Role of Eosinophilic Inflammation in Disease Severity among Egyptian COVID-19 Patients

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Background and study aim: The coronavirus disease pandemic of 2019 (COVID-19) has created global health and economic implications. All potential biomarkers, risk factors, therapy and preventative measures of the disease has been thoroughly investigated. This study examined the relationship between eosinophils and COVID-19 severity, as well as other clinical and laboratory markers of the disease.

Patients and Methods: In this retrospective study, we collected data from 162 patients' medical records including baseline complete blood counts with differential total leucocytic counts (TLC). Descriptive and comparative statistics were performed.

Results: Regarding disease severity, TLC significantly increased (p=0.019) and lymphocyte count significantly decreased (p=0.003) with more severe disease but eosinophil count showed no significant differences (p= 0.864). Patients admitted to the ICU showed no significant difference in eosinophil count (p=0.551), they had significantly higher TLC (p≤0.001) and significantly lower lymphocyte count (p≤0.001). No significant correlations (p > 0.05) were found between eosinophil count and any of the laboratory markers of the disease, age of patients, or length of hospital stay.

Conclusion: Eosinophil count had no correlation with COVD-19 severity, while lymphopenia was a poor prognostic marker.

INTRODUCTION

The novel coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), is unprecedentedly causing the worldwide coronavirus disease 2019 (COVID-19) pandemic. All efforts are directed towards the understanding of its diagnostic biomarkers, risk factors, and novel strategies for its prevention and treatment **[1]**.

Eosinophils arise from pluripotent progenitor cells in the bone marrow under the control of granulocyte-monocyte colony-stimulating factor (GMCSF), interleukin (IL)-3, and IL-5 **[2]**. They constitute a small portion of leukocytic pool in the blood. After being released to the circulation, they migrate to tissues. They are commonly increased in association with parasitic and allergic conditions to deliver inflammatory mediators like (histamine, leukotrienes, platelet-activating factor, adenosine, and bradykinin) to promote host defense, which is accompanied by collateral tissue damage **[3]**. The US National Institutes of Health Clinical Center Laboratory's normal range for blood eosinophils is 40–360 cells per microliter or 0.7–5.8% of the total leukocytic count. Eosinophilia is defined as > 500 eosinophils per microliter of blood, while eosinopenia is < 10 eosinophils per microliter of blood **[4]**.

The role of eosinophils in COVID-19 infection is being investigated. Eosinopenia, together with lymphopenia, were identified as diagnostic markers in COVID-19 **[5]**. Moreover, eosinopenia was correlated with severity and poor outcome. However, this finding is neither definitive nor pathognomonic for COVID-19 **[4]**.

This study aimed to determine the relationship between eosinophils and COVID-19 severity as well as the other clinical and laboratory markers in COVID-19 patients.

MATERIALS AND METHODS

Study subjects and settings

A total of 162 adult patients (age ≥18 years) recruited from COVID-19 isolation hospitals of Ain Shams University from September to November 2020 were enrolled in this study. Of them, only 159 patients were positive for SARS-CoV-2 by initial reverse transcription-polymerase chain reaction (RT-PCR) testing for nasal and pharyngeal swab specimens. Patients with aplastic anemia, lymphoproliferative disorders, immune deficiency disorders, or drug intake history that affects eosinophil counts like epinephrine, thyroxin, or corticosteroids were excluded. Ain Shams University Faculty of Medicine Research Ethics Committee (REC) approved this study.

In this retrospective study we collected data from patients' medical records, including age, gender, clinical presentations at the time of hospital admission, associated co-morbidities, duration of hospital stay, the requirement for ICU admission, and medications received.

Baseline routine laboratory data including complete blood count (CBC) parameters with differential white blood cell count, C-reactive protein (CRP), D-dimer, and ferritin that were tested according to the standard automated methods at Ain Shams Hospitals' Central Laboratories as well as findings of chest CT that were performed in the Radiology Department were also collected from the patients' hospital files. Patients were diagnosed and divided into mild, moderate, and severe groups based on the WHO interim guidance **[6]**. The mild and moderate groups had clinical signs of pneumonia (fever, cough, anorexia, malaise, and muscle pain). The severe group had respiratory distress, respiratory rate > 30 breaths/min in resting state, mean oxygen saturation < 93%, and arterial blood oxygen partial pressure (PaO2)/ oxygen concentration (FiO 2) ≤ 300 mmHg) **[6]**.

COVID-19 Reporting and Data System (CO-RADS):

According to the clinical findings and laboratory test results of patients, as well as CT records, COVID-19 infection suspicion level is graded by CO-RADS score. While CO-RADS 0 indicates negative infection, COVID-19 infection suspicion in CO-RADS 1: is highly unlikely, the CT is normal with findings of non-infectious disease; CO-RADS 2: is low, consistent CT findings with other infections; CO-RADS 3: is indeterminate, CT abnormalities indicate infection but are not specifically sure of COVID-19; CO-RADS 4: is high, most CT findings are suspicious but not extremely typical as multifocal consolidations, unilateral ground glass, or confluent; and CO-RADS 5: is high with typical CT findings; and CO-RADS 6 reflects positive RT-PCR SARS-Cov2 infection **[7]**.

Statistical analysis:

Medians and interquartile ranges were used to describe quantitative variables, while qualitative variables were described as frequencies and percentages. Comparisons were made using the Mann–Whitney U test, and the Kruskal-Wallis test was used. For the assessment of non-random associations between qualitative variables, Chi-squared or Fisher exact test was performed. Spearman's coefficients represented correlations. The statistical analyses were performed using SPSS version 20.0 software, a p-value < 0.05 was considered statistically significant.

RESULTS:

This study included 162 patients, 76 (46.9%) were male and 86 (53.1%) were female. The average age was 47.51 ± 16.30 years. According to disease severity at the time of admission, 68 (42.0%) patients were mild, 69 (42.6%) were moderate and 25 (15.4%) were severe. Hypertension (28.4%) and diabetes (17.9%) were the most common comorbidities. Only 11 (6.8%) patients were smokers. The most common complaints during ER visits were fever (40.1%) and dyspnea (27.2%), while 11 (6.8%) of all patients had no symptoms at all. Most of the patients (total number 120; 74.1%) reported a previous contact with a confirmed case of COVID-19. Of all studied subjects, 45 (27.8%) required ICU admission and only 10 (6.2%) died. The median hospitalization duration was 10 (IQR: 6 – 13) days. The baseline characteristics of the included patients are shown in Table 1.

Comparisons made according to total leucocytic count (TLC), lymphocyte and eosinophil counts of patients included in the study, revealed no significant difference in sex (p =0.585, 0.518 and 0.607, respectively) (Tables 2a,3a,4a). Regarding co-morbidities, the diabetic patients had statistically significant higher TLC compared to the non-diabetics (median: 8.00, (IQR: 6.90 – 13.00) $\times 10^3$/cmm vs. 6.30 (4.79 – 8.00) $\times 10^3$/cmm; p= 0.002). In addition, patients with chronic liver disease (CLD) had statistically significant higher TLC (median: 25.00; (IQR: 13.00 – 27.00) $\times 10^3$/cmm vs. 6.70 (4.80 – 8.81) $\times 10^3$/cmm; p= 0.008) (Table 2b) and statistically significant lower lymphocyte count (median: 0.64 (IQR:0.40 – 0.70) $\times 10^3$/cmm vs. 1.50 (0.80 – 2.18) $\times 10^3$/cmm; p=0.028) compared to those with no CLD. Also, hypertensive patients had statistically significant lower lymphocyte count compared to the non-hypertensives (median: 1.14 (IQR: 0.70 – 1.92) $\times 10^3$/cmm vs. 1.60 (0.90 – 2.24) $\times 10^3$/cmm; p=0.040) (Table 3b). On the other hand, eosinophil count did not show significant changes with any of the comorbidities ($p > 0.05$) (Table 4b).

Regarding disease severity, TLC was significantly increased (p=0.019) (Table 2a) and lymphocyte count significantly decreased (p=0.003) (Table 3a) with more severe disease but eosinophil count did not show any significant differences (p= 0.864) (Table 4a). Median (IQR) TLC was 5.80 (4.80 – 7.67) $\times 10^3$/cmm in mild, 6.90 (4.99 – 9.30) $\times 10^3$/cmm in moderate and 9.00 (4.80 – 17.00) $\times 10^3$/cmm in severe patients, while median (IQR) lymphocyte count was 1.78 (1.28 – 2.54) $\times 10^3$/cmm in mild, 1.17 (0.70 – 2.00) $\times 10^3$/cmm in moderate and 1.15 (0.64 – 1.92) ×103/cmm in severe patients.

By investigating the effect of drug intake on blood cell count we found that, eosinophil count was significantly increased in patients treated with Clexane (median: 0.04 (0.00 – 0.07) $\times 10^3$/cmm vs. 0.00 (0.00 – 0.00) $\times 10^3$/cmm; p=0.039) (Table 4c). On the other hand, TLC significantly decreased in patients treated with Plaquenil (median: 6.34 (IQR: 4.80 – 8.00) $\times 10^3$/cmm vs. 8.00 (5.90 – 15.20) $\times 10^3$/cmm; p=0.002), and significantly increased in patients treated with steroids (median: 7.10 (IQR: 4.81 – 10.11) $\times 10^3$/cmm vs. 5.80 (4.80 – 7.67) $\times 10^3$/cmm; p=0.039) (Table 2c). Besides, lymphocyte count was significantly decreased by intake of Actemra (median: 1.01 (IQR: 0.74 – 1.30) $\times 10^3$/cmm vs.1.58 (0.86 – 2.30) $\times 10^3$/cmm; p=0.014) and steroids (median: 1.17 (IQR: 0.70 – 2.00) $\times 10^3$/cmm vs. 1.78 (1.28 – 2.54) $\times 10^3$/cmm; p=0.001) (Table 3c).

Patients admitted to the ICU showed no significant difference in eosinophil count (median: 0.04 (IQR: 0.00 – 0.07) $\times 10^3$/cmm vs. 0.03 (0.00 – 0.07) $\times 10^3$/cmm; p=0.551) (Table 4a), they had significantly higher TLC (median: 8.00 (IQR: 6.00 – 16.70) $\times 10^3$/cmm vs. 6.00 (4.80 – 7.90) $\times 10^3$/cmm; $p \leq 0.001$) (Table 2a) and significantly lower lymphocyte count (median: 0.80 (IQR 0.58 – 1.60) $\times 10^3$/cmm vs. 1.70 (1.14 – 2.34) $\times 10^3$/cmm; $p \leq 0.001$) (Table 3a) when compared to patients who did not require ICU admission and were treated at the hospital ward. None of the TLC, lymphocyte and eosinophil counts has changed significantly according to the patients' outcome (p=0.125, 0.997 and 0.994, respectively).

In all included patients, TLC showed significant positive correlations with COVID-19 CO-RADS classification (rs=0.161; p=0.041), platelet count (rs= 0.211; p=0.007), CRP (rs= 0.324; $p \leq 0.001$), ferritin (rs= 0.232; p=0.003) and d-dimer levels (rs= 0.279; $p \leq 0.001$). In addition, Lymphocyte count showed a significant positive correlation with hemoglobin level (rs=0.331; $p \leq 0.001$) and significant negative correlations with age (rs= -0.187; p=0.019), COVID-19 CO-RADS classification (rs= -0.198; p=0.013), CRP (rs= -0.218; p=0.006), ferritin (rs= -0.314; $p \leq 0.001$) and d-dimer levels (rs= -0.260; p=0.001). In contrast, there were no significant correlations ($p > 0.05$) between eosinophil count and any laboratory test results, age of patients, COVID-19 CO-RADS classification or length of hospital stay. Table 5

Table (1): Baseline characteristics of the included COVID-19 patients (n=162).

	Total (n= 162)	
Demographics	**Age (years)**	Mean ± SD: 47.51 ± 16.30 Range: (17 – 86)
	Sex	Female: 86 (53.1%) Male 76 (46.9%)
Source of infection	**Unknown** **Contact**	42 (25.9%) 120 (74.1%)
Comorbidities	**SMOKING** **DM** **HTN** **CLD** **CKD** **On dialysis**	11 (6.8%) 29 (17.9%) 46 (28.4%) 3 (1.9%) 12 (7.5%) 2 (1.2%)
CO-RADS	**Median (IQR)** **Range**	4 (1 - 4) 1 – 5
Severity	**Mild** **Moderate** **Severe**	68 (42.0%) 69 (42.6%) 25 (15.4%)
Symptoms	**Asymptomatic** **Fever** **Cough** **Diarrhea** **Dyspnea** **Fever + Diarrhea** **Fever + Cough** **Fever + Cough + Dyspnea** **Fever + Dyspnea** **Cough + Diarrhea** **Fever + Bony aches**	11 (6.8%) 65 (40.1%) 11 (6.8%) 7 (4.3%) 44 (27.2%) 5 (3.1%) 2 (1.2%) 13 (8.0%) 2 (1.2%) 1 (0.6%) 1 (0.6%)
Drug therapy	**Plaquenil** **Zithro** **Clexane** **Tamiflu** **Avigan** **Remdesivir** **Steroids** **Actemra**	132 (82.0%) 162 (100.0%) 153 (94.4%) 34 (21.0%) 1 (0.6%) 4 (2.5%) 94 (58.0%) 19 (11.7%)
Laboratory	**Initial PCR**	Negative 3 (1.9%) Positive 159 (98.1%)
	HB gm/dl	Mean± SD: 12.38 ± 2.05 Range: 6.9 – 17.1
	PLT×10^3/µl	Mean: ± 230.81 ± 98.33 Range: 8 – 546
	Lymphocyte×10^3/µl	Median (IQR): 1.4 (0.77 – 2.18) Range: 0.12 – 7.58
	Eosinophils×10^3/µl	Median (IQR): 0.03 (0 - 0.07) Range: 0 – 1.1
	TLC×10^3/µl	Median (IQR): 6.73 (4.8 - 9) Range: 0.8 – 29.7
	CRP (mg/L)	Median (IQR): 15.5 (5 - 48) Range: 1 – 164
	Ferritin (ng/ml)	Median (IQR): 250 (60 – 717) Range: 1 – 2200
	D-dimer (mg/L)	Median (IQR): 0.63 (0.2 - 1.7) Range: 0.01 – 10
Fate	**ICU** **Death**	45 (27.8%) 10 (6.2%)
	Days of hospital stay	Median (IQR): 10 (6 - 13) Range: 2 – 51
Chest CT	**Free** **Unilateral ground glass opacities** **Bilateral ground glass opacities** **Bilateral ground glass opacities + pneumonia**	63 (38.9%) 5 (3.1%) 91 (56.2%) 3 (1.9%)

DM, diabetes mellites; HTN, hypertension; CLD, chronic liver disease; CKD, chronic kidney disease; CO-RADS, COVID-19 Reporting and Data System; HB, hemoglobin; PLT, platelets; CRP, C-reactive protein; TLC, total leucocytic count; ICU, intensive care unit; PCR, polymerase chain reaction. P-value <0.05 was considered significant.

Normal reference ranges: ***absolute eosinophil count****: 0.1-1×10^3/µl;* ***absolute lymphocyte count:*** *1.5-4×10^3/µl;* ***CRP****: 0.5 mg/L;* ***D dimer****: up to 0.5 mg/L;* ***Ferritin****: male 21.8-274.6ng/ml, female 4.6-204ng/ml;* ***HB****: male 13-17 gm/dl, female 12-15 gm/dl;* ***PLT****: 150-450×10^3/µl;* ***TLC****:4-10×10^3/µl*

Table (2): Comparison of patients' characteristics (demographic and clinical data, comorbidities, and drug therapy) according to the total leucocytic count (TLC).

Variable		TLC		P-value
		Median (IQR)	Range	
(A)Demographic and clinical				
Sex	**Female** **Male**	6.80 (4.81 – 9.30) 6.67 (4.80 – 8.91)	0.80 – 27.00 1.02 – 29.70	0.585
Severity	**Mild** **Moderate** **Severe**	5.80 (4.80 – 7.67) 6.90 (4.99 – 9.30) 9.00 (4.80 – 17.00)	2.20 – 25.00 2.12 – 29.70 0.80 – 27.00	**0.019**
ICU	**Negative** **Positive**	6.00 (4.80 – 7.90) 8.00 (6.00 – 16.70)	1.02 – 25.00 0.80 – 29.70	**≤0.001**
Out come	**Died** **Alive**	12.65 (4.52 – 19.00) 6.65 (4.80 – 8.75)	0.80 – 27.00 1.02 – 29.70	0.125
(B) Comorbidities				
SMOKING	**Non-smoker** **Smoker**	6.90 (4.81 – 9.30) 4.99 (4.52 – 6.30)	0.80 – 29.70 1.02 – 14.00	0.065
DM	**Negative** **Positive**	6.30 (4.79 – 8.00) 8.00 (6.90 – 13.00)	0.80 – 27.00 2.36 – 29.70	**0.002**
HTN	**Negative** **Positive**	6.59 (4.80 – 8.00) 7.05 (4.80 – 11.00)	0.80 – 27.00 1.02 – 29.70	0.201
CLD	**Negative** **Positive**	6.70 (4.80 – 8.81) 25.00 (13.00 – 27.00)	0.80 – 29.70 13.00 – 27.00	**0.008**
CKD	**Negative** **Positive** **On dialysis**	6.75 (4.80 – 8.81) 5.90 (4.60 – 11.65) 5.58 (3.95 – 7.20)	0.80 – 29.70 2.97 – 27.00 3.95 – 7.20	0.809
(C) Drug therapy				
Plaquenil	**Negative** **Positive**	8.00 (5.90 – 15.20) 6.34 (4.80 – 8.00)	0.80 – 27.00 1.02 – 29.70	**0.002**
Clexane	**Negative** **Positive**	6.50 (4.80 – 8.80) 6.75 (4.81 – 9.00)	4.80 – 27.00 0.80 – 29.70	0.804
Tamiflu	**Negative** **Positive**	7.00 (4.99 – 9.75) 5.45 (4.80 – 7.20)	0.80 – 29.70 2.40 – 17.00	0.075
Avigan	**Negative** **Positive**	6.70 (4.80 – 9.00) 7.81 (7.81 – 7.81)	0.80 – 29.70 7.81 – 7.81	0.571
Remdesivir	**Negative** **Positive**	6.70 (4.80 – 9.00) 8.00 (7.25 – 9.00)	0.80 – 29.70 6.50 – 10.00	0.248
Steroids	**Negative** **Positive**	5.80 (4.80 – 7.67) 7.10 (4.81 – 10.11)	2.20 – 25.00 0.80 – 29.70	**0.039**
Actemra	**Negative** **Positive**	6.70 (4.80 – 8.84) 6.89 (4.80 – 12.77)	0.80 – 27.00 2.36 – 29.70	0.315

DM, diabetes mellites; HTN, hypertension; CLD, chronic liver disease; CKD, chronic kidney disease; ICU, intensive care unit; IQR, interquartile range. P-value <0.05 was considered significant.

Table (3): Comparison of patients' characteristics (demographic and clinical data, comorbidities, and drug therapy) according to the lymphocyte count.

Variable		Lymphocytes		P-value
		Median (IQR)	Range	
(A)Demographic and clinical				
Sex	**Female**	1.59 (0.98 – 2.17)	0.36 – 7.58	0.518
	Male	1.37 (0.70 – 2.24)	0.12 – 4.60	
Severity	**Mild**	1.78 (1.28 – 2.54)	0.45 – 4.20	**0.003**
	Moderate	1.17 (0.70 – 2.00)	0.12 – 4.96	
	Severe	1.15 (0.64 – 1.92)	0.28 – 7.58	
ICU	**Negative**	1.70 (1.14 – 2.34)	0.28 – 4.96	**≤0.001**
	Positive	0.80 (0.58 – 1.60)	0.12 – 7.58	
Out come	**Died**	1.37 (0.72 – 2.11)	0.64 – 7.58	0.997
	Alive	1.50 (0.77 – 2.18)	0.12 – 4.96	
(B)Comorbidities				
SMOKING	**Non-smoker**	1.40 (0.75 – 2.13)	0.12 – 7.58	0.367
	Smoker	1.85 (1.14 – 2.90)	0.28 – 4.00	
DM	**Negative**	1.55 (1.00 – 2.24)	0.12 – 7.58	0.058
	Positive	0.97 (0.64 – 1.92)	0.20 – 4.96	
HTN	**Negative**	1.60 (0.90 – 2.24)	0.12 – 7.58	**0.040**
	Positive	1.14 (0.70 – 1.92)	0.20 – 3.50	
CLD	**Negative**	1.50 (0.80 – 2.18)	0.12 – 7.58	**0.028**
	Positive	0.64 (0.40 – 0.70)	0.40 – 0.70	
CKD	**Negative**	1.58 (0.77 – 2.30)	0.12 – 7.58	0.077
	Positive	1.10 (0.55 – 1.40)	0.36 – 2.89	
	On dialysis	0.73 (0.70 – 0.75)	0.70 – 0.75	
(C)Drug therapy				
Plaquenil	**Negative**	1.26 (0.64 – 1.92)	0.12 – 7.58	0.156
	Positive	1.50 (0.99 – 2.30)	0.20 – 4.96	
Clexane	**Negative**	1.15 (0.65 – 1.66)	0.40 – 2.16	0.210
	Positive	1.50 (0.77 – 2.24)	0.12 – 7.58	
Tamiflu	**Negative**	1.35 (0.73 – 2.21)	0.12 – 7.58	0.349
	Positive	1.59 (1.10 – 2.13)	0.60 – 4.60	
Avigan	**Negative**	1.40 (0.76 – 2.17)	0.12 – 7.58	0.383
	Positive	2.18 (2.18 – 2.18)	2.18 – 2.18	
Remdesivir	**Negative**	1.40 (0.77 – 2.18)	0.12 – 7.58	0.873
	Positive	1.87 (0.74 – 2.02)	0.74 – 2.02	
Steroids	**Negative**	1.78 (1.28 – 2.54)	0.45 – 4.20	**0.001**
	Positive	1.17 (0.70 – 2.00)	0.12 – 7.58	
Actemra	**Negative**	1.58 (0.86 – 2.30)	0.12 – 7.58	**0.014**
	Positive	1.01 (0.74 – 1.30)	0.20 – 2.00	

DM, diabetes mellites; HTN, hypertension; CLD, chronic liver disease; CKD, chronic kidney disease; ICU, intensive care unit; IQR, interquartile range. P-value <0.05 was considered significant.

Table (4): Comparison of patients' characteristics (demographic and clinical data, comorbidities, and drug therapy) according to the eosinophil count.

Variable		Eosinophils Median (IQR)	Eosinophils Range	P-value
(A)Demographic and clinical				
Sex	**Female**	0.04 (0.00 – 0.07)	0.00 – 1.10	0.607
	Male	0.03 (0.00 – 0.06)	0.00 – 0.80	
Severity	**Mild**	0.03 (0.00 – 0.07)	0.00 – 0.50	0.649
	Moderate	0.04 (0.00 – 0.09)	0.00 – 1.10	
	Severe	0.03 (0.00 – 0.06)	0.00 – 0.26	
ICU	**Negative**	0.03 (0.00 – 0.07)	0.00 – 0.80	0.551
	Positive	0.04 (0.00 – 0.07)	0.00 – 1.10	
Out come	**Died**	0.04 (0.00 – 0.06)	0.00 – 0.22	0.994
	Alive	0.03 (0.00 – 0.07)	0.00 – 1.10	
(B)comorbidities				
Smoking	**Non-smoker**	0.03 (0.00 – 0.07)	0.00 – 1.10	0.757
	Smoker	0.04 (0.00 – 0.12)	0.00 – 0.36	
DM	**Negative**	0.03 (0.00 – 0.07)	0.00 – 0.80	0.441
	Positive	0.05 (0.00 – 0.08)	0.00 – 1.10	
HTN	**Negative**	0.03 (0.00 – 0.08)	0.00 – 0.80	0.742
	Positive	0.04 (0.00 – 0.06)	0.00 – 1.10	
CLD	**Negative**	0.03 (0.00 – 0.07)	0.00 – 1.10	0.743
	Positive	0.00 (0.00 – 0.22)	0.00 – 0.22	
CKD	**Negative**	0.03 (0.00 – 0.07)	0.00 – 1.10	0.461
	Positive	0.02 (0.00 – 0.05)	0.00 – 0.50	
	On dialysis	0.01 (0.00 – 0.02)	0.00 – 0.02	
(C)Drug therapy				
Plaquenil	**Negative**	0.04 (0.03 – 0.06)	0.00 – 0.22	0.437
	Positive	0.03 (0.00 – 0.08)	0.00 – 1.10	
Clexane	**Negative**	0.00 (0.00 – 0.00)	0.00 – 0.09	**0.039**
	Positive	0.04 (0.00 – 0.07)	0.00 – 1.10	
Tamiflu	**Negative**	0.04 (0.00 – 0.08)	0.00 – 1.10	0.430
	Positive	0.03 (0.00 – 0.06)	0.00 – 0.80	
Avigan	**Negative**	0.03 (0.00 – 0.07)	0.00 – 1.10	0.894
	Positive	0.04 (0.04 – 0.04)	0.04 – 0.04	
Remdesivir	**Negative**	0.03 (0.00 – 0.07)	0.00 – 1.10	0.679
	Positive	0.05 (0.02 – 0.08)	0.00 – 0.09	
Steroids	**Negative**	0.03 (0.00 – 0.07)	0.00 – 0.50	0.663
	Positive	0.04 (0.00 – 0.08)	0.00 – 1.10	
Actemra	**Negative**	0.03 (0.00 – 0.07)	0.00 – 0.80	0.974
	Positive	0.04 (0.00 – 0.09)	0.00 – 1.10	

DM, diabetes mellites; HTN, hypertension; CLD, chronic liver disease; CKD, chronic kidney disease; ICU, intensive care unit; IQR, interquartile range. P-value <0.05 was considered significant.

Table (5): Correlations of TLC, lymphocyte and eosinophil counts with different laboratory test results, age of patients, COVID-19 CO-RADS classification and length of hospital stay in all studied cases.

Variable	Lymphocytes		Eosinophils		TLC	
	r	P-value	r	P-value	r	P-value
Lymphocytes	-	-	0.111	0.167	0.022	0.785
Eosinophils	0.111	0.167	-	-	0.077	0.332
TLC	0.022	0.785	0.077	0.332	-	-
Age	**-0.187**	**0.019**	0.140	0.076	0.078	0.321
CO-RADS	**-0.198**	**0.013**	0.046	0.561	**0.161**	**0.041**
HB	**0.331**	**≤0.001**	0.047	0.555	-0.069	0.382
PLT	0.130	0.108	0.032	0.684	**0.211**	**0.007**
CRP	**-0.218**	**0.006**	0.035	0.658	**0.324**	**≤0.001**
Ferritin	**-0.314**	**≤0.001**	0.030	0.705	**0.232**	**0.003**
D-dimer	**-0.260**	**0.001**	-0.006	0.938	**0.279**	**≤0.001**
Hospital stay (days)	0.036	0.655	0.019	0.808	0.005	0.950

TLC, total leucocytic count; CO-RADS, COVID-19 Reporting and Data System; HB, hemoglobin; PLT, platelets; CRP, C-reactive protein; r, correlation coefficient. P-value < 0.05 was considered significant.

DISCUSSION

This study aimed to correlate total blood counts, lymphocytic, and eosinophilic counts with the COVID-19 severity, hoping to predict future sequelae of the disease, thus allowing clinicians to make proper management strategies as well early as possible.

Total leucocytic count (TLC) and differential counts showed no statistically significant difference between males and females in our study. In contrast, Haitao et al. **[8]** reported that men are consistently more infected by SARS-CoV-2 and more prone to its severe outcomes and higher mortality rates due to gender-related behaviors, social and comorbid factors, as well as genetic and hormonal factors.

Our study revealed that diabetic patients with COVID-19 had statistically significant higher TLC and that hypertensive patients had statistically significant lower lymphocyte counts. Similarly, Anurag et al. **[9]** observed that both neutrophil lymphocytic ratio (NLR) and neutrophil monocytic ratio (NMR) were indicative of severe COVID-19 infection, and both were significantly increased in diabetic patients. In contrast, NLR alone was significantly increased in hypertensive patients. They explained their findings by the dysregulated glucose metabolism in diabetics, which can lead to dysfunctional immunity and hypercoagulability, and angiotensin-converting enzyme-2 (ACE-2) receptor inhibitors and blockers used in the treatment of hypertensives that might increase ACE-2 receptor expression, facilitating SARS-CoV-2 entry to cells.

Our work showed that COVID-19 patients with chronic liver disease (CLD) had statistically significant higher TLC and lower lymphocyte counts. Cerbu et al. **[10]** indicated that immunocompromised COVID-19 patients with CLD had a more severe disease course, comorbidities, and outcomes; also, those patients had abnormally high aminotransferases, LDH, CRP and coagulation tests and low albumin.

We concluded that eosinophil counts did not show any significant changes with any comorbidities associated with COVID-19 patients. However, Rosenberg and Foster **[4]** have listed some complications associated with eosinophilia in COVID-19 patients, including isolated pulmonary eosinophilic vasculitis, acute eosinophilic pneumonia, fatal eosinophilic myocarditis, eosinophilic granulomatosis, with polyangiitis.

Regarding COVID-19 disease severity in our patients, the TLC significantly increased, and the lymphocyte counts significantly decreased with more severe disease. Selim **[11]** stated that the COVID-19 virus attaches to ACE2 receptors on type 2 pneumocytes, lymphocytes, and renal epithelial cells, causing direct damage to these target cells. The virus also causes a cytokine storm, leading to acute respiratory distress syndrome and multiple organ failure. He confirmed that neutrophilia and/or lymphocytopenia related to the cytokine storm were associated with more severe cases of COVID-19 disease and poor prognosis.

Mao et al. **[12]** also found that critically ill patients had persistent leukocytosis, neutrophilia, monocytosis, lymphocytopenia, eosinopenia, and anemia. They suggested that the virus may directly damage the hematopoietic and immune system, with subsequent hypoplastic bone marrow and lymphopenia.

On the other hand, eosinophil counts observed in our study showed no significant changes with the severity of COVID-19. Koenderman et al. **[13]** have stated that although eosinopenia is a common finding in COVID-19 at hospital presentation, yet it is not specific for SARS-CoV-2 infection neither related to its severity. They realized that patients with negative SARS-CoV-2 PCR and suffering from either bacterial or another viral infection also showed less pronounced eosinopenia and assumed that acute systemic inflammation could lead to eosinopenia irrespective of the causative agent.

Also, Lippi et al. **[14]** agreed with our results as they found only a marginally significant difference in the count of eosinophils in severe COVID-19 patients compared to milder cases. Therefore, they doubted the efficiency of eosinophil count for predicting severe COVID-19 disease because of the biological variation in eosinophil counts and the current technical drawbacks in measuring eosinophils in whole blood.

Qian et al. **[15]** as well reported that the relation between eosinopenia and COVID-19 was questionable, attributing eosinopenia to the sequestration of eosinophils by chemokines into the local inflammatory sites or suppression in the bone marrow.

While Rosenberg and Foster **[4]** have reported a different finding as they concluded that eosinopenia could facilitate both the diagnosis and prognosis of severe COVID-19, but it is neither definitive nor pathognomonic for COVID-19. They also reported some algorithms which predict COVID-19 severity and include eosinophil counts like the risk stratification score (COVID-19-REAL) and PAthRIS score.

In our study, eosinophil counts were significantly increased in patients treated with Clexane. Similarly, Ari et al. **[16]** have linked high eosinophil count to lower anti-factor Xa activity in patients with COVID-19 receiving low molecular weight heparin (LMWH). That is why they suggested that eosinophil counts can be used as an alarm to adjust the anticoagulation dose.

Moreover, our study showed that TLC was significantly decreased in patients treated with Plaquenil. Sames et al. **[17]** listed that rare side effects of hydroxychloroquine (Plaquenil) included leukopenia due to agranulocytosis and bone marrow suppression. On the other hand, our study showed that TLC was significantly increased in patients treated with steroids. Eljaaly et al. **[18]** also stated that glucocorticoids could cause leukocytosis, neutrophilia, and lymphopenia.

In the same context, our results showed a significant decrease in lymphocyte counts with treatment with Actemra or tocilizumab. Keske et al. **[19]** stated that the early administration of tocilizumab in COVID-19 disease improved survival and decreased the hospitalization duration and the need for oxygen support. It is an anti-IL-6 receptor monoclonal antibody that inhibits signal transduction and suppresses the cytokine storm. The earliest alarming signs that permit Actemra use include elevated CRP, IL-6, and decreased % lymphocytes, which improve upon its administration.

In our study, eosinophil counts did not show significant changes either in patients admitted to the ICU or with the fate of the patients. In accordance, Le Borgne et al. **[20]** concluded that extreme eosinopenia was predictive of COVID-19 severity but not mortality. While a study by Yan et al. **[1]** highlighted that progressively worsening eosinopenia was a sign of critical disease and higher mortality because eosinopenia correlated with biomarkers of coagulopathy and kidney and liver damage. Also, another study by Nair et al. **[21]** found that patients with eosinophilia had a lower CRP level, milder clinical course, and better disease outcomes indicating a protective role of eosinophils against severe inflammation via an inhibitory mechanism.

In all patients in our study, TLC showed significant positive correlations with COVID-19 severity, CO-RADS chest CT imaging classification, CRP, ferritin, and D-dimer levels. Anurag et al. **[9]** also realized that increased TLC, neutrophilia, lymphopenia, high NLR, and high NMR were associated with COVID-19 severity. Zhu et al. **[22]** specified that TLC count

(≥ 6.16 × 10^9/L) should receive more caution during treatment because TLC count at admission was significantly correlated with death in COVID-19 patients. Similarly, a study by Amano et al. **[23]** showed that Chest CT imaging had a vital diagnostic role in COVID-19 due to its high sensitivity and correlation with disease severity, together with increased CRP, ferritin, D-dimer, and lymphopenia.

Our work showed that lymphocyte count had a significant positive correlation with hemoglobin level. Zhang et al. **[24]** also reported that lymphocytes and hemoglobin decreased gradually with disease progression. They explained lymphopenia by direct viral infection to lymphocytes, direct viral destruction of lymphatic organs, inflammatory cytokines release like tumor necrosis factor-alpha and IL-6, or lymphocytic inhibition by metabolic products. They also explained the decrease in hemoglobin by viral adherence to hematopoietic cells through the ACE 2 receptor releasing endotoxins and destroying the hematopoietic stem/progenitor cells.

We additionally found that lymphocytes had significant negative correlations with age, COVID-19 CO-RADS classification, CRP, ferritin, and d-dimer levels. Zhang et al. **[24]** as well found that older age was accompanied by more severe disease, probably due to the weakening of the body's immune system or the presence of underlying comorbidity. Hashem et al. **[25]** investigated the prognostic value of the available laboratory biomarkers and concluded that lymphopenia together with anemia and leukocytosis and inflammation-related proteins like CRP, ferritin, D-dimer, procalcitonin, and IL-6 played independent roles in identifying severe cases with poor outcomes.

Finally, our work revealed no significant correlations between eosinophil count and any of the laboratory test results. Karimi Shahri et al. **[26]** have also stated that although eosinophils could have an antiviral role yet, it remains controversial when it concerns COVID-19. And although eosinopenia has a bad prognostic value, its actual involvement in the inflammatory process of infectious diseases is yet to be established. Eosinopenia in SARS-CoV-2 infection has been explained by eosinophils migration, like lymphocytes, into the pulmonary parenchyma, with a subsequent decrease in the circulating eosinophil count. However, their role against COVID-19 infection is not pathognomonic.

CONCLUSIONS:

In our study, the eosinophil count showed no significant correlation with the disease severity or with the other typical biomarkers of COVID-19 disease, while lymphopenia was persistently a poor prognostic marker. Until the analytical imprecision and the functional sensitivity of the modern hematological analyzers is improved, depending on the eosinophil count for diagnosis, prognosis, and treatment of COVID-19 would hence remain questionable.

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ABBREVIATIONS:

ACE-2: Angiotensin-converting enzyme-2
CBC: Complete blood count
CLD: Chronic liver disease
COVID-19: Coronavirus disease 2019
IL: Interleukin
NLR: Neutrophil lymphocytic ratio
NMR: Neutrophil monocytic ratio
SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

ETHICAL CONSIDERATIONS

The study followed the Declaration of Helsinki's ethical research criteria. The Ain Shams University Faculty of Medicine Research Ethics Committee approved the current study procedure. All patient data was kept private, only used for the study.

HIGHLIGHTS:

- The total leucocytic count and absolute lymphocyte count may serve as predictive biomarkers for COVID-19 progression.
- Total leucocytic count significantly increases with COVID-19 severity, while the absolute lymphocyte count significantly decreases
- Absolute eosinophil count does not change significantly with COVID-19 severity

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