CD64 AND CD11B EXPRESSION IN COVID-19 PATIENTS

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Abstract:

Introduction: Coronavirus disease 2019 (COVID-19), is the highly contagious viral illness, resulting in more than 6 million deaths worldwide.

Aim of the work: evaluation of the expression of cell surface markers CD64 and CD11b and correlate their expression with the disease severity in COVID -19 patients.

Patients and Methods: This study conducted on 80 individuals divided into: ***Group I (Patients group):*** it included sixty (60) patients diagnosed COVID -19 patients by PCR and was subdivided into 2 subgroups: **Group Ia**: 30 (Non- ICU) COVID -19 patients with no respiratory symptoms. **Group Ib**: 30 COVID -19 patients who were admitted to ICU (intensive care unit) with respiratory complications. ***Group II (control group):*** It included twenty (20) apparently healthy individuals matched for age and sex. Measurement of percentage of expression of CD64 and CD11b on neutrophils and monocytes in peripheral blood by Flow cytometry. In addition to other investigations; complete blood count (CBC) with differential count, prothrombin concentration (PC), international normalized ratio (INR), random blood glucose, serum ferritin, D-dimer, liver function tests.

Results: There was high significant difference in CD64 expression on neutrophils and monocytes when comparing it among all groups with higher value patient's group (p = **< 0.001*, p = < 0.0001* respectively)** but no statistically significant difference when comparing group Ia and Ib. while, there was no statistically significant difference in CD11b expression on neutrophils when comparing it among all groups, between group Ia & Ib but There was statistically significant difference in CD11b expression on monocytes when comparing it among all groups with higher value in patients group (p = **0.010*)** but there was no statistically significant difference when comparing group Ia to group Ib

Conclusion: Both neutrophils and monocytes are activated during COVID -19 infection, and both show high upregulation of CD64. While CD11b is not upregulated on activated neutrophils, while it is upregulated on monocytes, and both can be used as elevated markers in COVID -19 but not useful for categorization of patients as regarding severity.

Key Words: COVID-19, ICU, CD64, CD11b

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Introduction:

In 2019, millions of individuals were impacted by the global pandemic caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection. The Coronavirus Disease 2019 (COVID-19) produces a wide range of symptoms, many of which are complex, initially involving the respiratory tract, but also affecting multiple organs in many cases and resulting in serious health problems or death **(1).**

Individuals in early stages of SARS-CoV-2 infection may present with no manifestations, until emergence of shortness of breath, severe pneumonia , organ damage and even death **(2).**

Severe COVID-19 is characterized by massive inflammatory response, including excessive cytokine expression, that can be detected in both the bloodstream and the lungs. This response involves a wide range of immune cells, including neutrophils and macrophages, which sense pathogens and damaged self-structures, and subsequently inducing inflammatory mediators **(3).**

Innate immunity's initial line of defense against an invading infection are neutrophils. Neutrophil CD64 (nCD64) is a useful indicator for infection and sepsis as expression of CD64 on dormant neutrophil is decreased and markedly up-regulated after few hours of activation **(4).**

CD64 is a high-affinity Fc-gamma receptor I (FcγRI) which is normally expressed on the monocytes. It is considered as an indicator of sepsis and could be associated with higher mortality in the

intensive care unit (ICU). Granulocyte colony stimulating factor (G-CSF) and Interferon γ (IFNγ) both upregulate CD64 in the neutrophil. Because of its reactivity to G-CSF and IFNγ the high expression of CD64 generally indicates a severe health problem **(5).**

CD11b is considered a cell surface receptor, which is important for both neutrophil and monocyte migration to the sites of infection / inflammation. The upregulation of CD11b has been considered in leucocytes from the peripheral blood of patients who suffer from pulmonary disease **(6).**

Neutrophil CD64, and also both neutrophil and monocyte CD11b expression, has shown good power in discrimination between severe septic and non-septic ICU patients (intoxication, trauma, operation, cerebral hemorrhage) and also in distinguishing infection from disease progress in patients with autoimmune disease. n CD64 is considered a useful marker in both diagnosis and prediction of survival in ICU patients with ventilator-acquired pneumonia and also superior to C reactive protein (CRP) and procalcitonin in diagnosis of sepsis in ICU patients. Strong upregulation of CD64 show that both neutrophils and monocytes are activated during severe COVID-19 infection **(7).**

The aim of this study was to evaluate the expression of cell surface markers CD64 and CD11b and correlate their expression with the severity of disease in Covid-19 patients.

Patients and methods:

Ethical consent:

This study was **approved by Academic and Ethical Committee of Minia University**. providing written informed concent from participants (Approval number : 134:11/2021, Date of approval : 29 November 2021) . All procedures in this study have been performed in compliance with the principles in the World Medical Association's Declaration of Helsinki on human research ethics.

This prospective cross-sectional study was carried out at the Clinical Pathology Department and chest department, Faculty of Medicine, Minia University, Egypt, through the period from November 2021 to June 2022. This study was conducted on 80 individuals divided into: ***Group I (Patients group):*** it included sixty (60) patients, diagnosed COVID -19 patients by PCR and was subdivided into 2 subgroups: **Group Ia**: 30 (Non- ICU) COVID -19 patients with no respiratory symptoms. **Group Ib**: 30 COVID -19 patients who admitted to ICU with respiratory complications. ***Group II (control group):*** It included twenty (20) apparently healthy individuals matched for age and sex.

All subjects included were subjected to careful history taking considering age, occupation, residence, duration of disease, presence of fever, cough, dyspnea and co-morbidities. Clinical data as the temperature by thermometer and oxygen saturation by pulse oximeter were recorded for each patient. All such clinical data were extracted from the hospital information system and patients' paper medical records. All patients included in this study received antibiotics

Inclusion Criteria: PCR positive RNA of COVID -19. **Exclusion Criteria**: vaccinated patients.

Blood sampling protocol: About 7.8 ml of venous blood samples were withdrawn from each subject under complete aseptic conditions:

- 2 ml of blood was evacuated in Ethylene Diamine Tetra acetic Acid (EDTA) containing tube for CBC and Flow cytometric analysis.
- 1.8 ml of blood in a tube containing 0.2 ml trisodium citrate 3.2% for detection of prothrombin concentration and D-dimer.
- 4 ml in plain tube. Blood was left to be clotted for 30 min in the incubator then centrifuged at 3000 rpm for 15 minutes. The expressed serum was used for determination of random blood glucose, liver function tests and serum ferritin.

Methodology:

Patients were diagnosed by RT-PCR on nasal swab using Rotor-Gene Q and QIA cube from QIAGEN (Made in Germany)

Routine investigations: Complete blood count (CBC) which was performed using 5-part diff Celltac G, NIHON KOHDEN CORPORATION, AUTOMATED HEMATOLOGY ANALYSER, Japan **(8). PC and INR** were determined by turbodensitomertic method using (coaDATA 2004, LABiTec GmbH, Germany, using LABiTec PT-Reagent kit according to the manufacturer's instructions. **D-dimer** was done using GENRUI, biotech Inc , kinetic assay, China using the commercially available kits according to the manufaturer's instructions**. Random blood glucose** and **liver function tests** (total bilirubin, direct bilirubin, Alanine transaminase (ALT) , Aspartate transaminase (AST) , and albumin) were performed using auto-analyzer SELECTRA PRO XL, ELITech Group, clinical chemistry automation systems, Netherlands, using the commercially available kits according to the manufaturer's instructions. **Serum ferritin** was done using a fully automated analyzer (Cobas e 411 analyzer , ROCHE DIAGNOSTICS GmbH, Mannheim, Germany) , by electrochemiluminescence technology for immunoassay analysis. Using commercially available kits supplied by ROCHE DIAGNOSTICS.

Special investigations: Measurment of the percentage of expression of CD64 and expression of CD11b on neutrophils and monocytes in

peripheral blood by Flow cytometry, **BD FACS canto II, USA (9).**

Flow cytometric assessment: Performed within 24 h of sample collection.

In brief : EDTA blood was used for evaluation of CD 64, CD11b expression. **For each sample**, two tubes were labeled 1&2, one was the test tube and the other tube was used for isotypic control then Hundred ul (100 ul) of blood samples were added in both tubes then 20 ul of phycoerythrin (PE) conjugated anti-CD64 antibody and 20 ul of allophycocyanin (APC) conjugated anti-CD11b antibody were added to the first tube (test tube) , and then both tubes were vortexed and then incubated in the dark at room temperature for 15-20 minutes then 2 ml of lysing buffer solution (diluted 1:10) were added to each tube then tubes were vortexed and then incubated for 10 minutes at room temperature in the dark then the tubes were centrifuged at 1200 rpm for 5 minutes and then supernatant was discarded then 2 ml phosphate buffered saline (PBS) was added to each tube and mixed well then the tubes were centrifuged at 1200 rpm for 5 minutes and then supernatant was discarded. Then Cells were suspended in 300 ul PBS, then tubes were ready for acquiring data by flow cytometric analysis. Neutrophils and monocytes population were defined and gated based on forward and side scatter. Results were expressed as percentage of cells positive for CD64 and CD11b. Analysis was carried out using a flow cytometry **(BD FACS canto II, USA)**. Data processing was carried out with the **Diva software**.

Statistical analysis:

The analysis of the data was carried out by using IBM SPSS 26.0 statistical package software (IBM; Armonk, New York, USA). Testing of normality of the data by using the Kolmogorov-Smirnov tests. Expression of data was as mean, standard deviation (SD), minimum and maximum of range for quantitative measures ,and expressed as median (IQR) for non-parametric quantitative data in addition to both number and percentage for categorized data. Independent sample t test for non-parametric data used for comparison between two independent groups, Concerning comparison of multiple independent groups, ANOVA test was used. To compare categorical variables ,the Chi-square test or Fisher's exact test were used. A p-value less than 0.05 was considered significant.

Results:

This work included sixty COVID- 19 patients divided into two subgroups (Group Ia: included 30 (Non- ICU) patients their ages ranged from 20 to 84 years with Mean ± SD: 56.2 ± 17.36. This group included 15 males (50%) and 15 females (50%). and Group Ib: included 30 ICU patients ,their ages ranged from 25 to 87 years with Mean ± SD: 60 ± 18.08. This group included 17 males (56.7%) and 13 females (43.3%). in addition to twenty apparently healthy volunteers were served as control group ,their ages ranged from 25 to 85 years with Mean ± SD: 52 ± 21.08. This group included 11 males (55%) and 9 females (45%).. There was no statistically significant difference between the groups regarding age and sex **(Table 1)**.

Table (1): Demographic data of the different studied groups:-

	Group Ia (n = 30)	Group Ib (n = 30)	Group II (Control) (n = 20)	p value Among all groups	p value G Ia vs G Ib	p value G Ia vs G II	p value G Ib vs G II
Age (years) **Range** **Mean±SD**	 20 – 84 56.2±17.36	 25 – 87 60 ± 18.08	 25-85 52 ±21.0	0.326	> 0.99	0.446	0.155
Sex: **Male N (%)** **Female N (%)**	 15 (50%) 15 (50%)	 17(56.7%) 13 (43.3%)	 11(55%) 9 (45%)	0.372	0.605	0.160	0.417

*: Significant difference at P value < 0.05

Statistically significant difference in hemoglobin level & Total leucocyte count was noted when comparing them among all groups (P- value 0.006*, 0.001*respectively) and between group Ia and control group (P- value **0.022*, 0.016*** respectively) and between group Ib and control group (P- value **0.001*** , < 0.001** respectively) with lower hemoglobin level and higher total leucocyte count in patients groups but no statistically significant difference between group Ia & group Ib . Statistically high significant difference in absolute lymphocytes count & absolute neutrophils counts when comparing them among all groups was noted with lower absolute lymphocytes count and higher absolute neutrophils count in patients groups (P- value < 0.001**, < 0.001**respectively) , but no statistically significant difference between group Ia & group Ib concerning absolute neutrophil count (P- value 0.936). Statistically significant difference in

absolute monocyte count and platelets count when comparing them among all groups (P- value 0.04*, 0.034* respectively) and between group Ib and control group (P- value 0.036*, 0.014*respectively) but no statistically significant difference between group Ia and both group Ib and control group. Statistically significant difference in PC & INR when comparing them among all groups was noted (P- value 0.001*, 0.001*respectively) and between group Ia and control group (P- value **0.023*, 0.023*** respectively) and between group Ib and control group (P- value **< 0.001****, **< 0.001**** respectively) but there was no statistically significant difference when comparing group Ia to group Ib **(Table 2)**.

Table (2): Comparison of the different studied groups as regarding hematological parameters:

Variable	Group Ia (n = 30)	Group Ib (n = 30)	Group II (Control) (n = 20)	p value Among all groups	p value G Ia vs G Ib	p value G Ia vs G II	p value G Ib vs G II
Hemoglobin (g/dl) Range Mean ± SD	9.3 - 16.4 12.4 ±1.971	6.7 – 17.3 12.5 ± 2.8	12.3 - 16.6 14.29 ± 1.27	**0.006***	0.723	**0.022***	**0.001***
Total leucocytes count ($x10^3$ /µl) Range Mean ± SD	4 – 24.6 12.63±4.79	1.7 – 40 12.3 ± 7.91	4.7 - 10.6 7.73 ± 2.03	**0.001***	0.778	**0.016***	< **0.001****
Absolute Lymphocytes count /µl Range Mean ± SD	560 – 4450 1601 ±792.9	170 – 3476 1184±735.5	1600 – 3400 2137 ± 457.9	< **0.001****	**0.011***	< **0.001****	**0.001***
Absolute neutrophils count /µl Range Mean ± SD	2760-19434 9971 ±3960	1394–22400 9145 ± 5050	2350 –7314 5062.4 ±1777	< **0.001****	0.936	**0.001***	< **0.001****
Absolute Monocytes count/ µl Range Median IQR	44 – 3560 355.5 (215-460.5)	15 – 1456 306 (196.5-588.5)	104 – 380 280 (200 - 310.5)	**0.04***	0.695	0.242	**0.036***
Platelets ($x10^3$ /µl) Range Mean ± SD	100 – 594 307 ± 118.9	38 – 446 236 ± 123.4	150 – 378 203.3 ± 65.8	**0.034***	0.095	0.859	**0.014***
PC (%) Range Mean ± SD	58 – 100 84.9 ±13.45	12 – 100 75.4 ± 20.6	78 – 100 94.65 ± 6.64	**0.001***	0.183	**0.023***	< **0.001****
INR Range Mean ± SD	1 - 1.49 1.13 ± 0.13	1 - 6.24 1.40 ± 0.9	1 - 1.18 1.04 ± 0.05	**0.001***	0.168	**0.023***	< **0.001****

Analyzed by ANOVA with post hoc tests and independent sample t test
***: Significant difference at P value < 0.05**
****: Highly significant level at P value < 0.001**
Statistically significant difference in the level of total Bilirubin was noted when comparing among all groups with high total bilirubin level in patients groups (P- value 0.012*) but no statistically significant difference when comparing it between group Ia and both group Ib and control group. Concerning ALT when comparing them among all groups (P- value 0.004*) with high ALT level in patients groups and between group Ib and control group (P- value 0.003*) but no statistically significant difference when comparing them between group Ia and both group Ib and control group. There was statistically significant difference in the level of both AST and serum albumin when comparing them between group Ia and control group (P- value 0.016*,0.018* respectively) but no statistically significant difference when comparing them among all groups and between group Ib and both group Ia and control group **(Table 3).**

Table (3): Comparison of the different studied groups regarding random blood glucose and liver function tests:

Variable	Group Ia (n = 30)	Group Ib (n = 30)	Group II (Control) (n = 20)	p value Among all groups	p value G Ia vs G Ib	p value G Ia vs G II	p value G Ib vs G II
RBG (mg/dl) Mean ± SD Range	141. ± 67.2 80 – 350	139. ±74.47 85 - 391	125.1 ±24.8 89 - 180	0.841	0.728	0.531	0.881
Total bilirubin (mg/dl) **Range** **Median** **IQR**	0.3 – 11.6 0.815 (0.6 – 1)	0.3 – 2.29 0.675 (0.5 – 1)	0.3 - 0.9 0.6 (0.4 – 0.77)	**0.012***	0.299	0.077	0.881
Direct bilirubin (mg/dl) **Range** **Median** **IQR**	0.1 - 4.8 0.23 (0.2 - 0.32)	0.1 - 1.35 0.21 (0.17- 0.32)	0.1 – 0.4 0.2 (0.1 - 0.3)	0.098	0.625	0.114	**0.031***
AST (U/L) **Range** **Median** **IQR**	13 – 365 42 (26.5-58.7)	11 – 209 32 (19.5- 59.2)	15-37 32 (25 - 34.75)	0.123	0.344	**0.016***	0.5
ALT (U/L) Range Mean ± SD	10 – 144 50.4 ± 31	14 – 128 38.5±23.93	18 – 35 27.6 ±5.67	**0.004***	0.224	0.239	**0.003***
Albumin (g/dl) Range Mean ± SD	- 4.7 3.75 ± 0.64	2.2 - 5.1 3.68 ± 0.65	3.7 - 4.6 4.075 ±0.34	0.07	0.750	**0.018***	0.09

Analyzed by ANOVA with post hoc tests and independent sample t test

***: Significant difference at P value < 0.05**

Statistically significant difference in oxygen saturation, ferritin and D- dimer was noted when comparing them among all groups (P- value < 0.001**, 0.03*, < 0.001**, respectively) and between group Ia and group Ib (P- value < 0.001**, 0.04*, 0.042*respectively) and between group Ia and control group (P- value 0.004*, 0.04*, < 0.001**respectively) and between group Ib and control group (P- value < 0.001**, 0.03*, < 0.001**, respectively) with low oxygen saturation and high ferritin level and D-dimer **(Table 4)** (**Figure 1) and (Figure 2)**.

Table (4): Comparison of the different studied groups as regarding to Oxygen saturation , ferritin &D-dimer.

Variable	Group Ia (n = 30)	Group Ib (n = 30)	Group II (Control) (n = 20)	p value Among all groups	p value G Ia vs G Ib	p value G Ia vs G II	p value G Ib vs G II
O2 Saturation (%) Range Mean ± SD	68%-98% 92% ±5.39	30% - 88% 69.5%±15.2	94% - 99% 96.7% ± 1.3	< **0.001****	< **0.001****	**0.004***	< **0.001****
Ferritin (ng/ml) Range Median IQR	11 – 981 188 (100-314.7)	17.5 – 1405 242.5 (109-362.5)	75 – 350 115 (90-200)	**0.03***	**0.04***	**0.04***	**0.03***
D-dimer (µg/ml) Range Median IQR	0.2 –12.9 0.9 (0.4-1.7)	0.4 – 10 1.35 (0.75-2.85)	0.2 – 0.6 0.4 (0.3-0.475)	**<0.001****	**0.042***	**<0.001***	**<0.001****

***: Significant difference at P value < 0.05**
****: Highly significant level at P value < 0.001**

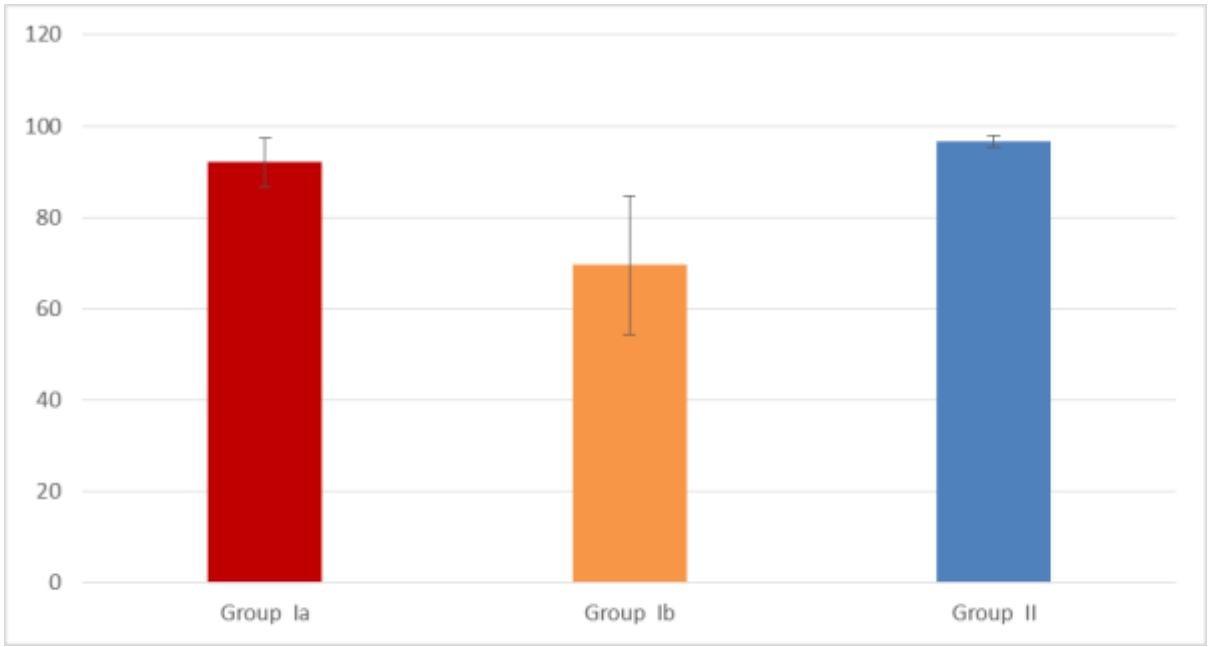

Figure (1): Comparison of the different studied groups as regarding to Oxygen saturation.

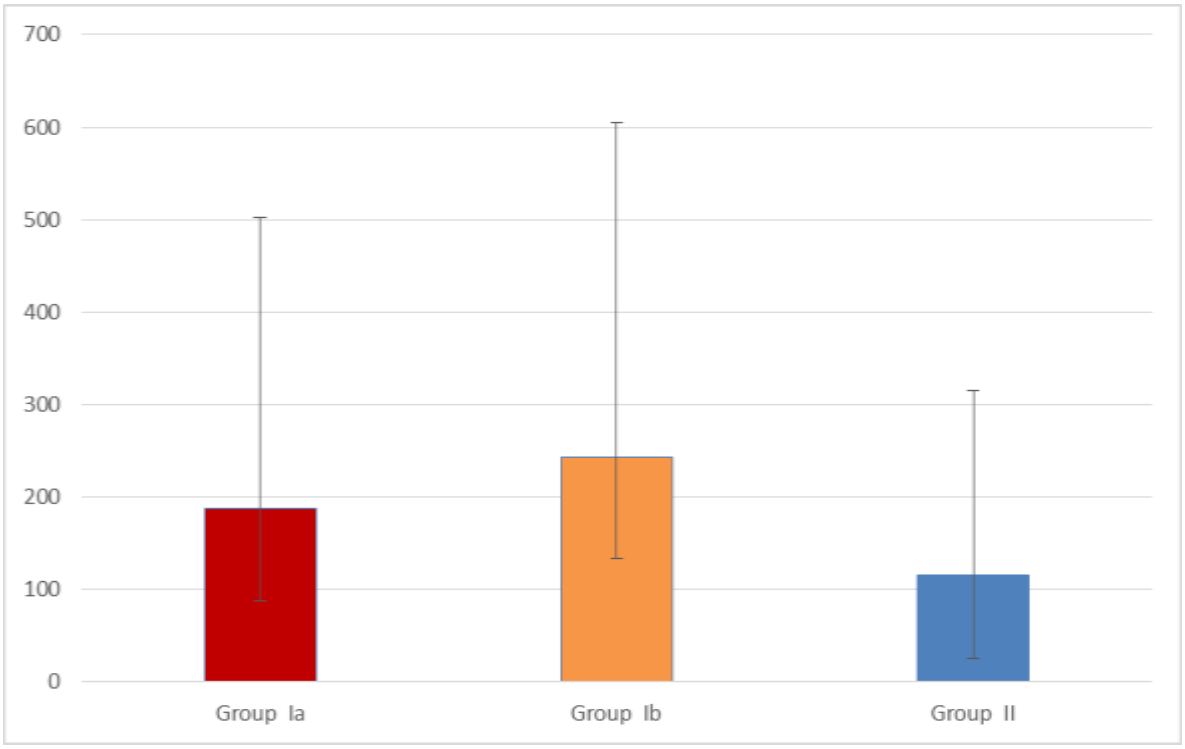

Figure (2): Comparison of the different studied groups as regarding to ferritin.

There was high significant difference in CD64 expression on neutrophils when comparing it among all groups and between control group and both group Ia and group Ib (P- value < 0.001**, < 0.001**, < 0.001**respectively) but no statistically significant difference when comparing group Ia and group Ib (P- value > 0.99). There was no statistically significant difference in CD11b expression on neutrophils when comparing it among all groups, between group Ia & Ib and between control group and both group Ia and group Ib **(Table 5) and (Figures 3 ,4, 5).**

Table (5): Comparison of the different studied groups as regarding to expression of CD64 &CD11b on neutrophils:

Markers	Group Ia (n = 30)	Group Ib (n = 30)	Group II (n = 20)	p value Among all groups	P valueG Ia vs G Ib	p value G Ia vs G II	p value G Ib vs G II
CD 64 (%) Range Mean ± SD	19.7 - 84.2 39.1 ± 17.55	24.1 - 76.6 43.6 ± 15.89	0.4 – 7 1.72 ±1.42	**< 0.001****	> 0.99	**< 0.001****	**< 0.001****
CD 11b (%) Range Mean ± SD	70.9 - 99.9 96.1 ± 5.753	75.5 - 99.7 96.3 ± 5.073	70.4 - 99.7 93.5 ±8.75	0.509	0.695	0.494	0.219

***: Significant level at P value < 0.05**
****: Highly significant level at P value < 0.001**

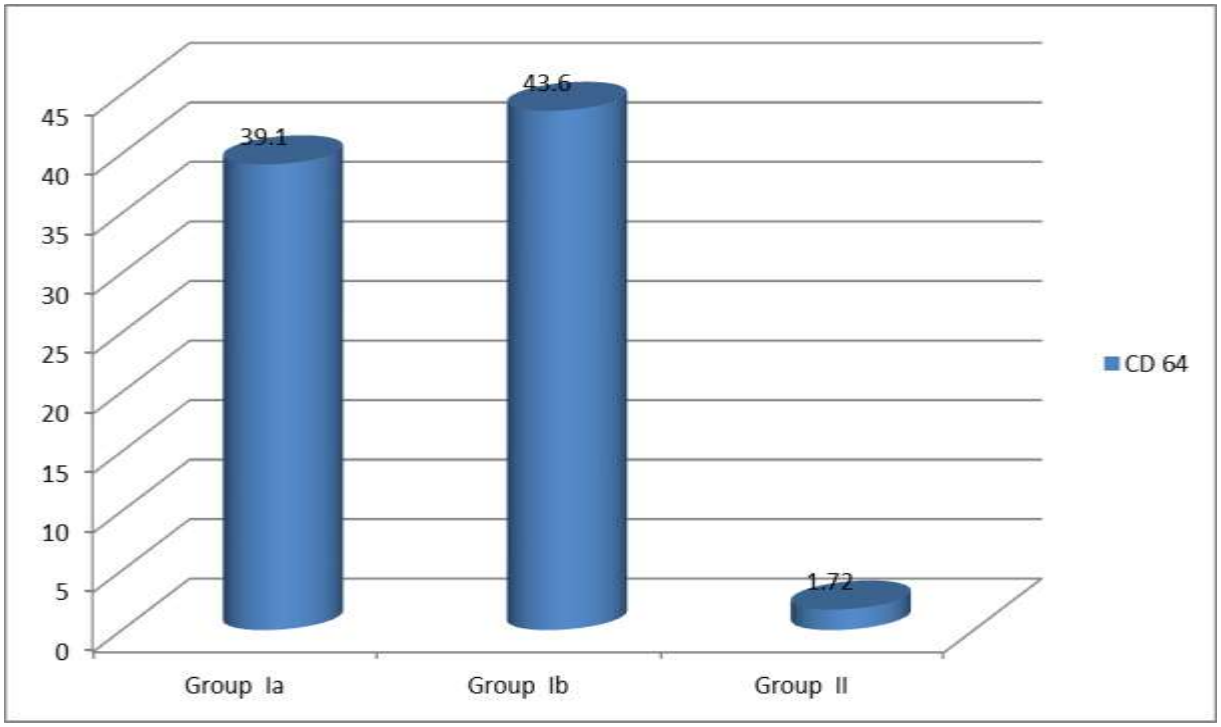

Figure (3): Comparison of the expression of CD 64 on neutrophils in different studied groups

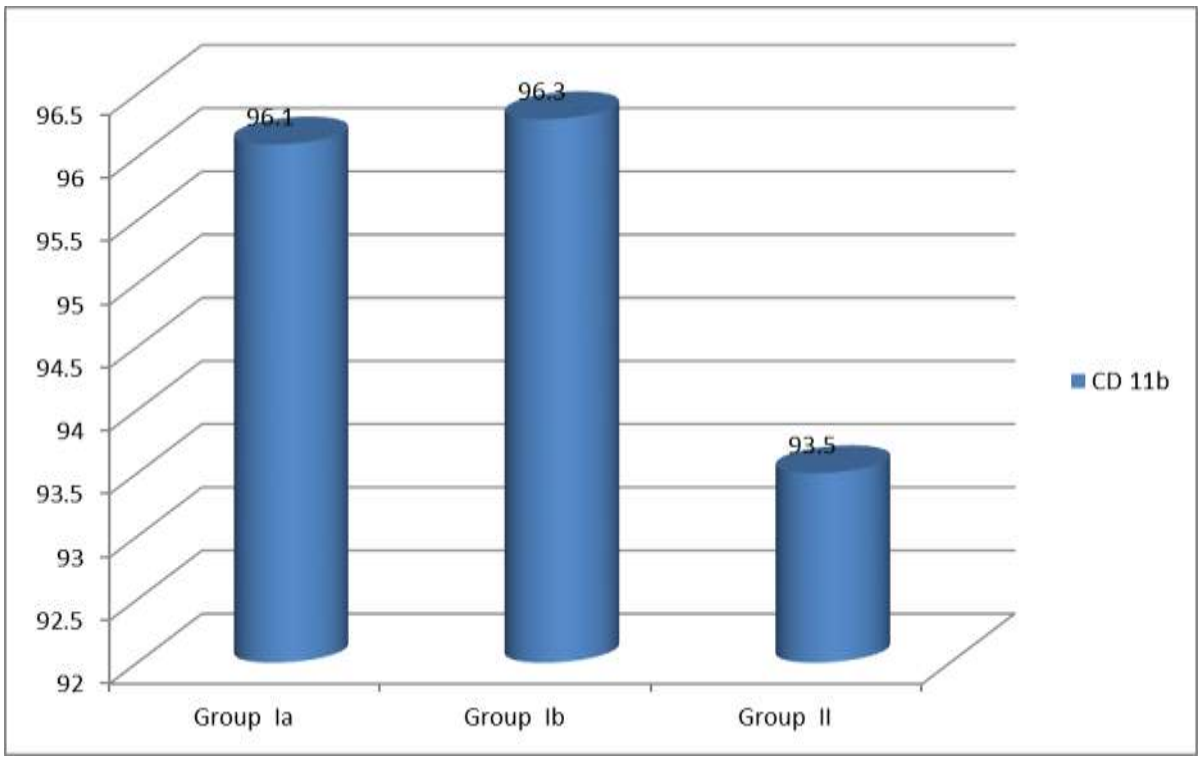

Figure (4): Comparison of the expression of CD 11b on neutrophils in different studied groups

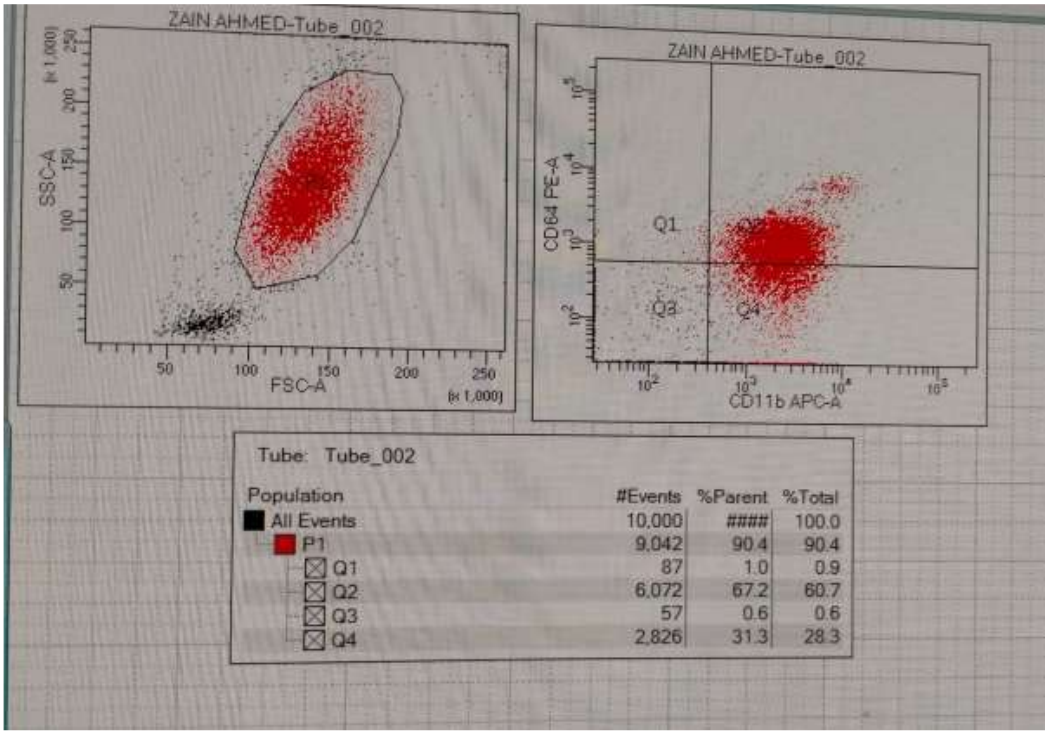

Figure (5): Flowcytometric analysis of CD64 and CD11b expression on neutrophils in one of the patient groups.

Significant positive correlation between CD64 percentage level on neutrophils and absolute lymphocytes count in group Ia (r = 0.399*, p value =0 .029*).

There was also significant positive correlation between CD64 percentage level on neutrophils and absolute monocytes count and direct bilirubin (D.B) in group Ib (r = 0.375*, p value = 0.041*) (r = 0.439*, p value = 0.015*) respectively **(Table 6)**.

Table (6): Correlations between CD64 expression in patient groups on neutrophils and studied laboratory parameters:

CD 64 marker Correlations	Group Ia (n = 30)		Group Ib (n = 30)	
	r value	p value	r value	p value
CD 11b (%)	- 0.245	0.191	0.112	0.557
Hemoglobin (g/dl)	- 0.116	0.542	- 0.355	0.054
Total leucocytes count ($x10^3$/µl)	0.257	0.171	0.013	0.945
Platelets ($x10^3$/µl)	- 0.079	0.679	0.103	0.586
Absolute lymphocytes count /µl	**0.399***	**0.029***	0.170	0.369
Absolute neutrophils count /µl	0.194	0.305	0.074	0.699
Absolute monocytes count /µl	0.295	0.113	**0.375***	**0.041***
PC (%)	- 0.174	0.356	- 0.224	0.234
INR	0.133	0.485	0.313	0.093
O2 saturation (%)	0.192	0.309	0.034	0.859
Ferritin (ng/ml)	0.027	0.887	- 0.021	0.914
D – dimer (ug/ml)	0.049	0.798	- 0.001	0.995

*** Correlation is significant at P value < 0.05.**
**** Correlation is highly significant level at P value < 0.001.**

Significant negative correlation between CD11b percentage level on neutrophils and (absolute lymphocytes count and absolute monocytes count) in group Ia was noted (r = - 0.676*, p value = 0.0001*) and (r = - 0.749*, p value = 0.0001*) respectively. There was significant negative correlation between CD11b percentage level and (total leucocytes count and absolute neutrophils count) in group Ib (r = - 0.440*, p value = 0.015*) and (r = - 0.446*, p value = 0.013*) respectively, significant negative correlation between CD11b percentage level on neutrophils and oxygen saturation in group Ib (r = - 0.413*, p value = 0.023*) **(Table 7).**

Table (7): Correlations between CD11b expression in patient groups on neutrophils and studied laboratory parameters:

CD 11b marker Correlations	Group Ia (n = 30)		Group Ib (n = 30)	
	r value	p value	r value	p value
CD 64 (%)	0.245	0.191	0.112	0.557
Hemoglobin (g/dl)	0.061	0.749	0.319	0.085
Total Leucocytes Count ($x10^3$/µl)	0.213	0.258	**- 0.440***	**0.015***
Platelets ($x10^3$/µl)	0.251	0.180	0.114	0.550
Absolute lymphocytes count /µl	**0.676***	**0.0001***	0.057	0.764
Absolute neutrophils count /µl	0.117	0.539	**0.446***	**0.013***
Absolute monocytes count /µl	**0.749***	**0.0001***	0.168	0.375
PC (%)	0.256	0.172	0.307	0.099
INR	0. 228	0.226	- 0.017	0.930
O2 saturation (%)	0.287	0.124	**- 0.413***	**0.023***
Ferritin (ng/ml)	0.278	0.136	- 0.056	0.767
D – dimer (ug/ml)	0.162	0.394	0.032	0.867

*** Correlation is significant at P value < 0.05.**
**** Correlation is highly significant level at P value < 0.001**

- There was high significant difference in CD64 expression on Monocytes and CD11b expression on monocytes when comparing them among all groups (P- value < 0.0001**, 0.010* respectively) and between group Ia and control group (P- value < 0.0001**, 0.003* respectively) and between group Ib and control group (P- value < 0.0001**, 0.044* respectively) but no statistically significant difference when comparing group Ia and group Ib **(Table 8).**

Table (8): Comparison of the different studied groups as regarding to expression of CD64 &CD11b on monocytes:

Markers	Group Ia (n = 30)	Group Ib (n = 30)	Group II (n = 20)	p value Among all groups	P value G Ia vs G Ib	p value G Ia vs G II	p value G Ib vs G II
CD 64 (%) Range Mean ± SD	23.60 – 76.20 43.66 ±15.29	20.00 –70.70 42.77 ±16.26	0.40 - 2.6 1.35 ± 0.71	**< 0.0001****	0.802	**< 0.0001****	**< 0.0001****
CD 11b (%) Range Mean ± SD	73.60 – 99.90 92.51± 7.64	73.30 –99.70 90.05 ± 7.06	60.40 – 98.00 85.37 ±9.48	**0.010***	0.233	**0. 003***	**0.044***

- **Data displayed as mean and standard deviation (SD)**
- **one way ANOVA test for quantitative data between the groups**
- **Post Hoc LSD of one way ANOVA test for quantitative data between the groups**
- **Significant level at P value < 0.05**

Significant positive correlation between CD64 percentage level on monocytes and absolute monocytes count and serum ferritin level in group Ia was noted (r = 0.409*, p value = 0.025*) (r = 0.432*, p value = 0.017*) respectively **(Table 9).**

Table (9): Correlations between CD64 expression in patient groups on monocytes and studied laboratory parameters:

CD 64 marker Correlations	Group Ia (n = 30)		Group Ib (n = 30)	
	r value	p value	r value	p value
CD 11 (%)	- 0.084	0.671	- 0.123	0.518
Hemoglobin (g/dl)	- 0.235	0.211	- 0.343	0.064
Total leucocytes count (x10^3/µl)	0.043	0.823	- 0.050	0.792
Platelets(x10^3/µl)	0.033	0.861	0.117	0.537
Absolute lymphocytes count /µl	0.270	0.149	0.194	0.304
Absolute neutrophils count /µl	0.085	0.657	0.015	0.936
Absolute monocytes count /µl	**0.409**	**0.025***	0.215	0.254
PC (%)	- 0.124	0.513	- 0.155	0.413
INR	0.100	0.600	0.266	0.155
O2 saturation (%)	0.281	0.132	0.294	0.115
Ferritin (ng/ml)	**0.432***	**0.017***	0.151	0.425
D – dimer(ug/ml)	0.032	0.868	- 0.037	0.845

*** Correlation is significant at P value < 0.05.**
**** Correlation is highly significant level at P value < 0.001.**

Significant negative correlation between CD11b percentage level on monocytes and absolute monocytes count in group Ia (r = -0.422*, p value = 0.020*), significant negative correlation between CD11b percentage level on monocytes and oxygen saturation in group Ib were noted (r = -0.415*, p value = 0.023*). **(Table 10).**

Table (10): Correlations between CD11b expression in patient groups on monocytes and studied laboratory parameters:

CD 11 marker Correlations	Group Ia (n = 30)		Group Ib (n = 30)	
	r value	p value	r value	p value
CD 64 (%)	- 0.081	0.671	-0.123	0.518
Hemoglobin (g/dl)	0.118	0.536	0.329	0.076
Total Leucocytes Count (x10^3/µl)	0.140	0.460	- 0.292	0.117
Platelets (x10^3/µl)	0.199	0.292	- 0.148	0.435
Absolute lymphocytes count /µl	- 0.038	0.840	0.072	0.705
Absolute neutrophils count /µl	0.144	0.484	- 0.231	0.219
Absolute monocytes count /µl	**- 0.422***	**.0020***	- 0.285	0.126
PC (%)	0.235	0.211	- 0, 069	0.717
INR	- 0.191	0.312	0.121	0.525
O2 saturation (%)	- 0.180	0.340	**- 0.415***	**0.023***
Ferritin (ng/ml)	- 0.192	0.309	-0.289	0.121
D – dimer (ug/ml)	0.023	0.868	- 0.143	0.450

*** Correlation is significant at P value < 0.05.**
**** Correlation is highly significant level at P value < 0.001.**

Discussion:

SARS-CoV-2 activates antiviral immune responses, and can also cause uncontrolled inflammatory responses characterized by marked proinflammatory release of cytokine in patients with severe COVID-19, leading to lymphocyte dysfunction, lymphopenia and granulocyte and monocyte abnormalities **(10).**

This study revealed significant affection in liver function tests (AST,ALT & Total bilirubin) in patients groups and this was similar to **(11)** who showed that SARS-CoV-2 may infect the liver resulting in liver impairment. In individuals with

COVID-19 , mechanisms of liver injury could result from cytokine storm-induced systemic inflammation , drug-induced liver dysfunctions and or pneumonia related- hypoxia.

.Regarding oxygen saturation, statistically significant difference between ICU and non-ICU patients was noted as oxygen saturation is lower in ICU cases than in those not admitted to ICU and this was in agreement with ***(12)*** who found that the severity of hypoxemia in COVID-19 cases, is independently related to in-hospital mortality and may be indicator for admission to ICU.

Regarding serum ferritin level, there was higher level in patients groups than control group and this was similar to ***(13)*** who showed that compared to control group, increased ferritin values were detected in the COVID-19 patients since serum ferritin, is an "acute phase reactant", reflects degree of both chronic and acute inflammatory reactions. Activated monocyte-macrophage system is indicated by increased ferritin level this is because ferritin synthesis inside monocytes and macrophages is responsive to change in the cytokine status at the translational and transcriptional levels.

Regarding D dimer, Statistically significant difference between ICU and non-ICU patients was noted as D-dimer is increased in ICU cases than in those not admitted to ICU which was in agreement with ***(14)*** who showed that levels of D dimer are frequently increased in SARS-CoV-2 individuals. Markedly increased levels are detected in those with severe disease. and can be utilized as a prognostic biomarker for in-hospital mortality. COVID-19 illness induces a hypercoagulable condition that results from the imbalance between the pro- and anti inflammatory response, endothelial dysfunction with excessive thrombin production, hypoxia and immobility

Neutrophilia observed in COVID-19 patients suggests leukocytes role in the pathology of the disease. Neutrophil and monocyte expression of CD64 and CD11b have an important role in the development of the inflammatory response **(7).**

So, this study aimed to evaluate the expression of cell surface markers CD64 and CD11b on neutrophils and monocytes in Covid-19 patients and correlate their expression with the severity of the disease to detect if they can be used as markers of ongoing inflammation or not.

Regarding CD64 (the high affinity receptor for IgG) expression on neutrophils and monocytes, this study showed that there was statistically significant increase in patient groups than control group and this was in agreement with **(15)** who showed that the neutrophil CD64 was virtually absent in healthy controls but highly expressed in moderate and severe COVID-19. Also it was in agreement with **(7)** who showed that in COVID-19 patient group, CD64 was strongly upregulated on monocytes and neutrophils compared with healthy controls, also very high neutrophil CD 64 has been reported in patients with bacterial infection **(16)** , lower grade CD64 expression was reported in influenza patients **(17)** so it may be due to activation of neutrophils by bacterial superinfection or by inflammation caused by **COVID** -19 but concerning bacterial super infection, there was broad spectrum antibiotics use. Monocytes CD 64 was highly upregulated in Covid-19 patients which may be have a predictive value for poor outcome, this is due to activated monocytes ability for cytokine production and regulating T- cells.

Regarding CD11b expression on neutrophil, there was no statistically significant difference between patients group and control group and this was in agreement with **(18)** who showed that neutrophil activation markers (CD11b, CD10, CD16 and CD62L) did not differ between COVID-19-negative patients and COVID-19-positive patients diagnosed with other bacterial /viral infections, or between COVID-19 severity groups, in the peripheral blood at hospital presentation, the expression of CD11b and CD62L on neutrophils did not significantly differ between the COVID-19 severity groups, Remarkably, expression of CD62L and CD11b were also not higher in COVID-19 patients when compared to healthy controls. Also it was in agreement with **(6)** who showed that CD11b and CD66b expression on neutrophils showed no differences between COVID-19 patients groups , but it was in disagreement with **(19)** who showed that neutrophils of COVID-19 patients expressed lower levels of CD11b as compared to healthy group, may be due to difference in number of patients and treatment. CD11b is a subunit of the αMβ2 (CD11bCD18) integrin involved in intercellular adhesion, transmigration, fibrinogen adhesion, and neutrophil-T cell crosstalk during infection. CD11b has been found to play a role in the inflammation resolution . Lack of upregulation of neutrophil CD11b may impair extravascular neutrophil transmigration to tissues and so lead to an increased number of neutrophils in peripheral blood. Neutrophilia is present in **COVID** -19 due to decreased migration of neutrophils from blood to tissues or increased production from bone marrow or both.

Significant positive correlation between CD64 percentage level on neutrophils and absolute lymphocytes count was noted in group Ia, also significant positive correlation between CD64 percentage level and CRP level in group Ia, this was in agreement with **(20)** who showed that the CD64N ratio was statistically correlated with the different parameters (WBC, frequency of lymphocytes and neutrophils , percentage of immature neutrophils, and CRP). There was significant positive correlation between CD64 percentage level and absolute monocytes count in

group Ib, this was in agreement with **(21)** who showed that the expression of some functional markers such as CD11b and CD64 increases on monocytes. Monocytes and macrophages are the main cells of the mononuclear phagocyte system (MPS) which play an important role in both innate & adaptive immune systems. The presence of these cell populations in phases of SARS-CoV2 infection can ameliorate infection or exacerbate it. They are considered the main players in the innate anti-viral immunity. They also can trigger pro-coagulant syndrome, systemic inflammation and cytokine release syndrome (CRS) leading to ARDS and multi organ failure in COVID-19 patients. Monocytes and tissue macrophages express many membrane receptors and secretory elements that mediate their role in both anti-viral and inflammatory responses and the contribution of other immunity mechanisms can determine the severity and outcome of SARS-CoV-2 infection**.**

Regarding CD11b expression on monocytes, this study showed that there was statistically significant difference between patient groups and control group but not neutrophils, which differ from which reported in influenza and bacterial infection but agree with which reported in COVID-19 **(22)** and this was in agreement with (**23)** who showed that monocytes and granulocytes in patients with COVID-19 and established respiratory failure show increased levels of integrins CD11b and CD18, which comprise complement receptor 3 (CR3) independently of absolute monocyte , granulocyte counts and peripheral oxygen saturation . Monocytes and granulocytes mediate initial immune responses to pathogens, central to inflammatory processes following tissue damage and regulate hemostasis at sites of injury. Also it was in agreement with (**7)** who showed that expression of CD11b increased significantly on monocytes but not on neutrophils in COVID-19 patients compared with healthy controls which increase monocyte migration even in the late stage of disease. This makes monocyte receptors act as interesting marker in the future studies as an independent marker for inflammation in **COVID** -19 .

Significant positive correlation between CD64 percentage level on neutrophils and direct bilirubin (D.B) was noted in group Ib (r = .439*, p value = .015*) this was in agreement with (**24)** who showed that COVID-19 patients with elevated bilirubin were associated with worse prognosis and severe disease and also showed a significantly higher level of bilirubin in intensive care unit (ICU) patients with COVID-19 than in non-ICU patients. An elevated bilirubin level is regarded as a vital marker of impaired liver function and may indicate liver damage. There was significant negative correlation between CD11b percentage level on both neutrophils & monocytes and oxygen saturation in group Ib (r = - .413*, p value = .023*) as CD11b is increased in ICU patients with decreased oxygen saturation this was in agreement with **(23)** who showed that levels of CD11b and CD18, which together comprise Complement Receptor 3 (CR3), were increased in granulocytes and monocytes from hypoxic COVID-19 patients. Granulocyte and monocyte activation as a biomarker for respiratory complications in COVID-19 patients, possibly by driving thrombotic microangiopathy due to increased binding of fibrinogen. CR3 is also responsible for cytokine production ,complement-mediated leukocyte recruitment , phagocytosis and also contribute to respiratory failure, all of which are increased in severe COVID-19,

Conclusion:

Both neutrophils and monocytes are activated during COVID -19 infection and both show high upregulation of CD64. While, CD11b is not upregulated on activated neutrophils, while it is upregulated on monocytes and both can be used as elevated markers in COVID -19 but not useful for categorization of patients as regarding severity. D-dimer, INR & O2 saturation increased in ICU patients
than non- ICU patients which indicates coagulopathy and severity of the disease.
Absolute lymphocytic count decreased in ICU patients than in those not
admitted to ICU which indicates the disease severity.

Recommendations:

1- Future studies of CD64 and CD11b expression in COVID- 19 patients with COVID-19 therapy protocols on large sample size.
2- Future studies of CD 64 and CD 11b expression in combination with other receptors to be used for prognosis.

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