Complete blood cells count abnormalities in COVID-19 patients and their prognostic significance

Single center study in Makkah, Saudi Arabia

Amal E. Abd El-Lateef, MSc, MD, Manar M. Ismail, MSc, MD, Gamal Thabet, MSc, MD, Nur-Anna Cabrido, BSc.

ABSTRACT

Objectives: To evaluate the role of different peripheral blood count parameters as a cheap and rapid test in determination of coronavirus disease -19 (COVID-19) severity and patients’ outcome.

Methods: The data of 462 confirmed COVID-19 patients who attended at the Security Force Hospital, Makkah, Saudi Arabia, from October 2020 to March 2021 was retrospectively reviewed and C. Patients with viral infection and respiratory diseases other than COVID-19 were excluded from the study. Complete blood count parameters were compared in accordance with the severity of the clinical presentation, age, and disease outcome.

Results: A total of 277 (60%) were male and 185 (40%) female. Clinically, 32 (6.9%) had severe illness and 430 (93.1%) showed moderate clinical disease. Organ failure occurred in 2.8% of the patients. There was significant leucocytosis, neutrophilia, lymphopenia, high neutrophil-lymphocyte (N/L) ratio, and anemia in patients with severe COVID-19 diseases as well as in non-survivors’ cases (p<0.001). Similarly, the inflammatory markers (C-reactive protein [CRP] and serum ferritin) were significantly elevated in the above-mentioned 2 groups (p<0.001). Significant decrease of the platelets count was detectable in clinically severe cases and non-survivors (p<0.01). Older age (>60 years) was associated with high leucocyte, neutrophil count, lymphopenia, anemia, organ failure, and poor outcome.

Conclusion: Leucocytosis, neutrophilia, lymphopenia, and high N/L ratio together with elevated serum level of ferritin and CRP are eminent features of COVID-19 severity. The inclusion of these parameters in the regimens for patients’ categorization on admission will enable early effective intervention and proper decision making during clinical case management.

Keywords: COVID-19, complete blood count, inflammation, prognostic significance

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From the Department of Clinical Pathology (Abd El-Lateef), Faculty of Medicine, Tanta University, Tanta; from the Department of Laboratory Medicine (Ismail, Thabet), National Cancer Institute, Cairo University, Cairo, Egypt; from the Laboratory Department (Abd El-Lateef), Faculty of Applied Medical Science, Um Al-Qura University, from the Laboratory Department (Thabet, Cabrido), Security Force Hospital, Makkah, and from the Laboratory Department (Ismail), Al-Bishry hospital, Makkah, Kingdom of Saudi Arabia.

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Address correspondence and reprint request to: Dr. Amal E. Abd El-Lateef, Department of Clinical Pathology, Faculty of Medicine Tanta University, Tanta, Egypt. E-mail: damalezzat@yahoo.com
ORCID ID: https://orcid.org/000-0002-3428-5775
Coronavirus disease-19 (COVID-19) is caused by a new coronavirus that belong to the beta subfamily; it was given the name acute respiratory syndrome-2 (SARS-Cov-2). Coronavirus disease-19 has been emerged by the end of 2019 and by April 2020 it became a global health pandemic.\textsuperscript{1} Human to human transmission occur through respiratory droplets and indirectly through contact with contaminated surfaces.\textsuperscript{2-3} Coronavirus disease-19 infection frequently shows flu-like symptoms and very commonly real-time polymerase chain reaction (RT-PCR) positive subjects remain without symptoms. Percentage of symptomatic patients remain very low.\textsuperscript{4} Clinical manifestations are variables, ranging from asymptomatic, mild acute upper respiratory manifestations (fever, cough, and body ache) to critical illness with multiorgan failure. Approximately 80% presented with mild symptoms, 13% showed severe illness, and only 5% were critical and required intensive care unit (ICU) admission.\textsuperscript{5,7} Intensive care unit admitted patients showed high level of inflammatory cytokines specially interleukins and tumour necrosis factor-\(\alpha\) which can affect different hematologic parameters.\textsuperscript{8}

Primarily, COVID-19 affects the lower respiratory tract, although many organs could be affected either directly through viral injury or indirectly by the elevated level of cytokines and hyperinflammatory state. Cardiovascular, gastrointestinal, genitourinary, immune system, and the bone marrow are target organs for SARS-Cov-2.\textsuperscript{9} Hematologic abnormalities have been documented in many COVID-19 pneumonia in the form of lymphopenia, thrombocytopenia, and haemostatic defects. They are linked to disease severity, ICU admission, and mortality.\textsuperscript{10} Many diseases condition have shown association with various blood parameters such as neutrophil/lymphocyte ratio (NLR). Neutrophil/lymphocyte ratio is associated in patients with ulcer colitis, NLR is suggested as an active parameter or a marker of inflammation and prognosis in many diseases including inflammatory bowel disease.\textsuperscript{11} Elevated NLR, and platelet/lymphocyte ratio (PLR) could be marker of worse diabetic control in men with type 2 diabetes mellitus, thyroiditis, and irritable bowel syndrome.\textsuperscript{12-14} Inflammatory markers like NLR, PLR, and C-reactive protein (CRP) were found to be higher in COVID-19 positive patients than COVID-19 negative subjects.\textsuperscript{15} Hemogram indexes were considered novel inflammatory markers in many recent studies in the literature.\textsuperscript{13-16} The aim of this study is to investigate the complete blood count (CBC) parameters and the inflammatory parameters in COVID-19 patients and their relation to disease severity and prognostic significance.

**Methods.** This study is a retrospective record-based study that was carried out at Security Force Hospital, Makkah, Saudi Arabia, between March 2021 and June 2021. Data was collected after taking ethical approval form the Institutional Review Board of Security Force Hospital (IRB# 0394-040121). This study included 462 cases all were proved to be COVID-19 positive by molecular detection of the viral gene by RT-PCR in nasal and throat swabs. Patients were classified into mild to moderate and sever according to their clinical characteristics as published in the sixth edition of the Guideline for Diagnosis and Treatment of SARS-CoV-2 released by the National Health Commission of the People’s Republic of China.\textsuperscript{17} Approximately 10 ml venous blood were collected from the patients on admission and divided between ethylenediamine tetraacetic acid, 3.2% citrated blood tubes, and plane tube for serum separation. Complete blood count was carried out using Sysmex XN2000 (Wakihomaha-Kaigandori Chuo-ku, Kobe, Japan) and Backman (UniCel® DxH 800 Coulter® Cellular Analysis System, USA, Brea, CA) coulter. Serum ferritin and CRP were carried out using Cobas® 6000 analyzer series (Roche Diagnostics International Ltd CH-6343 Rotkreuz Switzerland). Laboratory analysis was carried out following manufacture recommendation and standard operating procedure of the laboratory.

**Statistical analysis.** Data were analyzed using Statistical Package for the Social Sciences, version 20.0 (IBM Corp., Armonk, NY, USA). Numerical data were expressed as mean ± standard deviation (SD) or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Normality test was carried out as per the sample size, Shapiro-Wilk test was considered, and a \(p\)-value of <0.05 denoted non-normal data. Comparison of the quantitative normally distributed data between the 2 groups was carried out using Student \(t\)-test and comparison between the 3 groups was carried out using ANOVA test followed by paired comparisons using Tukey’s test. The non-normally distributed data were compared by either Mann-Whitney or Kruskal-Wallis for 2 and 3 group median comparisons consecutively. Two binary logistic

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regression model were used to identify the predictors of severity, organ failure, ICU admission, and death. Results were considered significant if the \( p \)-value was <0.05. All tests were 2-tailed.

**Results.** This study included 462 newly diagnosed COVID-19 cases. The median (minimum-maximum) age of the study group was 52.0 (1-112) years. A total of 277 (60%) were male and 185 (40%) were female. Regarding the clinical conditions of this cohort, 31 (6.7%) cases were admitted to the ICU, 13 (2.8%) cases suffered from organ failure (11 cases had respiratory failure and 2 cases had renal failure), 22 (4.8%) cases died, and the rest of the cases were discharged after clinical improvement and negative PCR results. Complete blood count and inflammatory parameters on admission are shown in **Table 1**.

The study group was classified according to the severity of their cases into sever cases (6.9%) and mild to moderate illness (93.1%). The mean±SD of the CBC parameters were compared; data are shown in **Table 2**. Nucleated red blood cells (RBCs) were detected in 10 (2.2%) cases. The cases that had a severe form of the disease were significantly older in age \( (p=0.002) \). Also, they had a significant leucocytosis, neutrophilia, lower lymphocyte count, and high NLR. The eosinophile and basophile count was significantly lower in the sever than the moderate group but still both groups had lower values than the normal reference range. Furthermore, anemia was significant in the cases with severe illness where the hemoglobin (Hb) levels and the RBCs counts were both significantly lower \( (p<0.001) \). The platelets count was significantly lower too \( (p=0.01) \). Inflammatory markers (CRP and serum ferritin) were significantly higher in the severe cases (**Table 2**).

There was a significantly high mortality rate in elderly patients \( (p=0.002) \). There was a significant association between high total leukocyte count (TLC), neutrophil, basophil count, NLR, CRP, serum ferritin, low platelet, Hb levels, and patients’ mortality (**Table 2**).

The study group was classified according to the age into 2 age groups as follow: 323 (69.9%) young age (<60 years) cases (62.8% male and 37.1% female), 139 (30.1%) old age (>60 years) cases (53.2% male and 46.8% female). This gender distribution in the

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**Table 1** - Complete blood count and inflammatory parameters on admission.

| Parameters          | Mean±SD | Median (min-max) |
|---------------------|---------|------------------|
| TLC                 | 7.60±4.70 | 6.53 (2.27-33.63) |
| Absolute neutrophiles | 5.52±5.21 | 4.14 (0.23-53.45) |
| Absolute lymphocytes  | 1.63±0.82 | 1.45 (0.09-6.43) |
| Absolute monocytes   | 0.52±0.23 | 0.52 (0.04-1.86) |
| Absolute eosinophile | 0.06±0.11 | 0.02 (0.01-2.32) |
| Absolute basophile   | 0.02±0.02 | 0.02 (0.01-0.19) |
| Hb                  | 12.9±2.1 | 13.11 (5.89-18.64) |
| RBCs                | 4.92±1.9 | 4.93 (1.78-41.33) |
| platelets           | 241.9±99.82 | 226.23 (25.21-763.11) |
| N/L ratio           | 4.75±7.14 | 2.90 (0.11-75.52) |
| ESR                 | 5.7±5.23 | 4.13 (0.24-53.44) |
| CRP                 | 1.6±1.82 | 1.47 (0.09-6.41) |
| Ferritin            | 0.53±0.22 | 0.56 (0.04-1.86) |

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**Table 2** - Comparison of the complete blood count and other studied parameters according to the severity of the disease and patients’ outcome.

| Parameters          | Severity of the disease | \( P \)-value | Recovered (n=439) | Outcome | \( P \)-value |
|---------------------|-------------------------|-------------|------------------|---------|-------------|
|                      | Mild to moderate (n=430) | Sever (n=32) |                  |         |             |
| Age                 | 50.3±18.2 | 61.0±19.52 | 0.002 | 50.3±18.2 | 61.0±19.53 | 0.002 |
| TLC                 | 6.9±1.3 | 16.3±9.73 | <0.001 | 7.1±3.5 | 18.9±9.23 | <0.001 |
| Absolute neutrophiles | 4±8±4.0 | 14.3±9.71 | <0.001 | 4.9±4.23 | 17.3±9.13 | <0.001 |
| Absolute lymphocytes  | 1.6±0.8 | 1.2±0.84 | 0.006 | 1.6±0.81 | 1.1±0.53 | 0.002 |
| N/L ratio           | 3.7±3.5 | 18.1±19.23 | <0.001 | 3.8±4.23 | 21.9±19.64 | <0.001 |
| Absolute monocytes   | 0.6±0.3 | 0.6±0.42 | 0.764 | 0.5±0.24 | 0.6±0.33 | 0.525 |
| Absolute eosinophile | 0.06±1±0.09 | 0.1±0.43 | 0.04 | 0.06±0.13 | 0.1±0.42 | 0.018 |
| Absolute basophile   | 0.02±0.02 | 0.03±0.03 | 0.003 | 0.02±0.02 | 0.04±0.04 | 0.001 |
| Hb                  | 13.1±2.0 | 10.2±2.72 | <0.001 | 13.2±2.00 | 9.3±2.61 | <0.001 |
| RBCs                | 5.0±1.9 | 3.7±0.91 | <0.001 | 5.0±1.91 | 3.4±0.9 | <0.001 |
| platelets           | 245.2±95.4 | 198.1±141.15 | 0.01 | 246±97.84 | 160.7±106.42 | 0.01 |
| ESR                 | 37.6±26.9 | 56.6±28.40 | 0.008 | 38.3±27.43 | 41.6±20.01 | 0.723 |
| CRP                 | 4.1±5.6 | 11.1±8.82 | <0.001 | 4.2±5.82 | 11.7±9.52 | <0.001 |
| Ferritin            | 65.5±1866.14 | 2653.8±4723.63 | <0.001 | 671.3±185.1 | 3152.1±553.43 | <0.001 |

Data are presented as mean \( ± \) standard deviation (SD). TLC: total leukocyte count, Hb: hemoglobin, RBCs: red blood cells, N/L: neutrophil-to-lymphocyte, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein.
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younger age and old age groups showed significant higher prevalence in the male gender (p=0.009).

Regarding the disease severity and gender of the patients, 8.3% of male were admitted to ICU versus 4.3% female (p=0.01). Also, significant higher death rate was recorded in the male group; 6.5% versus 2.2% female (p=0.01). There were significant higher percentages of organ failure, ICU admission, and death in the old age group (>60 years) than the other age groups. It is worthy to mention that none of the patients aged <18 years and diagnosed with COVID-19 experienced organ failure or required ICU admission and all of them were cured and discharged without complication (Table 3).

The CBC findings were compared among these 2 age groups and a significant difference was noted in the TLC, absolute neutrophiles, lymphocytes, monocytes counts, Hb, NLR, and RBCs (Table 4). The TLC, neutrophiles, and monocytes showed significant higher values at the old age group than the young age group (p<0.001). No significant difference was detected between the age group. However, there was significant lower lymphocyte count in old age group (p=0.001). The Hb level and the RBCs count were both significantly low at the old age group compared to the young and adult age groups. Nucleated RBCs was detected in 7 cases of the old age group, 3 of the young and adult groups without significant difference (p=0.07).

Two binary logistic regression models were used; one to determine the association between TLC, differential count, and the disease severity. First of these models reveals the significance of TLC only with 96.1% overall correction in predicting the patient to his group according to the TLC (Table 5). Second model determine the strength of association of the acute phase reactant (CRP, ESR, and ferritin) and it reveals the significance of CRP only with 95.4% overall correction in predicting the patient to his group according to the CRP level (Table 5).

**Discussion.** This study demonstrated the abnormalities seen in CBC parameters together with some inflammatory markers in relation to disease severity and outcome in COVID-19 patients. In regards to the gender distribution in this study, males were more affected by COVID-19 than females. Vahidy et al18 reported higher rate of COVID-19 positivity, more severe clinical manifestations and poor outcome among male patients when compared to female patients and more severe disease in male than female patients.19,20

The current study demonstrated that leucocytosis, neutrophilia, high NLR were associated with more severe disease.20 These findings were similar to Taj et al21 who reported high NLR and leucocytosis in 101 SARS-Cov-2 infected patients. Yan et al22 showed that high NLR was associated with COVID-19 severity. Lio et al23 found high NLR in patients with severe COVID-19 and was significantly associated with higher mortality rate. Our study showed that lymphopenia was in most of the COVID-19 patients with significant lower values in the severe cases (p=0.006) similar to the earlier finding.21 Huang et al24 and the centre for disease control in the United States of America reported lymphopenia as the most frequent hematologic abnormality among SARS-Cov-2 infection.8,22,23 Many studies reported significant association between lymphopenia and COVID-19 severity and mortality.24-27 This study showed significant lower platelet count among severe cases compared to the moderate cases (p=0.01).

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**Table 3** - The association of the clinical severity with the different age groups.

| Parameters            | <60 years (n=323) | >60 years (n=139) | P-value |
|-----------------------|-------------------|-------------------|---------|
| Organ failure         | 1 (0.3)           | 12 (8.2)          | <0.001  |
| Admission to ICU      | 13 (4.0)          | 18 (12.9)         | 0.004   |
| Death                 | 5 (1.5)           | 17 (12.2)         | <0.001  |

Data are presented as a number and percentage (%). ICU: intensive care unit, *Organ failure include both respiratory failure (11 cases) and renal failure (2 cases).

**Table 4** - Comparison of the hematological parameters according to the age groups.

| Parameters          | <60 years (n=323) | >60 years (n=139) | P-value |
|---------------------|-------------------|-------------------|---------|
| TLC                 | 6.95±3.84a        | 9.17±6.01a        | <0.001  |
| Absolute neutrophiles | 4.89±4.41b       | 6.93±6.00b        | <0.001  |
| Absolute lymphocytes | 1.65±1.15c       | 1.49±0.78c        | 0.001   |
| N/L ratio           | 3.75±4.36a        | 6.92±10.57a       | <0.001  |
| Absolute monocytes  | 0.55±0.30a        | 0.64±0.30a        | 0.001   |
| Absolute eosinophile| 0.06±0.08         | 0.07±0.21         | 0.49    |
| Absolute basophile  | 0.02±0.02         | 0.02±0.02         | 0.14    |
| Hb                  | 13.21±1.61c       | 12.21±2.32c       | <0.001  |
| RBCs                | 5.11±0.41c        | 4.62±1.16c        | 0.046   |
| Platelets           | 238.79±75.60      | 244.16±114.29     | 0.68    |
| ESR                 | 35.04±22.96b      | 46.2±27.42b       | 0.002   |
| CRP                 | 4.03±4.98b        | 5.8±6.93b         | 0.013   |
| Ferritin            | 777.82±137.26     | 810.52±180.07     | 0.698   |

Data are presented as mean ± standard deviation (SD). Groups having the same letters (a or b) showed no significant variation. TLC: total leucocyte count, Hb: hemoglobin, RBCs: red blood cells, N/L: neutrophil-to-lymphocyte, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein.
Furthermore, on comparing the patient’s outcome, platelets count was significantly lower in non-survivors. This could be explained by the fact that our severe cases also included the critical patients. Similarly, Liao et al. reported thrombocytopenia in both severe and critical COVID-19 patients and mild thrombocytopenia was reported in 20% of COVID-19 patients in a study carried out by Fan et al. On the other hand, Taj et al. found no association between platelet count and disease severity. However, COVID-19 infection or COVID vaccines may trigger autoimmune thrombocytopenia.

In regards to patient’s outcome, non-survivors showed significant leucocytosis, neutrophilia, high NLR, lower platelet, RBCs count, and lower Hb concentration when compared to those who recovered. On the same context, a study reported higher neutrophil count in patients who died of COVID-19. This study revealed that non-survivors showed leucocytosis and absolute neutrophilia. Neutrophilia in patients who died of COVID-19 could be explained by the cytokine storm which is a prominent manifestation in severe SARS-Cov-2 infection. Elevated NLR, decreased lymphocyte count have been previously reported in other works as well. The current study showed lower RBCs count and Hb concentration in severe COVID-19 patients when compared to those with moderate clinical disease. Similar finding was reported by Arya et al. The increased risk of anemia in severe COVID-19 disease and the association between anemia and poor patients’ outcome was reported in various studies. It was observed that anemic patients are at risk of developing severe form of COVID-19. However, Cai et al. found no association between Hb level and severity of COVID-19. The current study showed correlation between CBC abnormalities in COVID-19 patients and age. The overall magnitude of CBC abnormalities including leucocytosis, neutrophilia, increased NLR, lymphopenia, and anemia were associated with old age (>60 years). These findings agreed with previous studies that reported an association between neutrophilia and age >50 years old. They also reported lower platelet count in this age group which was not found in the current study.

Binary logistic regression models reveals the significance of TLC and CRP with 95.4 and 96.1 overall correction. However, in both models the odds ratio were 1.35 and 1.16 that denotes a low risk despite being significant. Data were adjusted and revised for sever
cases, which indicate a markedly elevated TLC which continued until patients’ death which mostly related to the development of cytokine syndrome (Table 5). In the current study values of serum ferritin and CRP were significantly higher in severe COVID-19 patients when compared to moderate and in non-survivors when compared to survivors. Similar findings reported that high serum ferritin and CRP were associated with ICU admission and mortality in COVID-19 patients. Taj et al. reported increased serum ferritin, lactate dehydrogenase, and CRP level in severe and critical COVID-19 patient when compared to mild clinical disease.

Study limitations. This study is a retrospective study that described the laboratory changes we found in the COVID-19 sero-positive cases. Most of the described changes were explained by cytokine storm syndrome but no laboratory investigation prove this explanation. For this purpose, measurement of serum cytokine levels such as IL-6 would be helpful and recommended in future studies. In the same context, thrombocytopenia was detected in sever cases that could be due to either autoimmune destruction or consumption in disseminated intra vascular coagulopathy process that needs further coagulation studies.

In conclusion, a simple, rapid, and cheap test such as CBC can reveal major abnormalities in COVID-19 infected cases that includes leukocytosis, neutrophilia, lymphopenia, and high NLR. Monitoring all the haematological parameters including novel hemograms NLR, and acute phase reactant (CRP, serum ferritin, and ESR) can aid in identification of potentially severe cases at early stages, enabling early effective intervention and proper decision-making during patient’s therapy.

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