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Role of Eosinophil Count for early recognition and prognostication of Covid-19

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Abstract

Background: The Severe Respiratory Distress Syndrome caused by the COVID-19 virus has turned into a pandemic and has caused havoc worldwide and is still continuing to do so. In such times, there is a need for a diagnostic tool which eases the technique of detecting the disease and is also less time consuming. Thus an analogy was established between the Covid-19 positive RT-PCR cases and their blood eosinophil levels.

Aims: To study the diagnostic and Prognostic significance of eosinophil counts in Covid-19 infections.

Settings and Design: This study that was conducted in a tertiary health care centre was done by analysing the eosinophil counts of the patients and there by correlating them with the progression of the disease.

Materials and Methods: 105 Patients who tested positive by RT-PCR for COVID-19 were enrolled for this study and their complete blood count report was obtained.

Statistical Analysis: The data analysis was done in proportions and percentages. The statistical test of significance applied manually to the cross table was chi square test.

Result: Based on the outcome it was noted that 70.50% cases were eosinopenic on admission. Also drop in eosinophil count(93.75% of the cases) or the absence of eosinophils on presentation correlated with poor prognosis.

Conclusion: Eosinopenia can help in early detection of Covid-19 infection and the variation in the eosinophil count can help in gauging the prognosis and the outcome of the disease.

Keywords: Covid-19, Complete blood count, Diagnostic, Eosinopenia, Prognostic.

Introduction

The novel Coronavirus (SARS-CoV-2) has taken the world by storm as a pandemic affecting 72,851,747 people (as on 17 december 2020) and caused fatal outcomes in 1,643,339 of them (mortality rate= 0.022%).[1]

Covid 19 belongs to the family Coronaviridae which causes severe acute respiratory syndrome.It enters the host cells via the angiotensin-converting enzyme 2 (ACE2) receptor,[2] which is expressed in various human organs and cells like the lungs, heart, nasal and oral mucosa, lymphocytes,enterocytes, endothelial cells.[3] Most patients are either asymptomatic carriers who despite being without symptoms have the potential to be infectious to others coming in close contact, or have a mild influenza-like illness which cannot be differentiated from a simple upper respiratory tract infection. Moderate and severe cases can require hospitalisation along with intensive therapy which includes non-invasive as well as invasive ventilation, along with antipyretics, antivirals, antibiotics and steroids.[4]

In the human body each cell plays a specific role and helps the body to stay functioning. So is the case of eosinophils, they are the main cells involved in the pathogenesis of mild to severe asthma and other allergic reactions. Though they normally account for nearly 1-6%,[5] they play vital roles in the mechanism of immunity and aid with the process of acute inflammation.[6] This task is accomplished by the assembly of an array of molecules like several endosomal toll-like receptors like TLR3, TLR7 AND TLR9 and cytokines, predominantly IL-12 and IFN-γ.[7&8] They also possess MHC-II molecules (used to detect intracellular viral pathogens) and act as antigen presenting cells.[9]

But their role in providing anti-viral immunity is less talked about,[10&11] especially in the context of the global pandemic causing virus, the SARs-CoV-19. Their counts may have a role in disease progression and may indirectly give us an idea of the prognosis of the patient.[12]

Moreover,the widely used PCR technique done on the nasopharyngeal swab remains unreliable as there is variable turn-around time and high chances of false negative.[13] Hence, there is a need for a rapid and cost effective method to be developed to hasten Covid diagnosis amidst the pandemic, especially in rural and underdeveloped areas.

Aim: To study the diagnostic significance of Eosinopenia and also the prognostic significance of changing Eosinophil counts during the course of hospital administration in SARS-CoV-2 infection.

Materials and Methods

Total of 105 Patients who presented with influenza-like symptoms between 14 July to 24 August 2020 and were tested positive for RT-PCR of the nasopharyngeal swab were admitted to the isolation ward and thereby enrolled in this study.

Ethical clearance was obtained from the institutional ethical committee. As is the hospital policy, consent is routinely taken from all patients. Venepuncture was done under aseptic conditions and a venous blood sample was collected in an EDTA tube and processed in a Transasia Sysmex cell counter machine (Model

no:XN-350) and their complete blood count report was obtained.

The trends in the eosinophil and AEC levels were compared among these patients with different severity and were statistically analysed using Microsoft excel software. Out of the 105, 34 patients were excluded from the analysis for the prognostication point of view due to a single CBC report.

Inclusion Criteria: All patients in the adult age group who were tested positive for RT-PCR were included in the study(>18yrs)

Exclusion Criteria: Patients of the Paediatric age group were excluded and patients with negative RT-PCR and positive CT findings were also excluded.

Sample size calculation: The studies by Tan Y, Zhou J et al[11] and Xie G,Ding F et al[14] were used as a reference to calculate the appropriate sample size using the formula, $N=4*SD^2/d^2$. In the above studies their sample size was 40 and 97 patients respectively. The standard deviation of which was calculated as 40.30 and taking into account the error percentage as 10%, the appropriate sample size calculated was **64.9** while the sample size for this study of diagnostic significance is **105** and for the study of prognostic significance is **71.**

Results & Discussion

Demographics: In this study a total of 105 patients were taken who satisfied the inclusion criteria. There were 67(63.80%) men and 38 (36.20%)women in the study.(Table:1) The Male:Female ratio was 1.76. The mean age at presentation being 51.13 years(20-86yrs).

Eosinopenia as a diagnostic tool: The first CBC obtained on the day of presentation revealed that 56 patients(53.33%) had 'zero' eosinophil count and 18 patients (17.14%) had a low eosinophil count [<40cells/cumm].That is, a total of 74 patients(70.50%) were eosinopenic on admission.(Fig:1) In our hospital's laboratory, the normal absolute eosinophil count range is 40-400 cells/μL.

Eosinophil variations as a prognostic tool: In this study it was observed that the increase in the eosinophil count over the subsequent days was related to good prognosis of the disease(79.31%) and a decrease in the count reflected poor prognosis(93.75%).(Table:2) It was found that the drop in eosinophil count or the absence of eosinophils on presentation correlated with a poorer prognosis. (Fig:2)

CHI SQUARE VALUE, χ^2=22.11 (Computed from Table:2)

P VALUE=0.000016 [Calculated at 2 degree of freedom at 0.05 level of significance]

Statistically significant P<0.05.

It was observed that on a routine complete blood picture report most of the RT-PCR positive cases consistently had a low eosinophil count.

The results of this study were correlating with that of the study done by Yingzheng Tan, et al which included a total of 40 patients with M:F ratio of 1.35 and mean age of presentation being 59.37years. In their study 33 patients (82.5%) had a significant decrease in the eosinophil count, 18 patients lacked eosinophils.[12]

Similarly the results of this study were also comparable to studies done by Fahmina Tanni, et al[13] and Guogang Xie, et al[14] and Xia Z.[15]

In Covid-19 infection,the extent of eosinopenia and its degree varies from patient to patient. Most of the patients had eosinopenia.The pathophysiology of eosinopenia in covid 19 infection remains unclear.It may be due to suppression of eosinophilopoiesis in the bone marrow to divert their common progenitor cells to form more and more neutrophils or can be due to direct eosinophil apoptosis induced by type 1 interferons

released during the acute infection. The viral attack on bone marrow blocks eosinophils from entering peripheral circulation.[16]

Other causes for this fall may be their infiltration into the infected tissue or organ [17] or there can be migration to organs like spleen, lymph nodes as well.[6] In covid-19, the affected organ is the lung as it is the entry portal for the virus. But in the pathologic autopsy, the cell constituents of the lung interstitium were found to be lymphocytes, neutrophils and mononuclear cells; and not eosinophils.[18,19] The specific cytokine- EOTAXIN is the key chemoattractant for eosinophils migration.[20] There is evidence that the lung produces eotaxin in small amounts normally,[21] but whether there is an increase in the production of eotaxin in covid infection from the lungs is not established. The absence of eosinophils on autopsy can be due to cytolysis of eosinophils post release of their granules as cytolysis with granule release has been seen in many diseases involving the lungs.[20] The mechanism of cytolysis of eosinophils involves many components of the necroptotic pathway, including receptor-interacting protein kinase 3 (RIPK3) and mixed lineage kinase-like (MLKL).[22] Cytokines like IL-5, GM-CSF, Platelet Activating Factor act both as weak degranulation inducer and degranulation enhancer.[23] These granules which are released contain preformed INF-γ,TNF, IL-4,IL-5,IL-6,IL-13, etc.[24,25] Elevation of circulating cytokines was significantly associated with presence of pneumonia and severe lung injury in COVID-19 infection.[26]

As there is more severe disease progression, there is more marked eosinopenia and this implies that there is more eosinophil infiltration in the lung. This accumulation of eosinophils in lungs can further be justified by briefly discussing the factors which regulate their proliferation and migration. As the disease worsens, there is more and more accumulation of eosinophil and their granular contents are released ie. The interferon and interleukins. More clinically relevant is the increased levels in the blood, esp IL-6; as it is the major culprit and causes the cytokine storm which is the main cause of poor prognosis and patient deterioration.[27]

The eosinopenia can also be caused due to secondary adrenal glucocorticoid stimulation due to inflammatory mediators[28] or due to therapeutic administration of corticosteroids to antagonise the cytokine storm.[29,30]

This parameter helps us to segregate patients who present with similar clinical picture into covid and non-covid patients and may help us promptly isolate the suspected cases to prevent further spread.It is particularly of use in underdeveloped rural areas and community clinics where simple blood tests may be the only available approach due to lack of specialised and modern equipment.It is easy to detect the level of eosinophil count and very cost-effective.

Further, data analysis revealed that persistent eosinopenia or further decrease in the eosinophil count correlated directly with the severity of the disease i.e. poorer prognosis and increase in the eosinophil count was related to better prognosis. This factor may help us to predict the course and anticipate the need for an ICU support for any patient and help the doctors and hospital management to be ready with any kind of arrangements to be made amidst this pandemic where the demand of such facilities may likely surge.

Eosinopenia is not a specific marker of covid, even though it was observed to be present in the majority of the patients. Whether the acquired eosinopenia associated with COVID-19 directly contributes to the disease course or is a marker of severe disease has not

yet been determined.[13] Also the present study is limited as it relies on observational data from a single hospital setup.

Further studies could try to increase the specificity of this parameter by correlating it with other factors like acute phase reactants or coagulation profile in COVID patients. What is the role of IL-6 induced cytokine storm in association with the eosinophil count and whether the exogenous steroid administration can help in preventing the same by impeding eosinophil production and subsequent degranulation.

Table 1: Age and Gender distribution

Sn.	Age Groups	Males (N=67) (63.80%)	Females (N=38) (36.19%)	Total (N=105) (100%)
1.	=<30Y	01	05	06
2.	31-40Y	18	05	23
3.	41-50Y	15	11	24
4.	51-60Y	15	06	23
5.	>60Y	18	11	29

Table 2: Patient Outcomes in cases with increase and decrease in AEC post admission (N=71 Patients)

	Increase in AEC N=24(53.33%)	Decrease in AEC N=21(46.66%)	Total N=45(100%)
Good Prognosis	23(79.31%)	06(20.60%)	29(64.44%)
Poor Prognosis	01(06.25%)	15(93.75%)	16(35.55%)
CHI SQUARE VALUE, χ^2=22.11 Calculated at 2 degree of freedom and 0.05 level of significance, P VALUE = 0.000016 Statistically significant P<0.05			

Figure 1: Based on AEC on Admission

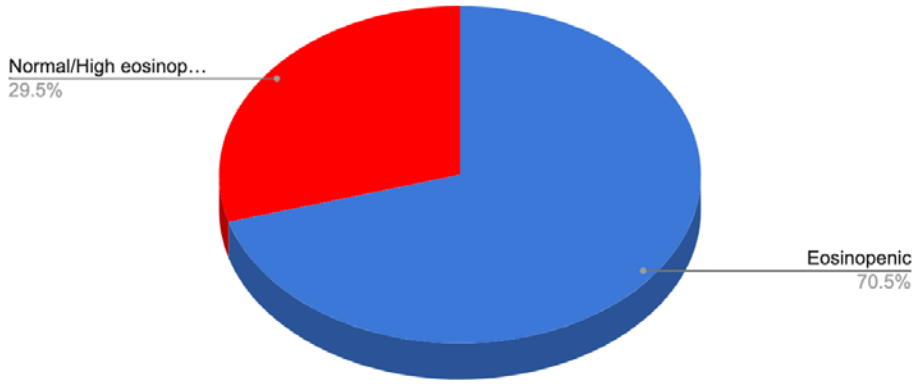
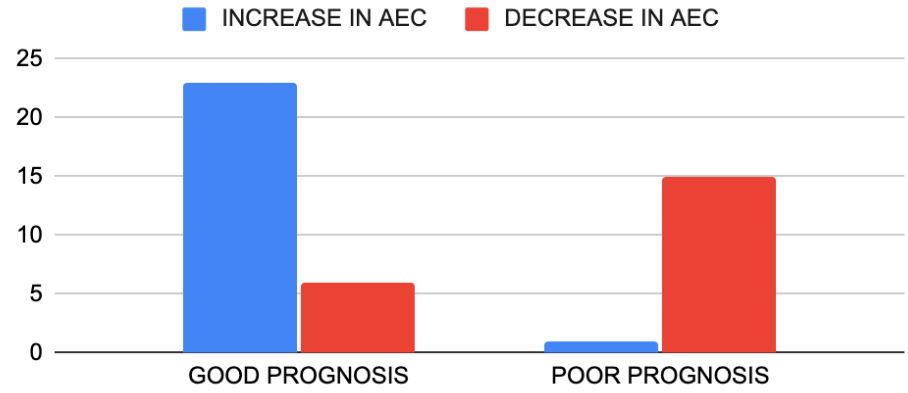


Figure 2: Prognosis based on change in AEC



Conclusion

Eosinopenia in a patient presenting with flu-like or respiratory symptoms can aid in early suspicion and immediate isolation till confirmatory tests are carried out for diagnosis of COVID-19. This may be especially useful in rural and underdeveloped areas where the latest medical technology may be unavailable.

Furthermore, persistent eosinopenia or falling eosinophil counts may act as a warning signal to the treating physician to be prepared for any eventuality and to take necessary measures by referring such patients to higher centres where the facilities of mechanical ventilation are available.

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