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# Age influences blood cell-based immune deregulation antibody response and unfavorable clinical outcomes in COVID-19 patients

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**COVID-19 is caused by SARS-CoV-2 and has a diverse spectrum of clinical presentations ranging from asymptomatic cases to severe and critical cases that result in the death of the patient. Alongside the viral factors host factors like Age, deregulation of the immune response and presence of comorbidity determine the patient's outcome. Here we sought to assess the impact of age on natural antibody response, CBC-based inflammatory markers, and outcome of COVID-19 patients. We divided the participants into three groups, young (≤ 35 years), middle-aged (40–60 years), and old (≥ 65 years) patients and collected and analyzed sociodemographic, clinical, and laboratory parameters. We found that elderly patients showed higher ($P < 0.05$) levels of inflammation like increased neutrophil percentages, NLR, lymphopenia, and low Hgb levels, compared to middle-aged and young patients. Interestingly these markers were also associated with mortality of COVID-19 patients. On the other hand, no significant difference was observed in ion concentration, lipid profile, and coagulation test between the three age groups. We also found that elderly patients showed significantly ($P < 0.05$) decreased levels of natural antibody response to SARS-CoV-2 infection compared with the two groups. Lastly, we assessed the effect of dexamethasone treatment, even if statistically not significant ($P > 0.05$) we observed a positive trend among patients under dexamethasone in the aspect of decreasing inflammatory markers. To conclude we showed that SARS-CoV-2 is characterized by an age-dependent deregulation of inflammatory markers that are associated with mortality among COVID-19 patients.**

**Keywords** Immune deregulation, Inflammatory markers, Natural antibody, Dexamethasone, Mortality

**Abbreviations**

| | |
|---|---|
| ALP | Alkaline phosphatase |
| AU | Arbitrary unit |
| Cl | Chloride |
| DBIL | Direct bilirubin |
| GOT | Glutamic oxaloacetic transaminase |
| GPT | Glutamic pyruvic transaminase |
| Hct | Hematocrit |
| HDL | High-density lipoprotein |
| Hgb | Hemoglobin |
| INR | International normalised ratio |
| K | Potassium |
| LDL | Low-density lipoprotein |
| LMR | Lymphocyte to monocyte ratio |
| MCH | Mean cell (corpuscular) hemoglobin |
| MCHC | Mean cell hemoglobin concentration |
| MCV | Mean cell (corpuscular) volume |
| MPV | Mean platelet volume |

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| | |
|---|---|
| Na | Sodium |
| NLR | Neutrophil to lymphocyte ratio |
| PT | Prothrombin time |
| PTT | Partial thromboplastin time |
| RBC | Red blood cell |
| RDW | Red cell distribution width |
| TBIL | Total-value bilirubin |
| TG | Triglycerides |
| WBC | White blood cell |

Coronavirus disease–2019 (COVID-19) is caused by SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2), an enveloped non-segmented positive-sense RNA virus that belongs to the family of Coronaviridae. The virus was first identified in late 2019 in Wuhan, Hubei province, China[1,2]. COVID-19 is one of the worst pandemics witnessed in the twenty-first century killing more than 7 million people across the globe (as of Dec 24, 2024)[3]. Successful speed-up of vaccine development and effective preventive measures by the global community helped in decreasing the transmission and control of the disease. Yet, the different variants of SARS-CoV-2 are still surging in the population[4]. Thus full understanding of pathophysiological changes associated with SARS-CoV-2 infection is crucial.

SARS-CoV-2 infection impacts the hematopoietic system and also induces an immune impairment and deregulation in patients resulting in the release of inflammatory mediators[5]. Immunopathology like tissue infiltration of macrophage/monocyte, lymphopenia, neutrophilia, activation of platelet, increased cytokine release, enhanced interferon production, increased capillary permeability and over-activation of the complement system have been reported in patients with COVID-19. These changes were linked with poor clinical outcomes in COVID-19 patients[6–8]. Especially cytokine storm due to the deregulation of the immune-inflammatory response is linked with multiorgan failure among COVID-19 patients. Besides the respiratory system SARS-CoV-2 affects other organ systems resulting in diverse symptoms and different biochemical profiles[9,10].

The spectrum of clinical presentation of COVID-19 is dissimilar, ranging from asymptomatic cases to severe disease resulting in mortality. Both viral factors and host factors determine the overall outcome of the patient. One host factor that influences disease severity is age, compared to pediatric patients, elderly patients tend to develop a severe form of the disease with a high mortality rate[11]. Likewise, other host factors like the presence of comorbidity, and male sex, were also associated with predicting mortality in COVID-19 patients[7].

Aging is one of the risk factors that is associated with disease severity and mortality of COVID-19 patients. With age molecular and physiological changes occur throughout the body including in the immune system[12,13], this age-dependent immune deregulation has a significant impact on the spectrum of COVID-19 and the outcome of the disease[8]. Mortality due to COVID-19 is mainly due to pneumonia resulting from hyper-inflammatory processes like the release of cytokines from immune cells leading to acute respiratory distress syndrome (ARDS)[14]. In the aspect of COVID-19 treatment the use of low-dose dexamethasone (anti-inflammatory glucocorticoid that inhibits inflammatory chemokines by immune cells) has been shown to decrease 28-day all-cause mortality among COVID-19 patients[15] as well it also decreases the overall mortality in critically ill patients[16,17]. In overall this suggests the existence of an immune-deregulation event in COVID-19 patients, thus it is important to assess hematological parameters like CBC (complete blood count) based inflammatory markers and other simple biomarkers in COVID-19 patients that are associated with mortality in an age-dependent manner.

Here We enrolled 240 patients and divided them into three groups, Young (age ≤ 35y), Middle age (40–60 years), and Old (≥ 65 years). We measured different parameters and followed them until discharged or death (see the details in the method part) (Fig. 1). Our main intent was to asses age-dependent immune deregulation and its association with mortality rate during the follow-up period of hospital stay. We also analyzed the effect of age on the natural antibody response to SARS-CoV-2 and the impact of dexamethasone treatment among COVID-19 patients. Our finding is especially important in employing these simple parameters to determine the risk of COVID-19 patients in an age-dependent manner in different communities and resource-limited settings.

## Methods and materials

### Study setting

The study was conducted at St. Paul's Hospital Millennium Medical College (SPHMMC), a referral-specialized hospital located in Addis Ababa, Ethiopia. By delegating 260 beds, it served as the main isolation and treatment center for COVID-19 patients. During the study period (From 1 October 2020 to 16 September 2021) we used the national Ethiopian Public Health Guideline (EPHG)[18] and recruited adult patients 18 years old and above who met the eligibility criteria for the study.

### Study design

A time series cross-sectional study was conducted for one year, from 1 October 2020 to 16 September 2021.

### Study participants

Eligible patients with any acute respiratory illness and having at least one symptom like fever, cough, shortness of breath, etc. were used as a source population of the study. All COVID-19 suspected patients were tested for SARS-CoV-2 real time-PCR (RT-PCR), and those who tested positive, met the eligibility criteria, and volunteered to participate were enrolled in the study. Study participants were isolated and followed until death or discharge (patients were discharged only when tested negative for the SARS-CoV-2, RT-PCR test). The average time of hospital stay was 2 weeks for mild/moderate COVID-19 patients and 6 weeks for severe/critical patients. Patients were excluded from the study if they were discharged or died before the second set of measurements

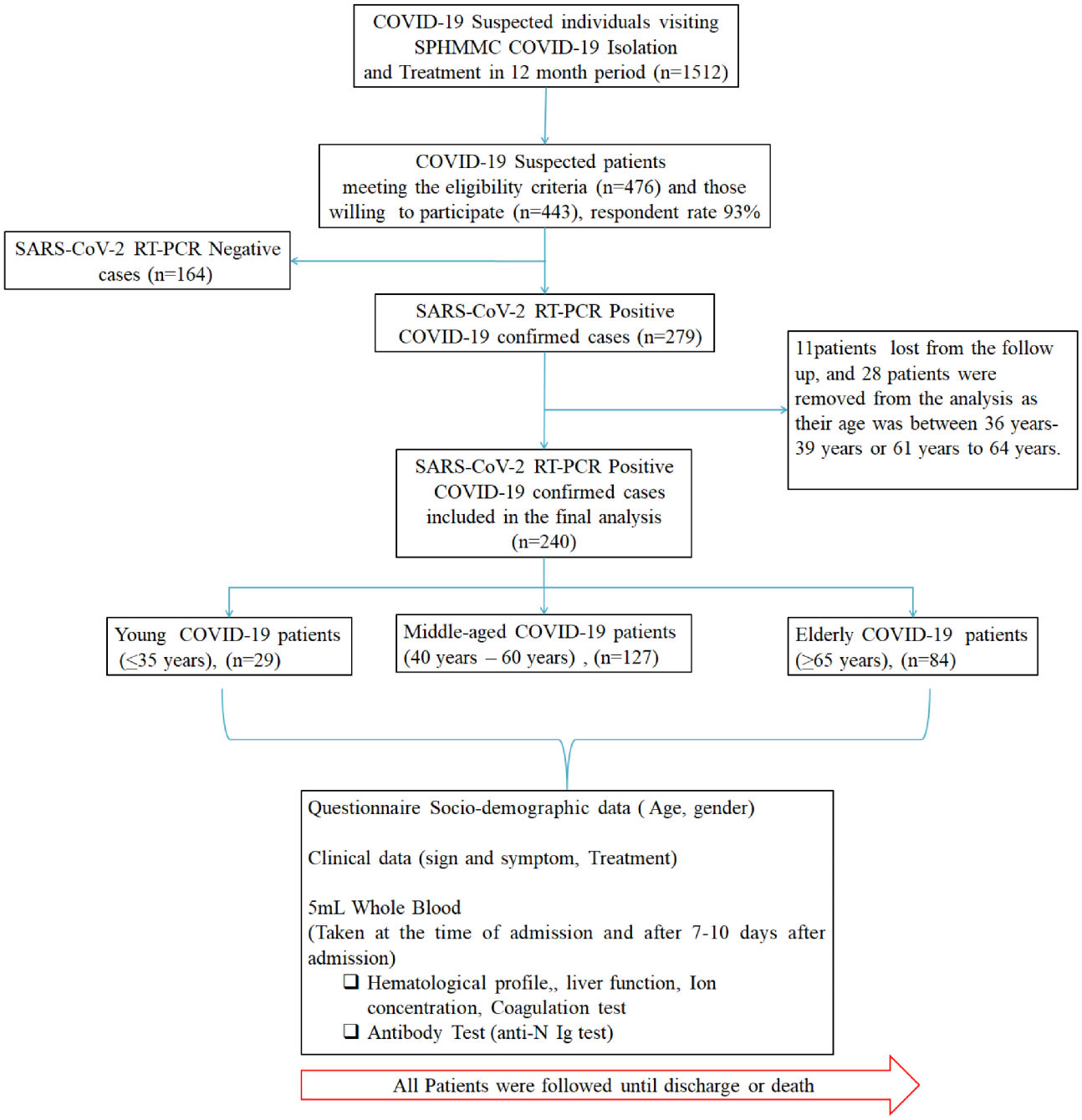


**Fig. 1**. General framework of the study. Data spanning aspects of the socio-demographic, clinical data, and laboratory parameters were collected and age-dependent changes in these parameters were assessed with the final outcome of COVID-19 patients during their hospital stay.

taken (Fig. 1). Clinical diagnosis was made based on the WHO interim guidance into mild (no evidence of viral pneumonia or hypoxia), moderate ($SpO2 \geq 90\%$ on room air and a sign of pneumonia), severe (signs of pneumonia and having additional signs of respiratory rate > 30 breaths/min; severe respiratory distress; or $SpO2 < 90\%$ on room air) and critical cases (patients with ARDS, septic shock or multi-organ dysfunction)[19]. This cohort was previously used to assess how changes in laboratory parameters can predict mortality in COVID-19 patients[20]. We extended our investigation by incorporating previously unreported measurements (like effect of dexamethasone treatment) and assessing the impact of age on different measured parameters.

## Sample size and sampling procedure

We used a convenient sampling technique and collected samples at the peak of the first COVID-19 wave. During the study period, a total of 1512 patients suspected of COVID-19 visited the center of which 476 met the eligible criteria and 443 were willing to participate with a response rate of 93%. Oropharyngeal (OP) and/or

nasopharyngeal (NP) samples were tested for SARS-CoV-2 RNA by RT-PCR and we excluded 164 patients who tested negative, and 279 patients tested positive of whom 11 participants were lost from the follow-up. Finally, we enrolled 240 COVID-19 patients (28 patients were excluded due to their age were not within the cut-off value) into three groups young (age ≤ 35y, n = 29), middle age (40–60 y, n = 127), and old (≥ 65y, n = 84), and collected socio-demographic, clinical, and two-point laboratory measurements (within 24 h of patient admission and 7–10 days after hospital admission) and followed (on average patients were followed for 2 weeks to 6 weeks) until discharged or death (Fig. 1).

## Socio-demographic and clinical data

We used a pre-tested questionnaire and collected socio-demographic data (Age, sex), and collected clinical (clinical presentation, comorbidities, and treatment type and clinical outcomes) and laboratory (WBC Count, Electrolyte, Liver function test) data were integrated using unique patient code that kept the privacy of the participant. Socio-demographic data were collected at the time of admission. Within 24 h of patient admission, blood sample, Oropharyngeal (OP) and/or nasopharyngeal (NP) swabs were collected and transported to SPHMMC laboratory for analysis and a second blood sample was collected between 7 and 10 days after admission for further assessment of the patient progress. Continuous OP and NP swabs were taken to check the COVID-19 status of the patient. The collected data were merged and cleaned for further analysis between the three age groups.

## RNA extraction and RT-PCR analysis

Oropharyngeal (OP) and/or nasopharyngeal (NP) swabs were collected and transported to the SPHMMC COVID-19 laboratory using VTM (viral transport media) (in a cold chain of 2–8 °C) for RT-PCR analysis of SARS-CoV-2. The viral RNA was extracted using a nucleic acid isolation Kit (Da'an Gene Corporation) by mixing 200µL of the NP or OP swab with 50 µl proteinase K and 200 µl lysis buffer and then eluted with 60µL elution buffer (Da'an Gene Corporation). Two PCR primer and probe sets, which target the open reading frame 1ab (ORF 1ab/N) (FAM reporter) and nucleocapsid protein genes and VIC reporter genes were added to the same reaction mixture. Positive and negative controls were used for each run and a reliable signal with ≤ 40 CT value was considered positive. RT-PCR amplifications took place at 50 °C for 15 min, 95 °C for 3 min, followed by 45 cycles at 95 °C for 15 s and 60 °C for 30 s. All three cycles were accomplished within an hour and 35 min of the reaction being started.

## Anti-N Ig antibody test

The natural antibody response to SARS-CoV-2 infection was determined by the Elecsys anti-SARS-CoV-2 assay (Elecsys Anti-N; Roche Diagnostics, International Ltd, Rotkreuz, Switzerland) that can detect the presence of anti-nucleocapsid antibodies against the virus. The kit can detect both immunoglobulin M (IgM) and G (IgG) as early as day 5 after symptom onset. With the median seroconversion being observed within 10–13 for IgM and day 12–14 for IgG. We included 141 patients that met the criteria for antibody detection. A cut of value below 1 (COI < 1.0) was interpreted as non-reactive (negative) while a cut of index values greater than or equal to 1.0 (COI ≥ 1.0) was interpreted as reactive (positive).

## Hematological and biochemical tests

Using di-potassium EDTA–anti-coagulated vacutainer tubes (Becton Dickinson) a fresh (˂4 h from collection) whole blood sample (8-10 ml) was collected aseptically within 24 h and 7–10 days after patient admission. Twenty-eight different hematological parameters like shape, size, and complexity were measured using the Beckman Coulter DxH 800 Hematology Analyzer (California, USA). Plasma or serum sample from coagulated blood was used for liver and kidney function tests using Cobas C 501 Chemistry Analyzer (Roche). Quantitative measurement of total cholesterol (CHOL), triglycerides (TG), high-density level cholesterol (HDL-C), and low-density level cholesterol (LDL-C), was done using Roche Cobas C311 (Roche/Hitachi). The level of sodium, potassium, chloride, Magnesium, calcium, and Phosphorus concentration of the patient was determined using the Roche-Cobas-C311 (Roche/Hitachi), clinical chemistry analyzer. Coagulation parameters prothrombin time (PT), partial thromboplastin time (PTT; also known as activated partial thromboplastin time (aPTT)), and an INR (international normalized ratio) were measured using Stago STA Compact Coagulation Analyzer (Diagnostica Stago).

## Data management and analysis

We followed a strict procedure to reduce errors by pre-testing our questionnaires and making the necessary amendments when needed. Laboratory analysis positive and negative controls were used for each set of samples, as well pre-analytical, analytical, and post-analytical procedures were performed as per the Standard Operating Procedures (SOPs) of the lab to maximize the quality of collected data. After filling in all the socio-demographic, clinical, and laboratory data, it was pooled together and entered into an Excel spreadsheet. Participants with incomplete information were removed from the analysis. We used the Shapiro–Wilk test using R software (V 4.2.2, R Core Team, 2021) to check the distribution of the data. Means and Standard Deviation (SD) were used to describe contentious data with normal distribution, whereas median and interquartile ranges were used for contentious data without normal distribution and the percentage was used for a categorical data set. Percentage increase was calculated by subtracting the second time measurement from the first point measurement divided by the first point measurement and multiplied by 100. A statistical test was done using SPSS version 23.0 (SPSS, Inc, Chicago, Ill). For univariate analysis, Mann–Whitney U test, Chi-square, and Fisher's Exact test were used to compare between two groups. We used the R 4.2.2 package (RcmdrPlugin.KMggplot2)(R Core Team, 2021) to generate the graphs, and a 95% CI and $P \leq 0.05$ were considered statistically significant.

## Result

### Socio-demographic status of the study participant

We enrolled a total of 240 SARS-CoV-2 PCR-positive COVID-19 patients during the study. We divide the participants into three age groups to assess the effect of age on parameters including immune deregulation, antibody response, disease progression, and the fatal outcome of COVID-19 patients. We categorized COVID-19 patients ≤ 35 years as Young (n = 29), those with the age group between 40 and 60 years as Middle age (n = 127), and those ≥ 65 years of age as Old (n = 84) (Fig. 1). The median age of the young patients was 30 years (IQR 25y-34y), the median age of the Middle-aged adults was 52 years (IQR, 46–58y) and the median age of old patients was 70 years (68–76.5 y). We find similar clinical presentation between the three age groups except sneezing which was significantly common in middle age adults compared to the other two age groups (Supplementary Table 1). The proportion of BCG vaccination was significantly lower ($P < 0.05$) in old patients compared to young and middle-aged patients. Whearas other socio-dmographic characters showed no significant difference ($P > 0.05$) between the three age groups (Supplementary Table 1).

### Comparison of biochemical and hematological parameters between the three age groups at the time of admission (within 24 h)

We assessed the association of different measured parameters with a specific age group at the time of admission. We observe no significant vital clinical signs associated with a specific age group, indicating all COVID-19 patients in our study showed a similar trend in vital clinical signs (Table 1). Similarly, the level of different hematological parameters was not different between the three age groups (Table 1) but even if it is not significant we observe an increased trend in NLR with age that is 7.6 AU (2.17 AU—18.32 AU), 8.01 AU (5.14 AU -14.65 AU), and 11.3 AU (6.18 AU—22.08 AU), in young, middle-aged adults and old COVID-19 patients respectively. Similarly, an increased number of neutrophil percentage was also observed with an increase in age that is 80.9% (65.3%—91.1%), 83.1% (77.3–88.4%), and 86.6% (78.5–90.9%), in young, middle-aged adults and old COVID-19 patients respectively (Table 1). This could indicate the presence of inflammation in middle-aged and elderly COVID-19 patients compared to young ones. We also observed that increased ($P < 0.05$) MCV levels of 85.65 fL (83.5 fL—89.3 fL), 88.2 fL (84.7 fL—91.3 fL), and 89.6 fL (87.6 fL—92.3 fL) in young, middle-aged adults and old COVID-19 patients respectively (Table 1). Significant ($P < 0.05$) increased levels of urea and ALP were observed in elderly COVID-19 patients compared to middle-aged, and young patients. On the other hand, no significant difference was observed in ion concentration, lipid profile, and coagulation test between the three age groups.

### Increased markers of immune deregulation at the second point measurement are associated with increased age

We then analyzed the measured parameters at the second point (7–10 days after patient hospital admission) measurement of COVID-19 patients (Supplementary Table 2). We observe that elderly COVID-19 patients showed a significantly ($P < 0.05$) decreased level of RBC count ($10^6$/μL), hemoglobin level (g/dL), and lymphocyte percentage (Fig. 2A–C), compared to young and middle-aged COVID-19 patients. The level of neutrophil percentage (Fig. 2D) and Neutrophil to Lymphocyte Ratio (NLR) (AU) (Fig. 2E) were significantly ($P < 0.05$) higher in elderly COVID-19 patients compared to young and middle-aged COVID-19 patients. Interestingly the level of RBC count ($10^6$/μL) was inversely related ($P < 0.05$) with the level of NLR (AU) (Fig. 2F). This could indicate that in elderly COVID-19 patients, there is an increased level of inflammation. We hypothesized that specific activation of the complement system or viral-induced hemolysis of RBC could be linked with the decreased number of RBC, others also showed the presence of complement-mediated RBC lysis[21]. Further, in vivo experiments could give important insight into our findings.

We also assessed individual percentage increase in measured variables (subtracting the second point measurement from measurement at the time of admission divided by measurement at the time of admission and multiplied by 100) and observed a similar trend where older patients have a lower percentage increased in RBC count ($10^6$/μL), hemoglobin level (g/dL), monocyte percentage and lymphocyte percentage (Fig. 3A–D) and higher percentage increase of neutrophil percentage and NLR (AU) (Fig. 3E,F). which could indicate that older patients tend to resolve inflammation slowly.

### Decreased response of natural antibody against nucleocapsid (N) antigen in elderly COVID-19 patients

We looked into the anti-nuclear antibody natural antibody response to SARS-CoV-2 in different age groups in 141 patients that met the criteria for antibody measurement. A significantly ($P < 0.05$) lowered level of antibody production was observed in elderly patients compared to middle-aged and young patients (Fig. 4A). Taking the cut-off index value of ≥ 1 as positive (COI ≥ 1), the positivity to natural antibodies in young patients was 60% (9/15), in middle-aged was 48.1% (37/77) and in old patients was 37.5% (18/48) ($P < 0.05$) (Fig. 4B). The natural antibody response was not influenced by sex, as antibody positivity was 35.7% (10/28) in old, 48.1% (13/27) in middle-aged, and 66.7% (2/3) in young female COVID-19 patients ($P < 0.05$) (Fig. 4C). The positivity in natural antibody response was 40% (8/20) in old, 48% (24/50) in middle-aged, and 58.3% (7/12) in young male COVID-19 patients ($P < 0.05$) (Fig. 4C).

### Increased age and markers of inflammation associated with the incidence of mortality

Aging affects the final disease outcome and disease severity in COVID-19 patients. Similar to other cohorts in our case we observed significantly ($P < 0.05$) higher mortality and disease severity among elderly COVID-19 patients during the hospital stay. The mortality ($P < 0.05$) in the young age group was 13.8% (4/29), in the middle-aged group was 18.1% (23/127), and in elderly patients, the mortality rate was 31% (26/84) (Fig. 5A).

| Variable | Young X(75% IQR) | Middle age X(75% IQR) | Elderly X(75% IQR) | chi-squared | *P*-value |
|---|---|---|---|---|---|
| Age | 30 (25–34) | 52 (46–58) | 70 (68–76.5) | 193.4 | 2.20E-16 |
| Vital clinical signs | | | | | |
| BodyTemp ($^0$C) | 36.8 (36.5–36.9) | 36.9 (36.5–37.2) | 36.9 (36.4–37.2) | 0.502 | 0.7778 |
| Pulse rate (BPM) | 109 (91–109.75) | 87 (79.5–99) | 92.5 (79.5–103.5) | 4.732 | 0.09382 |
| Respiratory rate (BPM) | 26 (22–32) | 28 (23.75–36) | 26 (21.75–30.5) | 3.444 | 0.1787 |
| $O_2$ Satutarion (%) | 94.5 (92.5–95.75) | 93 (88.5–94) | 92 (88.5–94) | 3.209 | 0.2009 |
| Systolic BP (mmHg) | 110 (104–123) | 120 (110–139.5) | 128 (105.25–149.5) | 2.131 | 0.3445 |
| Diatolic BP (mmHg) | 71 (65–80) | 72 (65–80.5) | 77 (68–87.7) | 1.711 | 0.425 |
| Random sugar (mg/dL) | 163 (154–184) | 177 (130.5–242) | 172 (140–218) | 0.332 | 0.847 |
| Leukocyte | | | | | |
| WBC ($10^9$/L) | 6.95 (5.65–11.2) | 8.6 (6.1–12.5) | 9.05 (6.45–12.8) | 1.455 | 0.483 |
| Lymphocyte (%) | 16 (6–17.75) | 6.6 (3.65–15.65) | 7.05 (3–10) | 3.764 | 0.1523 |
| NLR (AU) | 7.6 (2.17–18.32) | 8.01 (5.14 -14.65) | 11.3 (6.18–22.08) | 4.881 | 0.08711 |
| Neutrophil (%) | 80.9 (65.3–91.1) | 83.1 (77.3–88.4) | 86.6 (78.5–90.9) | 2.562 | 0.2777 |
| Eosinophil (%) | 0.1 (0–0.2) | 0.1 (0–0.3) | 0.1 (0–0.4) | 1.458 | 0.4823 |
| Monocyte (%) | 4.7 (3.4–7.8) | 5.5 (3.9–7.9) | 5.8 (2.9–8.8) | 0.634 | 0.7282 |
| Basophil (%) | 0.15 (0.1–0.3) | 0.2 (0.1–0.4) | 0.2 (0.1–0.4) | 0.266 | 0.8752 |
| RBC Indices | | | | | |
| RBC ($10^6$/µL) | 4.81 (4.08–5.74) | 4.83 (4.5–5.12) | 4.66 (4.17–5.02) | 2.754 | 0.2523 |
| RDW (%) | 13.3 (12.9–15.5) | 13.7 (13–14.3) | 14 (13.6–14.8) | 5.724 | 0.05723 |
| MCHC (g/dL) | 34.1 (33.7–34.5) | 34 (33.3–34.6) | 33.9 (33.1–34.4) | 1.41 | 0.4926 |
| MCH (pg/Cell) | 29.6 (28.1–30) | 30 (28.9–31.4) | 30.4 (29.5–31.5) | 5.739 | 0.05672 |
| HCT (%) | 43.9 (35–49.02) | 41.7 (39–45.1) | 40.45 (37.6–44.5) | 1.099 | 0.577 |
| Hgb (g/dL) | 15 (11.5–16.8) | 14.3 (13.2–15.8) | 13.95 (12.6–15.4) | 0.895 | 0.6391 |
| MCV (fL) | 85.65 (83.5–89.3) | 88.2 (84.7–91.3) | 89.6 (87.6–92.3) | 8.751 | 0.01258 |
| Coagulation test | | | | | |
| Platelet ($10^3$/µL) | 202 (146–417) | 251 (169–149) | 248 (148–181) | 1.775 | 0.4116 |
| PTT (sec) | 36.8 (35.2–38.4) | 27.4 (23.15–29.6) | 28.9 (23.1–34.1) | 3.557 | 0.1689 |
| PT (sec) | 18.5 (16.15–18.95) | 14.95 (13.7–16.52) | 16.45 (14.1–19.7) | 1.266 | 0.5309 |
| INR (AU) | 1.4 (1.21–1.47) | 1.11 (1.02–1.24) | 1.18 (1.02–1.31) | 0.949 | 0.6222 |
| MPV (fL) | 8.6 (8–9.5) | 8.8 (8–9.3) | 8.6 (8.1–9.4) | 0.0398 | 0.9803 |
| Kidney function test | | | | | |
| Urea (mg/dL) | 30 (21.7–38.45) | 27.2 (19.5–43.5) | 37.6 (26.5–68.8) | 11.46 | 0.003235 |
| Creatinine (mg/dL) | 0.78 (0.67–0.99) | 0.79 (0.64–1.04) | 0.88 (0.68 -1.23) | 3.684 | 0.1584 |
| Liver function test | | | | | |
| ALP (IU/L) | 66 (44–72) | 71.5 (61–88) | 79 (60–103.5) | 7.046 | 0.0295 |
| GPT (IU/L) | 32.4 (14.8–55.9) | 31.9 (21.6–48.3) | 24.5 (15.5–40.5) | 5.434 | 0.06606 |
| GOT (IU/L) | 43.8 (28.6–48.9) | 37.1 (26–53.4) | 35.2 (26.5–58.9) | 0.347 | 0.8404 |
| DBIL (mg/dL) | 0.24 (0.12–0.3) | 0.155 (0.11–0.22) | 0.205 (0.155 -0.28) | 4.94 | 0.08453 |
| TBIL (mg/dL) | 0.59 (0.227–0.715) | 0.44 (0.245–0.291) | 0.55(0.447–0.7605) | 4.44 | 0.1083 |
| Albumin (g/dL) | 2.4 (2–2.76) | 2.75 (2.39–3.15) | 2.43 (2.34–2.5) | 1.138 | 0.5659 |
| Ion Con | | | | | |
| Cl (mmol/L) | 100.6 (98–102) | 97.6 (95.1–101.3) | 97.2 (93.4–101.1) | 4.766 | 0.09224 |
| Na (mmol/L) | 138 (136–141.5) | 137 (134–141) | 136 (133–140) | 3.464 | 0.1769 |
| Ca (mmol/L) | 2.04 (1.95–2.17) | 2.02 (1.9–2.12) | 1.87 (1.77–2.01) | 3.226 | 0.1993 |
| K (mmol/L) | 4.69 (4.18–4.78) | 4.42 (3.99–4.8) | 4.67 (4.1–5.02) | 2.591 | 0.2737 |
| Mg (mmol/L) | 0.84 (0.8–0.87) | 0.82 (0.7–0.88) | 0.885 (0.8–0.95) | 1.088 | 0.5802 |
| P (mmol/L) | 1.05 (0.89–1.12) | 0.9 (0.66–1.05) | 0.99 (0.97–1.07) | 2.559 | 0.2781 |
| Lipid profile | | | | | |
| LDL (mg/dL) | 126.3 (125.8–145) | 93.5 (68.1–122.9) | 70.95 (55–85.45) | 5.563 | 0.06192 |
| TG (mg/dL) | 140 (115.5–164.5) | 187 (138–322) | 138 (119–138) | 2.771 | 0.2502 |
| HDL (mg/dL) | 31.5 (30.1–42.1) | 31.25 (24.9–41.8) | 38.5 (36.7–44) | 0.918 | 0.6318 |
| Cholesterol (mg/dL) | 126.9 (111.4–142) | 150(138.1–157.1) | 162.6 (153.9–170.3) | 3.1005 | 0.2122 |

**Table 1**. Comparison of biochemical and hematological parameters between different age groups (n = 240) at the time of admission.

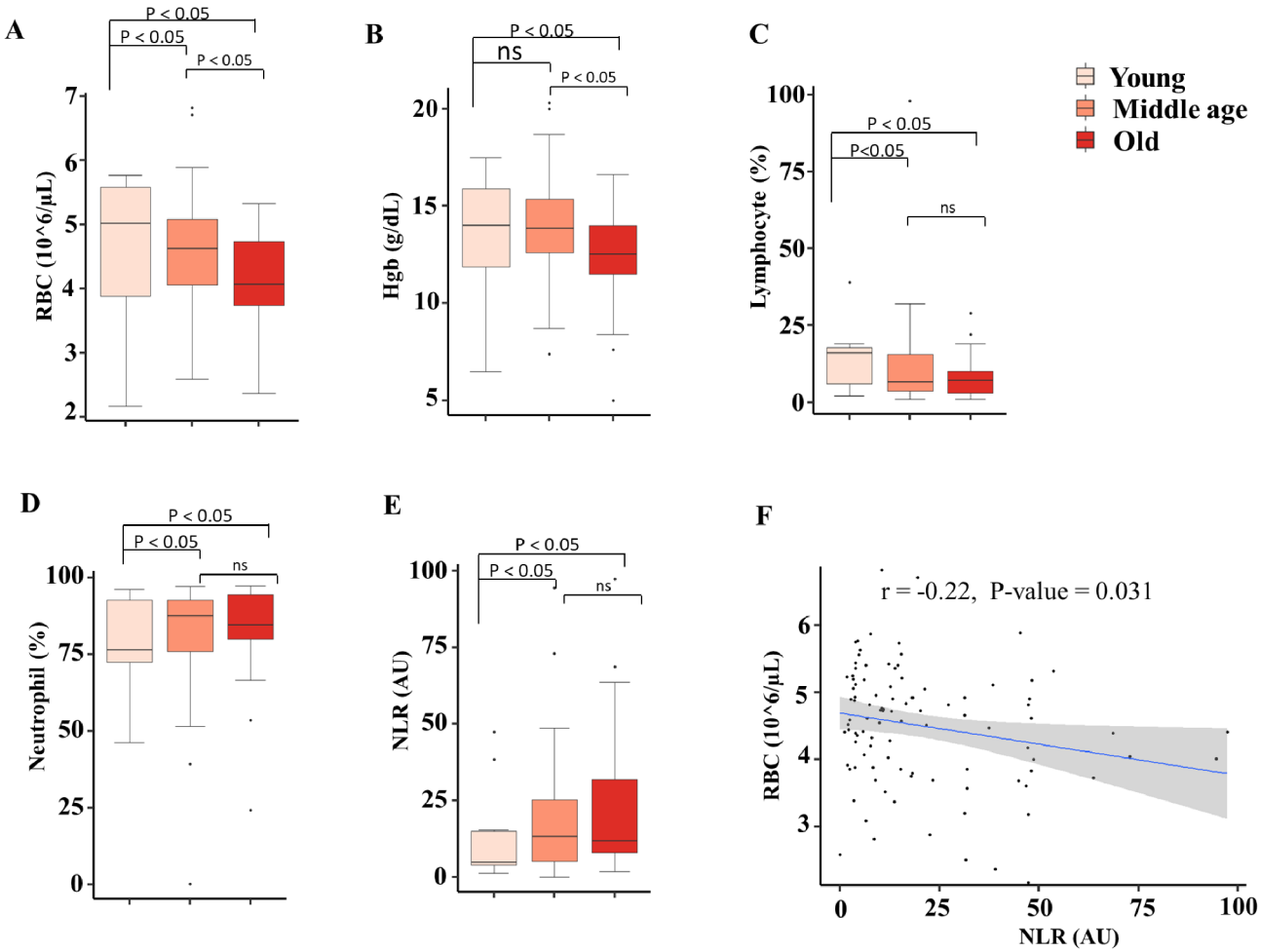


**Fig. 2**. Differences in CBC-based inflammatory markers in different age groups at the second point measurement: (**A**) level of RBC ($10^6$/uL), (**B**) Hemoglobin level (g/dL), (**C**) Lymphocyte percentage, (**D**) Neutrophil percentage, (**E**) Neutrophil to lymphocyte ratio (NLR), and (**F**) Inverse correlation of RBC count with NLR. P-values were calculated using the non-parametric Kruskal Wallis test and Mann Whitney u test for comparing more than two groups and for comparing between two groups respectively and the Spearman correlation test (r indicates Spearman's rho) was used to correlate RBC Count ($10^6$/uL) with NLR (AU) (ns = $P > 0.05$, and AU = Arbitrary Unit).

The clinical course of patients in their hospital stay showed that 76.2% (64/84) of older aged patients, 62.2% (79/127) of middle-aged patients, and 65.5% (19/29) of young patients develop severe/critical features (Fig. 5B).

When analyzing the second point (7–10 days after hospital admission) measurements, we found the levels of RBC count ($10^6$/uL), (Fig. 5C) and lymphocyte percentage (Fig. 5D) were significantly ($P < 0.05$) higher in COVID-19 survivors compared to those that were deceased within each group. Whereas the level of neutrophil percentage (Fig. 5E) and NLR (Fig. 5F) was significantly higher in older patients and in those who deceased within each group compared to middle and young-aged patients and those that survived within each group. We also observed a decrease in RBC count ($10^6$/uL) (Fig. 5G), lymphocyte percentage (Fig. 5H), and an increase in neutrophil percentage (Fig. 5I) and NLR (Fig. 5J) in severe/critical patients within each group compared to mild/moderate cases.

## Effect of dexamethasone treatment among COVID-19 patients

Of the total 240 participants, based on the clinical assessment 126 patients have been given treatment including different antibiotics and corticosteroids whereas 114 patients were only given supportive medication (advised to rehydration and given anti-pain). Among 126 patients, 92.8% (117/126) were treated with dexamethasone and antibiotics whereas 7.2% (9/126) were treated with different antibiotics but dexamethasone was not given. When assessing the hospital mortality rate was not significantly different ($P > 0.05$), which was 33.3% (3/9) and 32.4% (38/117) between those that were not treated and those treated with dexamethasone respectively and there was no significant difference in age between the two treatment groups (median age was 58 years and 60 years for dexamethasone-treated group vs for dexamethasone non treated group, respectively). We also observed changes in measured laboratory parameters like an increased number of RBC counts, hemoglobin level, percentage of lymphocytes, and decreased level of monocyte percentage (Fig. 6A–D) in the dexamethasone treatment group

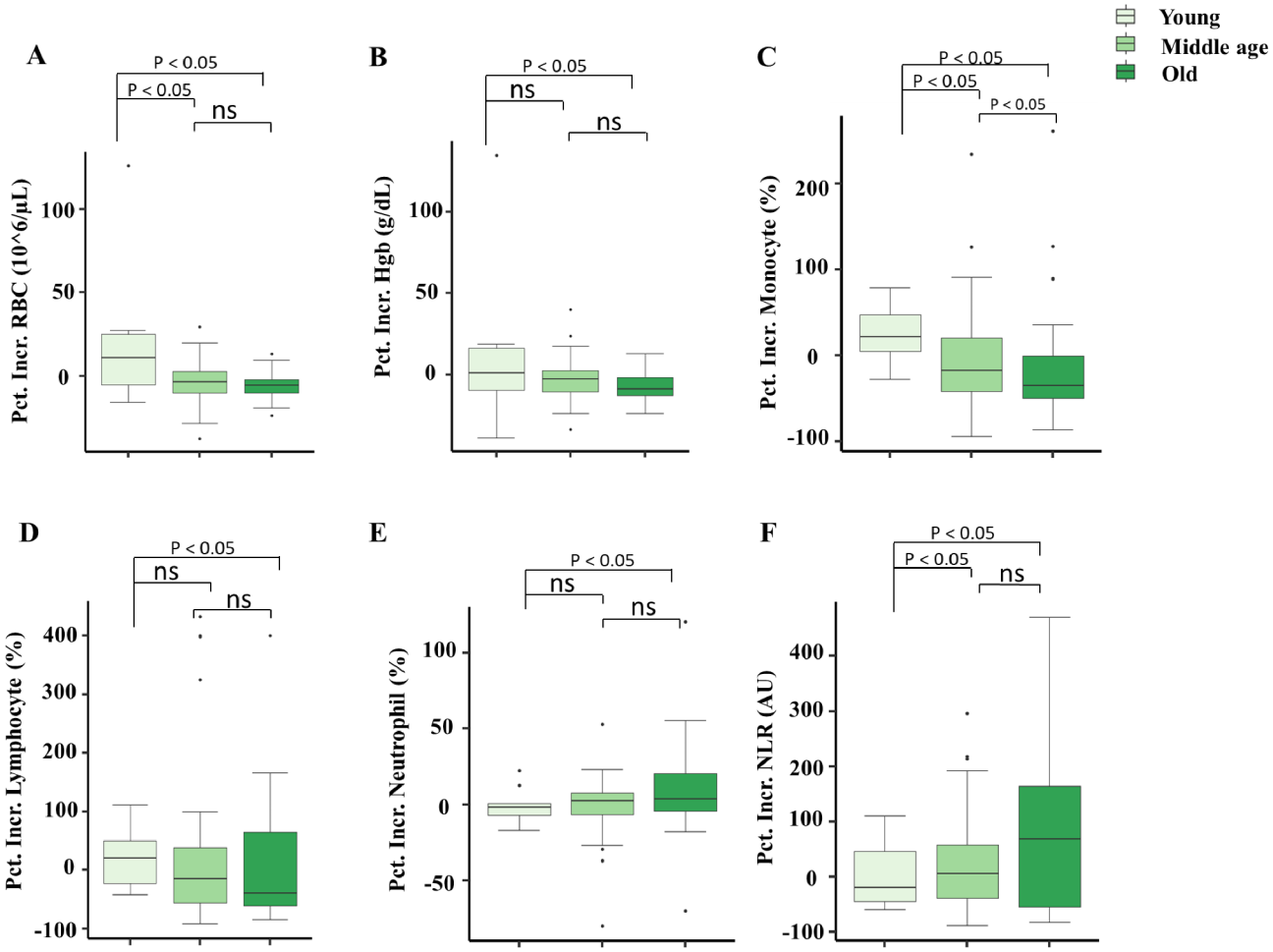


**Fig. 3**. Percentage increase of CBC-based inflammatory markers in different age groups (n = 240): (**A**) Percentage increase of RBC ($10^6$/uL), (**B**) Percentage increase of Hemoglobin level (g/dL), (**C**) Percentage increase of Monocyte percentage, (**D**) Percentage increase of Lymphocyte percentage, (**E**) Percentage increase of Neutrophil percentage, (**F**) Percentage increase of Neutrophil to lymphocyte ratio (NLR) (AU). Pct. Incr. (Percentage increase) was done by subtracting the second time measurement from the first point measurement divided by the first point measurement and multiplied by 100. P-values were calculated using the non-parametric Kruskal Wallis test and Mann Whitney u test for comparing more than two groups and for comparing between two groups respectively. (ns = $P > 0.05$ and AU = Arbitrary Unit).

compared to the non-treated group, but these differences were not statistically significant ($P > 0.05$). Similarly, there was no statistically significant ($P > 0.05$) difference in the NLR (Fig. 6E) and neutrophil percentage (Fig. 6F) between the two groups. As the number of individuals in the non-treated group is small (which could be one reason we lost statistical power) which is one of the limitations of our study thus, further large cohorts are needed to validate our result. As other studies showed, dexamethasone use has no effect[22] and others have shown to decrease mortality among COVID-19 patients[16,17,23].

## Discussion

The aging process affects all organs and systems of the body including the respiratory system. The structure and functionality of the lung alter with age and the response to infection varies with age. These changes with age result in lower response and tolerance of the lung to infection like pneumonia in elderly patients compared to young ones[24]. Similar to our finding previous studies have shown that the mortality due to SARS-Cov-2 is higher in older patients[20,25,26] indicating that aging is an important determinant factor of mortality among COVID-19 patients. We didn't find a significant association between comorbidity and mortality in all age groups, Others also showed that not comorbidity rather, frailty was associated with COVID-19 mortality[27]. Similar to our finding clinical symptoms of cough, and fever showed comparable prevalence in different age groups whereas unlike our finding dyspnea was common among elderly COVID-19 patients[28]. Vital clinical signs like Pulse rate, body temperature, and respiratory rate showed no significant difference between the three age groups others also showed similar findings[29]

Deregulation of the immune responses to SARS-CoV-2 has been linked with disease severity and fatal outcomes in COVID-19 patients. For instance, compared to healthy donors, neutrophils from COVID-19

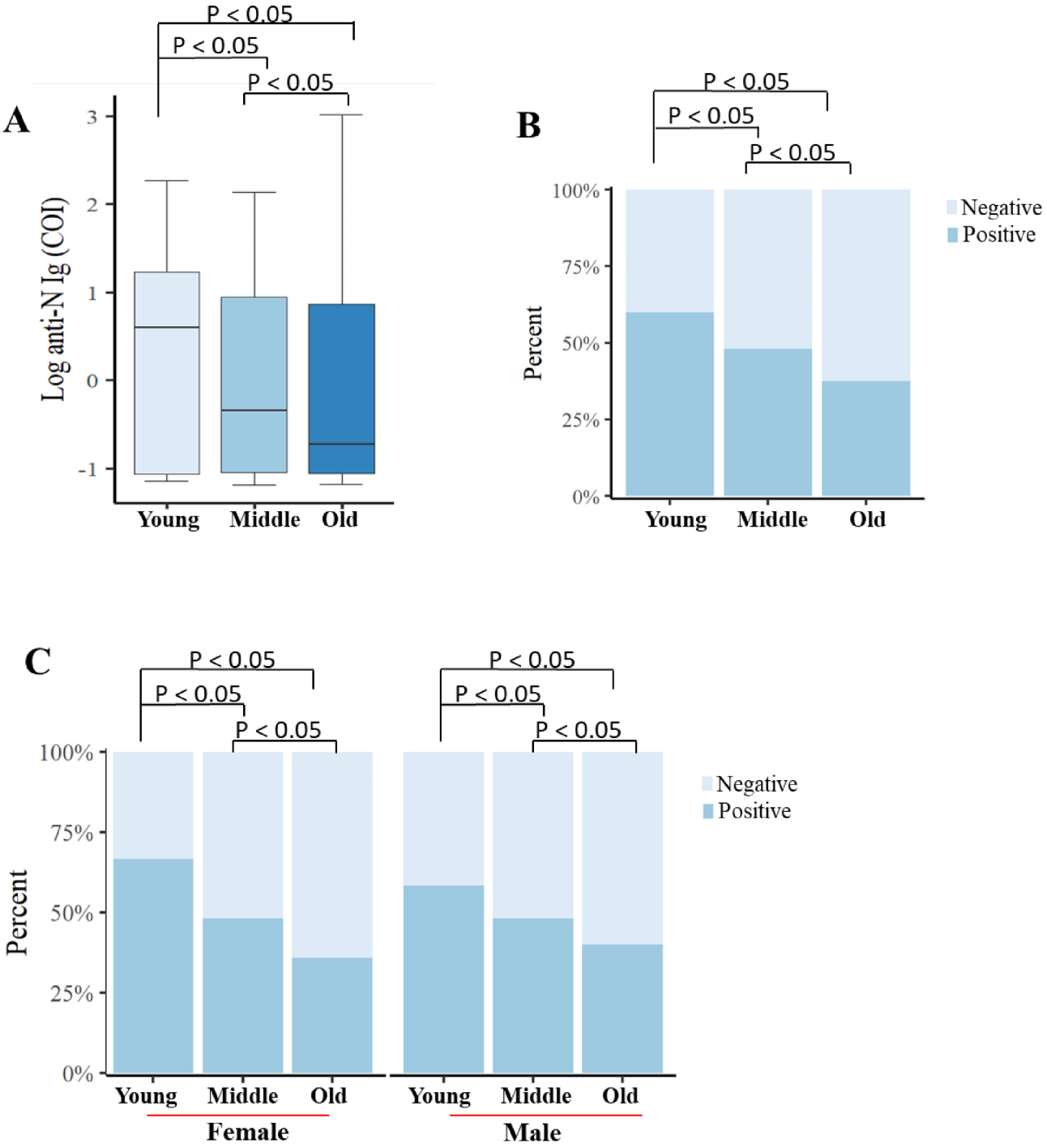


**Fig. 4**. Comparison of natural antibody response to SARS-CoV-2 in different age groups (n = 141): (**A**) lower level of ($P < 0.05$) natural antibody against SARS-CoV-2 in old patients compared to middle-aged and young patients (**B**) using the cutoff index value of ≥ 1, a lower percentage of response was detected in old COVID-19 patients compare to middle aged and young patients (**C**) lower antibody response ($P < 0.05$) was observed in both sex among old patients. P-values were calculated using the non-parametric kruskal wallis test and Mann Whitney u test for comparing for more than two groups and for comparing between two groups respectively and chi-squared test was used when comparing proportion between groups (ns = $P > 0.05$).

patients were demonstrated to have enhanced degranulation like increased release of myeloperoxidase and neutrophil elastase as well as increased release of IL-18 indicating the activation of neutrophils in COVID-19 patients. Similarly when stimulated with TLR ligands neutrophils from COVID-19 patients produce higher inflammatory molecules like IL-6, IL-1β, and TNFα compared to healthy donors[30]

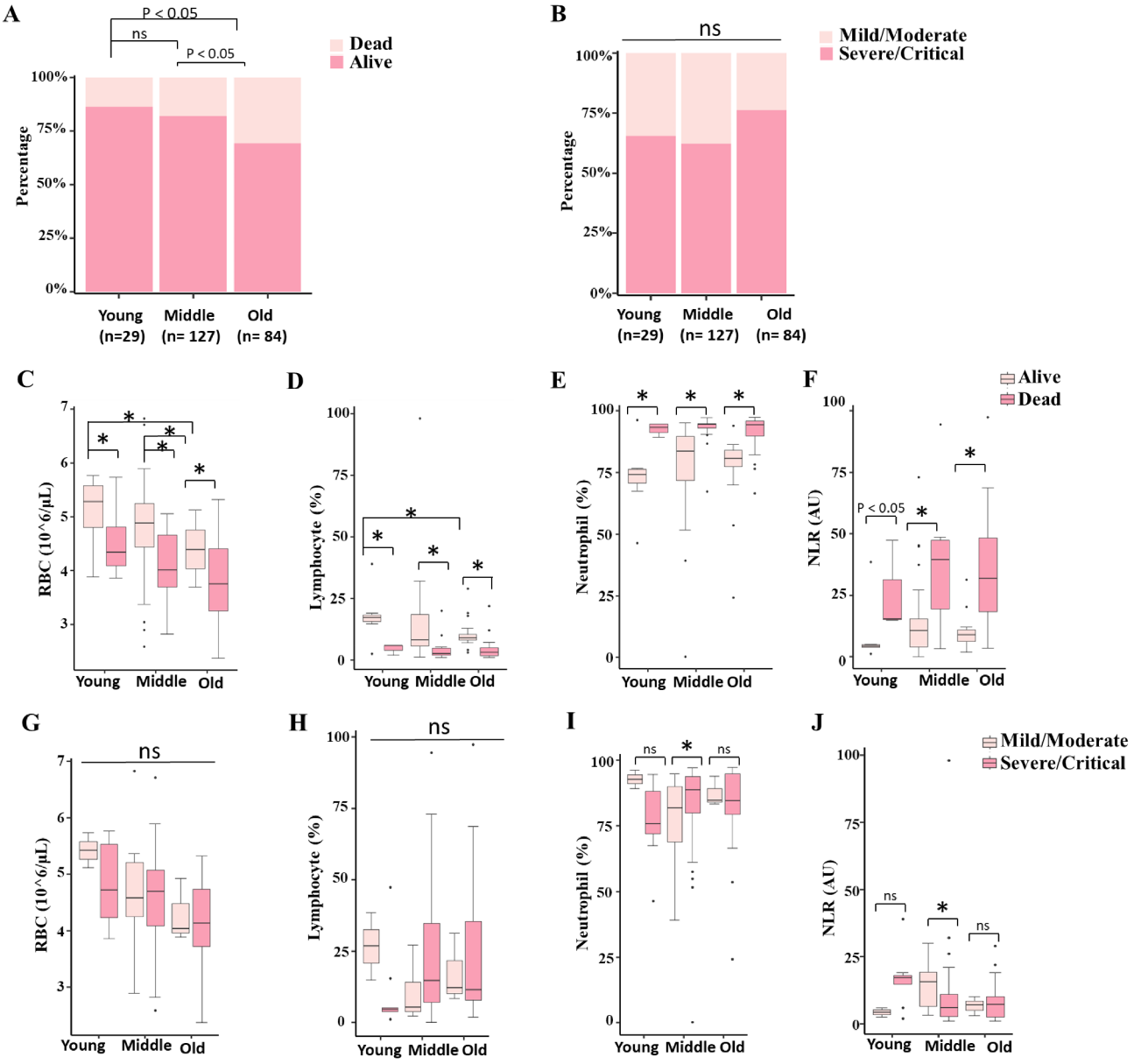


**Fig. 5**. Comparison of deceased and Alive COVID-19 patients between the three age groups (n = 240) : Significantly ($P < 0.05$) higher mortality rate in elderly COVID-19 patients compared to middle-aged and young patients (**A**), comparison of disease severity between the three groups (**B**), comparison of RBC count ($10^6$/uL) (**C**), Lymphocyte percentage (**D**), Neutrophil percentage (**E**), NLR (AU) (**F**) between deceased and alive patients in the three age groups. Comparison of RBC count ($10^6$/uL) (**G**), Lymphocyte percentage (**H**), Neutrophil percentage (**I**), NLR (AU) (**J**) between Mild/Moderate and severe/Critical cases between the three age groups. P-values were calculated using non-parametric kruskal wallis test and Mann Whitney u test for comparing for more than two groups and for comparing between two groups respectively and chi-squared test was used when comparing proportion between groups (ns = $P > 0.05$, * $P < 0.05$, and AU = Arbitrary Unit).

In our case, we found that there was an age-dependent increase in neutrophil percentage and NLR and a decrease in the RBC count and there was no significant difference in the percentage of basophils, monocyte, eosinophil, and platelet count between the three age groups COVID-19 patients, whereas other studies showed that elderly COVID-19 patients showed a lower percentage of peripheral blood basophils and eosinophils[31]. Whereas others have shown lowered median monocyte, platelet counts[32], and eosinopenia in deceased patients[33]. We also found that neutrophilia, lymphopenia, and high NLR were observed in deceased COVID-19 patients and in older COVID-19 which in line with other studies[34] and neutrophilia, lymphopenia, and high NLR was also significantly high in ICU-admitted COVID-19 patients[35]. This could indicate that COVID-19 is associated with immune deregulation, especially in elderly patients and severe COVID-19 patients. Neutrophils are one of the crucial cells involved in inflammatory response and have been linked with the development of cytokine storm and further in the development of acute respiratory distress syndrome (ARDS) in COVID-19 patients[36].

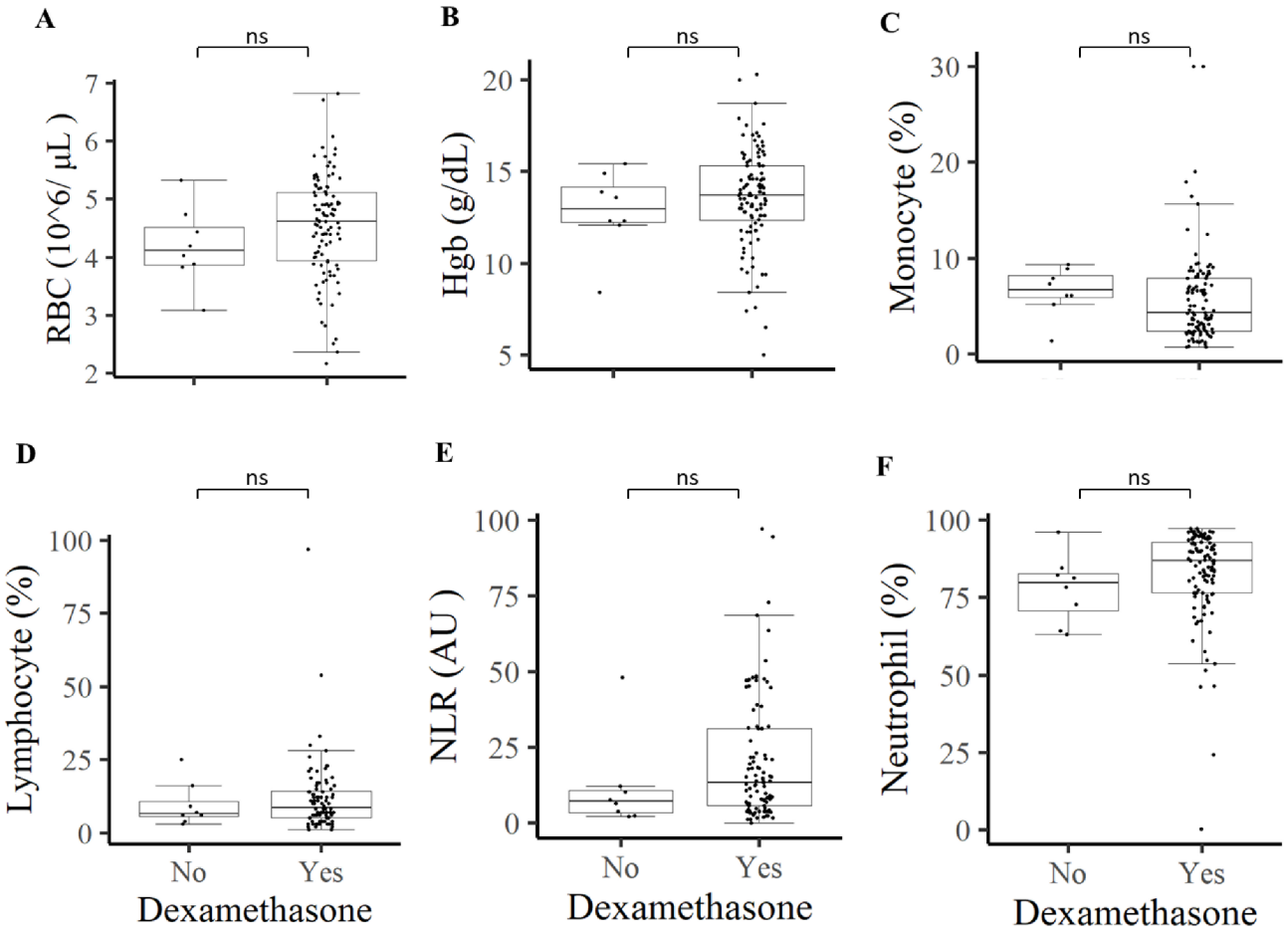


**Fig. 6**. Effect of dexamethasone treatment among COVID-19 patients (n = 126): Comparison of the level of RBC count ($10^6$/uL) (**A**), Hgb(g/dL) level (**B**), Monocyte percentage (**C**), Lymphocyte percentage (**D**), NLR (AU) (**E**), and Neutrophil percentage (**F**), between patients under dexamethasone treatment (n = 117) and those that are not under dexamethasone treatment (n = 9). P-values were calculated using Mann Whitney u test for comparing for more than two groups (ns = $P > 0.05$ and AU = Arbitrary Unit).

Ma A et al. also showed that an increased NLR > 11 predicts the development of ARDS[37] and Liu J et al., showed NLR value of > 3.13 predicts the occurrence of critical illness in COVID-19 patients aged > 50 years[38]. Alongside the degranulation of the immune system, there was also a decrease in the number of RBC count and level of hemoglobin especially in elderly patients. One reason for this could be that SARS-CoV-2 infection has a direct cytopathic injury or indirect induction of auto-antibodies targeting the RBC[39,40] or complement-mediated lysis of RBCs[21].

Assessing the natural antibody response has great value in that, it helps to identify the protection of individuals from reinfection[41]. When we determined the anti-Nuclocapsid natural antibody response in the three age groups, old COVID-19 patients showed decreased response which could be linked with the overall impact of age on the immune system. Others also showed that the seroconversion after a positive COVID-19 PCR test was low in old patients[42]. In our case, the response to natural antibodies was not influenced by sex but others have shown that female COVID-19 patients have a robust response immune response and less severe disease course[43,43]. Finally, the use of dexamethasone decreased some parameters of inflammation which was not statistically significant. As we clearly stated the number of samples was not conclusive and this is one limitation of this study. Previous studies have shown that the use of dexamethasone especially in critically ill patients lowers hospital stay mortality rate[16,17] whereas others showed no impact on mortality when using dexamethasone in COVID-19 patients[22]. Factors like study design, number of participants, disease severity, and other factors could be the reasons for the observed difference in the outcome of dexamethasone usage in COVID-19 patients.

Overall we found age-dependent immune deregulation in COVID-19 patients, where elderly patients showed higher markers of immune deregulation, similarly, this immune deregulation was linked with disease severity and mortality in all age groups. This age-dependent immune deregulation could be one reason for the observed difference in clinicopathology between young and old COVID-19 patients. Likewise, elderly COVID-19 patients showed a decrease in natural antibody response. One prominent feature of aging is the decreased production of antibodies to infection and vaccines, which is one reason elderly patients are more prone to reinfection. This study has its limitations, due to the capacity of our facility we were forced to enroll a small sample size of

COVID-19 patients, as well we did not enroll healthy controls for each group. The other being due to the setting of our laboratory we did not measure the level of inflammatory cytokines and perform immunophenotyping. To conclude we showed that simple parameters of inflammation can be effectively used as biomarkers of age-dependent disease progression, severity, and mortality in COVID-19 patients. This is especially important in resource-limited settings where advanced laboratory testing of inflammation like cytokines using flow-cytometer and ELISA are not available.

## Data availability

All relevant data supporting the manuscript are included in the manuscript. Upon reasonable request, raw data of this article will be made available by the corresponding author.

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## Author contributions

AMT conceived the study. AMT analyzed and interpreted the data and drafted the manuscript. AG and SA monitored the data collection and facilitated the setup of the COVID-19 testing laboratory and diagnosis units. AMT, AG, and SA reviewed and edited the manuscript. All authors read the manuscript and approved the submission.

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## Declarations

## Ethics approval and consent to participate

The institutional review board (IRB) of St. Paul's Hospital Millennium Medical College, in Addis Ababa, Ethiopia has reviewed the detailed procedure and protocol of the proposal and approved the study. The data collection and experimental protocol were carried out in accordance with the ethical guidelines of the Helsinki Declaration. All participants were included voluntarily and they were informed that they have the right not to participate or withdraw at any time and at any stage from the study. The objectives of the study were clearly explained to the participants in the local language. Participants were informed that their willingness to participate or not to participate has no impact on their normal treatment procedure. A consent form was written both in English and Amharic (the local language) and detailed information was written so that participants would have a full grasp of the study. For illiterate participants, the consent form was read (in the local language) in the presence of a family member and an impartial witness who had no connection with the study. Whenever they agreed to participate, signatures were taken from the family member, and the impartial witness and the thumbprint of the illiterate participant were taken on the consent form. Written informed consent was taken from every participant of the study. The confidentiality of all participants was kept by a unique identification code and timely communication was done with assigned medical doctors for COVID-19-positive patients.

## Competing interests

The authors declare no competing interests.

## Additional information

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-95722-3.

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