean absolute lymphocyte count was higher than ICU groups which is 1.75.

One Way ANOVA						
ALC	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared
Between groups	17.008	2	8.504	17.986	.000	.215
Within groups	61.936	131	.473			
Total	**78.943**	**133**				

Table 3. Variability in Absolute Lymphocyte Count between Groups

The above table (table 3) shows that 21.5 % of variability in absolute lymphocyte count is accounted for groups like ICU alive, ICU death and non-ICU that is severity.

ALC Tukey HSD Post HOC Test						
(I) Severity	(J) Severity	Mean Difference (I - J)	STD. Error	SIG.	95 % Confidence Interval	
					Lower Bound	Upper Bound
ICU alive	ICU Death	.2264	.2594	.658	- .389	.841
	Non-ICU	- .7152*	.1409	.000	- 1.049	- .381
ICU death	ICU Alive	- .2264	.2594	.658	- .841	.389
	Non-ICU	- .9416*	.2400	.000	- 1.511	- .373
Non-ICU	ICU Alive	.7152*	.1409	.000	.381	1.049
	ICU Death	.9416*	.2400	.000	.373	1.511

Table 4. Tukey HSD Post Hoc Test for ALC

*The Mean Difference is Significant at the 0.05 Level

When mean values of absolute lymphocyte count are compared between ICU alive group with ICU death & non-ICU groups, ICU alive and non-ICU groups showed significance. When ICU death group is compared with ICU alive & non-ICU groups, ICU death and non-ICU groups showed significance. We did not get any significance between ICU death & ICU alive groups. When Non-ICU group is compared with ICU alive & ICU death groups, both groups showed significance.

DISCUSSION

COVID-19 is primarily manifested as a respiratory tract infection, emerging data indicates that it should be regarded as a systemic disease involving multiple systems including cardiovascular, respiratory, gastrointestinal, neurological, hematopoietic and immune system.[8-10] Although the pathogenesis of SARS – COV - 2 is not yet fully understood, extensive lung damage in COVID-19 patients appears to be associated with high initial viral load, neutrophil infiltration in the lungs concomitant with elevated levels of serum proinflammatory cytokines and chemokines with a rapid and precipitous decrease of peripheral T lymphocytes.[1] Clinical deterioration and tissue damage during SARS – COV - 2 infection may result from direct virus induced cytopathic effects along with maladjusted immune responses. One of the key features of SARS–COV-2 infection is the global reduction of peripheral T lymphocyte and impaired immune responses during acute infection.[11,12] Many articles have found evidence that lymphocytopenia may correlate with disease severity.[13-16] However, most of the data in these articles are obtained from patients in China, thus data is lacking regarding trends in other parts of the world. In our hospital-based study, we found that lymphopenia is associated with poor outcomes, there was clear correlation between old age, lymphopenia and severe COVID-19 infection.

In our present study, ICU patients were older than non-ICU patients, mean age of patients admitted in ICU was 58.9 years and non-ICU was 49.9 years (P = 0.0002). This is in comparison with Fan et al.[13] study, in which median age of ICU patients was 54 years old while the median age of non-ICU patients was 42 years old(P = 0.02) and Wang DW[14] et al. study, in which median age of ICU patients was 66 years and non-ICU patients was 51 years (P < 0.001). There is no significant difference in gender between ICU and Non-ICU groups in our study (P = 0.6) which is similar to Fan et al.[13] study (P = 0.72) and Wang DW[14] et al. study (P = 0.34).

Our study results revealed that lymphopenia was observed in ICU patients when compared with non-ICU patients, indicating a dysfunctional inappropriate immune response of ageing individuals during the progression of COVID-19 disease. In ICU group, mean absolute lymphocyte count was 1.0 and in non-ICU group as 1.8 (P < 0.0001). Mean absolute lymphocyte count was lowest in ICU death group, 0.81. These results were consistent with the following studies. In Guan et al.[15] study, severe cases presented with lymphocytopenia more frequently (96.1 %, 147/153) vs non severe cases (80.4 %, 584/726); P < 0.001. Huang et al.[16] study showed that 85 % (11/13) of patients needing ICU care presented with low lymphocyte count vs. 54 % (15/28) of patients that did not need ICU care (P = 0.045). Wang DW et al.[14]study found that ICU cases presented with lower lymphocyte count (median: 0.8, IQR: 0.5 - 0.9) vs. non-ICU cases (median: 0.9, IQR: 0.6 - 1.2) ; P = 0.03. Elhassadi E et al.[17] study shows that there was a statistically significant difference in the lymphocyte count between the General Medicine (GM) admission cohort and the ICU / RIP combined cohorts with a P value of < 0.001. Fan et al.[13] study also found that on serial monitoring, the median nadir absolute lymphocyte count in the ICU group was $0.4x10^9$/L compared to 1.2 x 10^9/L in Non-ICU group (P < 0.001). Zhou et al.[18] evaluated risk factors for mortality in a retrospective cohort study involving 191 patients and showed that baseline lymphocyte count was significantly higher in survivors than non - survivors (1.1 x 10^9/L vs 0.6 x 10^9/l, P < 0.0001). In our study there was no significant difference of absolute lymphocyte counts in ICU alive and ICU death groups (table 4) which is similar to Yang et al. study,[19] in which lymphocytopenia occurred in 44 (85 %) of critically ill

patients, with no significant difference between survivors and non survivors. Lymphopenia was frequently encountered in patients requiring ICU care, ranging from 67 % to 85 % in various case series.[19-21]

Lymphocytes and their subsets play an important role in maintaining immune homeostasis and inflammatory response throughout the body. Adaptive immune response plays a critical role to restrict viral infections. The adaptive immune response to viral infections is exerted through the effector function of cytotoxic T lymphocyte (CTL)[22] response which specifically recognize and kill virus infected cells. Unlike the conventional immune responses against viruses, Depletion of T cells and NK cells was seen in patients suffering from COVID - 19.[13,23-25] Jiang et al.[26] evaluated lymphocyte subsets in 103 patients, which revealed that CD3+, CD4+, and CD8+T cells and NK cells were significantly decreased in COVID-19 patients with a more severe decrease in CD8+T cells compared with CD4+T cells. Liu et al.[27] also found that the higher the RNA load in the nasopharynx, the lower the CD4+ and CD8+T lymphocyte count and these changes were closely related to the severity of COVID - 19.

Several factors may contribute to COVID-19 associated lymphopenia. Lymphocytes express the corona virus receptor ACE2 on their surface;[28] thus SARS–COV-2 may directly infect lymphocytes and ultimately lead to lysis. The cytokine storm which is characterized by markedly increased levels of interleukins(mostly IL - 6, IL - 2, IL - 7, granulocyte colony stimulating factor, interferon - γ inducible protein 10, MCP - 1, MIP1 - a) and tumour necrosis factor (TNF) - alpha may also promote lymphocyte apoptosis.[29-31] Substantial cytokine activation may also be associated with atrophy of lymphoid organs, including the spleen, and further impairs lymphocyte turnover.[32] Autopsy of patients who died of COVID-19 showed markedly shrunken spleen with reduced lymphocyte, macrophage proliferation and phagocytosis.[33] All hematopoietic cell lineages were reduced in the bone marrow. Coexisting lactic acid acidosis,[34] which may be more prominent among cancer patients who are at increased risk for complications from COVID-19, may also inhibit lymphocyte proliferation.[35] Viral infections would lead to the activation of hypothalamic – pituitary - adrenal axis under stress, resulting in up regulation of endogenous corticosteroids, which might be involved in immuno pathogenetic mechanisms of lymphopenia of COVID-19.[36] Further research is required in this field as multiple mechanisms may be involved to cause lymphopenia in COVID-19 patients.

In our study, we found that mean HB in ICU group was significantly lower when compared to non-ICU group (P = 0.03) similar to Guan WJ et al.[15] and Zhou et al.[37] studies. In Guan WJ et al.[15] study, the haemoglobin level of 128.0 g/L (111.8 - 141.0) in severe group was lower than that of 135.0 g/L (120.0 - 148.0) in non-severe group (P < 0.001). It is noteworthy that reduction of haemoglobin was more pronounced in patients who reached composite endpoint (included admission to ICU, requirement of invasive ventilation and death) than in those who did not (125.0 g/l, (105.0 - 140.0) vs 134.0 g/l (120.0 - 148.0), P = 0.012). In Zhou et al.[37] study, although there was no difference in the incidence of anaemia, the haemoglobin of the patients with severe cases decreased more significantly (125.42 g/l (97 - 144) vs 145.24 g/l (111 - 162), P = 0.002). In contrast, Huang CL et al. study[16] with 41 patients of COVID-19 pneumonia showed that the haemoglobin level of severe patients was lower, although the difference was not marked (122.0 g/l (111.0 - 128.0) vs 130.5 g/l (120.0 - 140.0), P = 0.20). Inflammatory changes caused by SARS–COV-2 infection could interfere with erythropoiesis, resulting in a decrease in haemoglobin. The low incidence of anaemia in COVID-19 may relate to the long-life span of erythrocyte and the compensatory proliferation of erythrocyte induced by pneumonia associated hypoxia.[38] Reduced haemoglobin levels could be an indicator of COVID-19 disease progression, it would be more worthy to focus on decline of haemoglobin rather than anaemia.

In our study there was no significant difference in total leucocyte count (table 1) in ICU and Non-ICU groups (P = 0.4) similar to Jason Wagner et al. study[39] (P = 1.0) and Fan et al.[13] study (P = 0.87).

We found that mean absolute neutrophil count was higher in ICU group than in non-ICU group with significant difference (P = 0.03). In comparison with our study, Huang CL et al.[16] also showed that median absolute neutrophil count (ANC) in ICU cases was 10.6 (5.0 - 11.8) x 10^9/L, much higher than the 4.4 (2.0 x 6.1) x 10^9/L in Non-ICU cases (P = 0.00069). In Wang DW[14] et al. study, median ANC of ICU and Non-ICU patients was 4.6 (2.6 - 7.) x 10^9/L and 2.7 (1.9 - 3.9) x 10^9/L, respectively. In Liu J-Y et al.[40] study, the median neutrophil count of common type was 2.4 (1.9 - 3.4) x 10^9 /L, while median neutrophil was higher in severe or critical type, 2.8 (2.3 - 4.4) x 10^9/L, (P = 0.025). In Fan et al. study,[13] study of 69 confirmed cases of SARS–COV-2 infection from Singapore showed that ICU patients tend to develop neutrophilia with a median peak absolute neutrophil count of 11.6 x 10^9/L, compared to 3.5 x 10^9/L in the Non-ICU group. In Wu CM et al.[41] study, 34.5 % of COVID-19 patients demonstrated neutrophilia and patients with ARDS developed higher neutrophil count than those without ARDS (P < 0.001). The available literature suggests that neutrophilia is an expression of the cytokine storm and hyper-inflammatory state which have an important pathogenetic role in COVID-19 and related infections such as SARS.[16,42,10,23] neutrophilia may also indicate a superimposed bacterial infection.[43]

In our study, there was no significant difference in platelet counts between ICU and Non-ICU patients (P = 0.8) in comparison with Wang DW et al.[14] Huang et al.[16] and Fan et al.[13] studies. In Wang DW et al.[14] study, no significant difference (P = 0.78) was noted in platelet count between ICU cases (median: 142; IQR: 119 - 202) vs Non-ICU cases (median: 165; IQR: 125 - 188). In Huang et al. study[16], 8 % (1/13) of patients needing ICU care presented with low platelet count vs 4 % (1/27) of patients that did not need ICU care (P = 0.45). Even in Fan et al. study[13] also, low platelet counts were not associated with ICU care either at admission (P = 0.67) or as a nadir during hospital stay (P = 0.69). In contrast, study done by Guan et al.[15], showed thrombocytopenia (platelet count < 150 x 10^9/L) on admission was more commonly seen in severe (57.7 %) than

non-severe (31.6 %) patients (P < 0.001)[15,44] and Zhou et al.[18] reported that 20 % of non survivors had platelet counts less than 100 x 10^9/L on admission compared to only 1 % in survivors (P < 0.0001). Several mechanisms by which COVID-19 causes thrombocytopenia has been proposed which include - 1) Reduction in platelet production by direct viral infection of bone marrow cells by the virus, destruction of bone marrow progenitor cells by cytokine storm, and indirect effect of lung injury, 2) increased platelet destruction by autoantibodies and immune complex 3) platelet aggregation in the lungs, resulting in microthrombi and platelet comsumption.[45] Cytokine storm of severe disease may lead to secondary hemophagocytic lymphohistiocytosis, which can also result in thrombocytopenia.[10]

The limitations of this study include smaller sample size, focused only on one community hospital, did not include other haematological parameters like eosinophil count and monocyte counts. Some of the laboratory examination records were not available as it is a retrospective study. The strengths of this study include the use of an easily obtained laboratory parameter that is associated with clinical outcomes.

CONCLUSIONS

In our study we found that older age, lymphopenia, neutrophilia and reduced haemoglobin levels are more pronounced in ICU patients. Lymphopenia defined as absolute lymphocyte count less than 1.1 x 10^9/L appears to be effective and reliable indicator of COVID-19 disease severity, particularly in elderly individuals. Further studies are needed to focus on lymphocyte changes in COVID-19 to confirm the predictive ability of lymphopenia in COVID-19.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
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