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Assessment of absolute eosinophil count (AEC) as a prognostic indicator in patients with coronavirus disease (covid-19): A cross sectional study

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Abstract
Coronaviruses are non-segmented positive-stranded RNA viruses with a roughly 30 kb genome surrounded by a protein envelope. Most coronaviruses cause diseases in their particular host species[1]. Those coronaviruses that can infect humans through cross-species transmission have become an important threat to public health. Two serious coronavirus disease outbreaks have happened in the past two decades: severe acute respiratory syndrome (SARS) in 2003[2] and Middle East respiratory syndrome (MERS) [3] in 2012. Since December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) - a segmented RNA virus - has been recognized as the causal factor in a series of severe cases of pneumonia originating in - Wuhan in Hubei province, China - which panned out into a pandemic[4] This disease has been named coronavirus disease 2019 (COVID-19) by WHO. SARS-CoV-2 has been shown to cause disease via a mechanism analogous to the SARS coronavirus, with potential damage to vital organs such as lung, heart, liver, and kidney, and infection poses a considerable risk to patients by the high prevalence of pneumonia[5]. Accumulated evidence suggests that a subgroup of patients with severe COVID-19 could have a dysregulation of the immune response that allows the development of viral hyperinflammation[6] This state of hyperinflammation is referred to as the 'Cytokine storm', which has been linked to as one of the mechanisms causing severe COVID-19 infection[7]. Absolute Eosinophil Count (AEC) is an established inflammation marker, whose increase in number that reflect systemic inflammatory response, and decrease in number indicates decreased ability of the eosinophilic proteins to fight overwhelming viral response [8] The mechanism of eosinopenia has not been completely understood in COVID-19. It is assumed that eosinopenia is the redistribution result of circulating eosinophils to the infection locus due to the chemotactic effects of increased cytokines [9] Hence this study was done to assess the use of Absolute Eosinophil Count (AEC) as a tool to predict disease severity and prognosticate COVID-19 infection.

Keywords: COVID-19, absolute eosinophil count, hyperinflammation

Introduction

Aims of the Study

1. To study the association between disease severity in COVID-19 patients and Absolute Eosinophil Count (AEC).
2. To study the relation between Absolute Eosinophil Count (AEC) and prognosis (discharge/death) in COVID-19 patients.

Materials and Methods

A cross sectional study was conducted at the hospital attached to Bangalore Medical College and Research Institute from March 2020 to June 2020 involving 150 subjects. The study proposal was approved by the Institutional Ethics Committee. This study included consenting adult patients who presented to our hospital with COVID 19 infection confirmed by real time RT-PCR done as per ICMR Protocol. Patients who didn't give consent were excluded from the Study.

Of the 150 subjects included in the study, 66 patients were admitted in the ICU and 84 were admitted in the ward. The following details were documented in the study proforma for the subjects included in the study: symptoms, duration of disease, duration of hospital stay, co-morbid conditions like metabolic disorders, endocrine disorders, renal disorders, cardiac disorders, respiratory disorders history of treatment, relevant clinical findings along with oxygen saturation. Patients were categorised as mild, moderate, severe and critical as follows Mild Illness*:* Individuals who have any of the various signs and symptoms of COVID-19 (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell) but who do not have shortness of breath, dyspnea, or abnormal chest imaging. Moderate Illness*:* Individuals who show evidence of lower respiratory disease during clinical assessment or imaging and who have an oxygen saturation (SpO_2) ≥94% on room air Severe Illness: Individuals who have SpO_2 <94% on room air and respiratory frequency >30 breaths/min Critical Illness*:* Individuals who have respiratory failure, septic shock, and/or multiple organ dysfunction [10]. Patient underwent the following blood investigations which included complete blood count with differential leucoctye count, quantitative CRP and serum ferritin. Absolute Eosinophil count was calculated using the formula shown below:

$$A.E.C = \frac{\text{TOTAL WBC COUNT (cells X } 10^9\text{/L) * EOSINOPHIL (in \%)}}{100}$$

Eosinopenia was classified as follows: Normal – 50 to 500 cells X 10^9/L, Mild eosinopenia 31-50 cells X 10^9/L, Moderate eosinopenia 16-30 cells X 10^9/L, Severe eosinopenia ≤ 15 cells X 10^9/L. Clinical profile and Absolute

Eosinophil Count (AEC) were correlated to assess the disease outcome.

Statistical Analysis

SPSS (Statistical Package for Social Sciences) version 20. [IBM SPASS statistics (IBM corp. Armonk, NY, USA released 2011)] was used to perform the statistical analysis. Data were entered in the excel spread sheet. Descriptive statistics of the explanatory and outcome variables were calculated by mean, standard deviation for quantitative variables, frequency and proportions for qualitative variables. Inferential statistics were assessed using Pearson's correlation. The level of significance was set at 5%

Results

Out of 150(100%) subjects, 43(28.7%) subjects were aged above 60 yrs followed by 40(26.7%) subjects aged between 31 to 45 yrs and between 46 to 60 yrs. Mean age of the subjects was 49.14 ± 17.24 yrs Out of 150(100%) subjects, Maximum were males- 89(59.3%).

The demographic distribution of the 150 subjects included in the study are shown in table 1, 2, 3

Table 1: Distribution of the Subjects Based On Age

AGE	Frequency	Percent
18 to 30 yrs	27	18.0
31 to 45 yrs	40	26.7
46 to 60 yrs	40	26.7
Above 60 yrs	43	28.7
Total	150	100.0

Table 2: Mean Age Distribution of the Subjects

	N	Minimum	Maximum	Mean	Std. Deviation
Age	150	19	85	49.14	17.24

Table 3: Distribution of the Subjects Based On Gender

Gender	Frequency	Percent
Female	61	40.7
Male	89	59.3
Total	150	100.0

Most of the subjects- 62(41.3%) had HTN followed by DM-51(34%), CKD-23(15.3%), IHD- 11(7.3%) as depicted in table 4

Table 4: Distribution of the Subjects Based On Co-Morbidities

Co-morbidities	Frequency	Percentage
DM	51	34
HTN	62	41.3
Bronchial asthma	3	2
COPD	1	0.6
Hypothyroidism	7	4.7
CKD	23	15.3
IHD	11	7.3
CVA	3	2
Malignancy	3	2

Table 5 shows the distribution of the subjects based on AEC. 87(58%) subjects were having Normal AEC levels followed by 26(17.3%) subjects having mild AEC, 19(12.7%) subjects having moderate AEC and 18(12%) subjects having severe AEC.

Table 5: Distribution of the Subjects Based On AEC

AEC	Frequency	Percent
Normal	87	58.0
Mild	26	17.3
Moderate	19	12.7
Severe	18	12.0
Total	150	100.0

Out of 150(100%) subjects, 55(36.7%) were mild, 41(27.3%) were severe, 33(2%) were moderate and 21(14%) were critical. Chi- square test was applied to associate the AEC with severity. Chi-square test showed statistical significant association ($\chi 2$=194.73; p=0.00) as depicted in table 6

Table 6: Cross-Tabulation Based On AEC and Severity

Severity		AEC classified				Total
		Mild	Moderate	Normal	Severe	
Mild	Count	2	0	53	0	55
	%	7.7%	0.0%	60.9%	0.0%	36.7%
Moderate	Count	6	0	27	0	33
	%	23.1%	0.0%	31.0%	0.0%	22.0%
Severe	Count	18	15	7	1	41
	%	69.2%	78.9%	8.0%	5.6%	27.3%
Critical	Count	0	4	0	17	21
	%	0.0%	21.1%	0.0%	94.4%	14.0%
Total	Count	26	19	87	18	150
	%	100.0%	100.0%	100.0%	100.0%	100.0%
Chi-square value- 194.73						
p value- 0.00*						

*significant

Absolute eosinophil count levels were compared with other inflammatory markers. Pearson's correlation showed weak, positive significant correlation between AEC and TLC(r=0.309; p=0.00) whereas negative, very weak, non-significant correlation was seen between AEC and Hb (r=-0.129; p=0.115). Weak, negative and significant correlation was seen between AEC and CRP (r= -0.262; p=0.001). Very weak, negative and significant correlation was seen between AEC and ferritin (r= -0.18; p=0.022).

Table 7: Pearson's Correlation of TLC, HB, CRP, Ferritin with AEC

		r value	p value
AEC	TLC	.309	.000*
	Hb	-.129	.115
	CRP	-.262	.001*
	Ferritin	-.187	.022*

*significant

Out of 150 subjects, 66(44%) were admitted in ICU out of which 18 were having severe eosinopenia 19 had moderate and 21 had mild eosinopenia. Whereas out of 84(56%) subjects admitted in ward, 79 had normal eosinophil count and 5 had mild eosinopenia. Chi- square test was applied to associate the AEC severity with ICU and ward patients. Chi-square test showed statistically significant association ($\chi 2$=104.12; p=0.00). (Table 8)

Table 8: Cross-Tabulation Based On Aec and ICU/Ward

ICU/WARD		AEC classified				Total
		Normal	Mild	Moderate	Severe	
ICU	Count	8	21	19	18	66
	%	9.2%	80.80%	100%	100.00%	44.00%
Ward	Count	79	5	0	0	84
	%	90.8%	19.20%	0%	0.00%	56.00%
Total	Count	87	26	19	18	150
	%	100.00%	100.00%	100.00%	100.00%	100.00%

Subjects having normal AEC stayed longer in hospital- 11.31 ± 3.332 days followed by subjects with mild AEC- 8.62 ± 3.534, moderate AEC- 6.42 ± 1.835 and severe AEC- 5.33 ± 4.229. (Table 9)

Table 9: Distribution of the Subjects Based On Mean Hospital Stay

	N	Minimum	Maximum	Mean	Std. Deviation
Mild	26	5	20	8.62	3.534
Moderate	19	3	10	6.42	1.835
Normal	87	3	23	11.31	3.332
Severe	18	1	20	5.33	4.229

Out of the total 150 subjects, 99 were discharged and 51 patients expired. 51(34%) were dead, out of which 16 were having mild and moderate eosinopenia, 14 were having Severe eosinopenia. Chi- square test was applied to associate the AEC with outcome. Chi-square test showed statistical significant association between AEC and Outcome ($\chi 2$= 76.45; p=0.00). (Table 10)

Table 10: Cross-tabulation based On AEC and Outcome

Outcome		Eosinopenia				Total
		Normal	Mild	Moderate	Severe	
Death	Count	5	16	16	14	51
	%	5.7%	61.5%	84.2%	77.8%	34.0%
Discharge	Count	82	10	3	4	99
	%	94.3%	38.5%	15.8%	22.2%	66.0%
Total	Count	87	26	19	18	150
	%	100.0%	100.0%	100.0%	100.0%	100.0%

Discussion

The severity of COVID-19 infection has been linked to the severity of the immune response elicited in the body. The 'Cytokine storm' being presumed to one important mechanism in causing the same. The interaction of the Spike protein of the virus with the ACE2 receptor followed by further downstream signaling principally involving T_H1 cells leads to the so called 'Cytokine Storm' [11] The pro-inflammatory immune responses of pathogenic Th1 cells and intermediate CD14 + CD16 + monocytes are mediated by membrane-bound immune receptors and downstream signaling pathways. This is followed by the infiltration of macrophages and neutrophils into the lung tissue, which results in a cytokine storm [12]. Particularly, SARS-CoV-2 can rapidly activate pathogenic Th1 cells to secrete pro-inflammatory cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-6 (IL-6). GM-CSF further activates CD14+ CD16+ inflammatory monocytes to produce large quantities of IL-6, tumor necrosis factor-α (TNF-α), Interferons and other cytokines [13] the cytokine storm in COVID-19 is characterized by high expression of IL-6 and TNF-α. Hirano *et al* [14] proposed a potential mechanism of the cytokine storm caused by the angiotensin 2 (AngII) pathway. Among the various inflammatory markers available, Absolute eosinophil count is found to be reduced in COVID 19 patients in various studies. The pathophysiology for eosinopenia in COVID-19 remains unclear, but it may be multifactorial. Mechanisms may include inhibition of eosinophil egress from the bone marrow, blockade of eosinophilopoiesis, reduced expression of chemokine receptors/adhesion factors, and/or direct eosinophil apoptosis induced by type 1 interferons released during the acute infection [15]. A study done by Lippi G, Henry B.M, Eosinophil count in severe coronavirus disease 2019, concluded that lower Absolute Eosinophil Count (AEC) was associated with higher ICU admissions and more was the severity of the COVID19 patient [16]. Liua F, Xua, Zhanga Y *et al* found in their study that patients of COVID-19 may benefit from sustained Lopinavir-combined regimen and the increase of Eosinophil may predict the outcome of COVID-19 progression, concluded that lower Eosinophil count was associated with increased severity, poor prognosis and outcome in COVID19 patients [17]. A study done by Lindsley A W, Schwartz J T, Rothenberg M E, Eosinophil Responses During COVID-19 Infections and Coronavirus Vaccination, concluded that lower the A.E.C more is the severity and is an independent risk factor of mortality in COVID19 patients [18]. Lescure F X, Bouadma L, Nguyen D *et al* with the clinical and virological data of the first cases of COVID-19 in Europe concluded that a lower number of Eosinophils were found in the severe group with COVID-19 and associated poor prognosis [19]. In a study done by Huang *et al*, AEC was zero in more than half of the COVID-19 patients who eventually transferred to the ICU [20]. This phenomenon described by Shaaban *et al* was called "the almost zero eosinophil effect," probably because of the overwhelming effects of cytokine storm caused by severe infection [21] Although Eosinopenia is not unique to COVID-19, eosinopenia could help to distinguish which patients likely have COVID-19 with actionable reliability [22].

In our study, 87% of the patients admitted to the ICU had Eosinopenia of which 27% of them had severe Eosinopenia. Also 90% of the patients who expired had eosinopenia. The limitations of our study were that the sample size studied was small and limited to a single tertiary care centre. It was a cross sectional study where in a follow eosinophil count wasn't done to study the trends of eosinophil count. Further studies are required to elucidate to the exact mechanism of the Eosinopenia and also to assess the sensitivity and specificity of the same

Conclusion

The results of this study revealed significant correlation between the clinical severity of COVID 19 illness and severity of Eosinopenia. Hence, Absolute eosinophil count which is already an established marker of inflammation, can also be used as a prognostic indicator in COVID 19 infection

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