Differential Leucocytes Count: An Independent Predictor of Clinical Outcomes in SARS-CoV-2 Patients

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Abstract

SARS-CoV-2 is a systemic infection that has a significant impact on the hemostasis and hematopoietic system. Lymphopenia may be considered a cardinal laboratory finding, with prognostic potential. The study aimed to determine the differential leukocyte count in SARS-CoV-2 among Sudanese patients, during the period from March to December 2020. A total of 787 subjects were enrolled 487 patients with COVID-19 and 300 healthy volunteers as a control group; their ages ranged from 29 to 89 years. 3 ml of EDTA venous blood samples were collected from each participant standard for CBC investigation and then analyzed by SPSS version 21 (Mean and Standard deviation). A significant association between leukocyte count among ICU, ER group, and control with (P. value 0.000), in addition, a significant association was revealed among mild group and control group in differential neutrophil count, differential lymphocyte count, and absolute neutrophil count (p. value 0.000), however non-significant in TWBC absolute mixed cell count, absolute lymphocyte count, and differential mixed cells count (value 0.7,0.2, 0.19) respectively. The study concluded that leukocytosis with neutrophilia and lymphopenia is associated with the severity of SARS-CoV-2 infection, and should be implicated as predict for a serious course of the disease as well as mortality.

Keywords: Leukocytosis, COVID 19 patients, CBC, Neutrophilia, Lymphocytosis

INTRODUCTION

Coronavirus is a zoonotic virus, a type of RNA virus in the family Coronaviridae of the order Nidovirales. It is a family of viruses that cause infections related to respiration, which were first isolated in 1937 and called coronaviruses because they looked like a crown under microscopy [1]. It is a highly pathogenic and transmittable viral infection that is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged in Wuhan, China, and spread across the world. Genomic analysis showed that severe acute respiratory syndrome coronavirus 2 is phylogenetically related to severe acute respiratory syndrome-like (SARS-like) bat viruses. Therefore, bats could be the possible primary reservoir. The intermediate source of origin and transfer to humans is not known, however, the rapid human-to-human transfer has been confirmed widely [2-4].

A rapid spread of the SARS-CoV-2, rapid changes in clinical features, and increased mortality is devastating globally [5]. Therefore, in the past 20 years, following severe acute respiratory syndrome coronavirus (SARS-COV) and Middle East respiratory syndrome coronavirus (MERS-COV), SARS-CoV-2 has been the third coronavirus that has caused infections worldwide. The health of the public was threatened by the outbreak of COVID-19, and overall, countries are allocating medical and scientific resources to fight against the COVID-19 pandemic [6, 7]. The Coronaviruses have been classified as supported genomic sequence, genomic organization, antigenic properties of viral proteins, D) replication strategies, and structural characteristics of virions, pathogenic, cytopathogenic, and physic-chemical properties. The Coronaviruses (CoVs) are species of virus that belong to the Nidovirales order, which has Roniviridae, Mesoniviridae, Coronaviridae, and Arteriviridae families [8]. The largest one of all four families is the Coronaviridae family, by its genomic sizes of Coronaviridae range, from 26 to 32 kb [9]. This virus family is subdivided into 2 subfamilies:

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Coronaviridae, and torovirinae. It is now divided into four genera, Alpha coronavirus, Beta coronavirus, Gamma coronavirus, and Delta coronavirus [10].

It is assumed that two main processes drive the pathogenesis of COVID-19. Early in the clinical course, the replication of SARS-CoV-2 primarily drives it. Later in the clinical course, it appeared that the disease is driven by a dysregulated immune/inflammatory response to SARS-CoV-2, which leads to damage to the tissue [11]. Based on this, it was anticipated that antiviral therapies would be the most effective early in the course of the disease, while immunosuppressive/anti-inflammatory therapies are likely to be more beneficial in the later stages of COVID-19 [12]. On the other hand, a Complete Blood Count (CBC) is one of the commonly used laboratory examinations for the evaluation of the clinical condition of the disease [13]. The leukocyte differential count is used by clinics to evaluate the disease status of patients, which is composed of five types of mature cells: neutrophil, lymphocyte, monocyte, eosinophil, and basophil [14, 15]. Hence current study aimed to determine the differential leukocyte count in SARS-CoV-2 among Sudanese patients, and its correlation with the clinical course of the disease.

**Materials and Methods**

From April to December 2020, we studied differential leukocytes count of a consecutive series of 487 confirmed COVID, through an analytical cross-sectional study. Patients with cardiopulmonary arrest were excluded. Patients were separated into subgroups according to disease severity at admission; intensive care unit (ICU), emergency group, (ER), and mild group. COVID-19 patients admitted in Khartoum state isolation center (Sudan) during the period from April to December 2020, and 300 healthy individuals as control. All patients were confirmed as positive for COVID-19. Based on the history of exposure to the virus, using lungs computed tomography (CT scan), clinical manifestations, and pharyngeal swab specimen’s nucleic acid amplification test by reverse transcription-polymerase chain reaction (RT-PCR), EDTA blood samples were collected from confirmed cases with COVID-19 patients and control group who has negative COVID-19 analyzed using hematology analyzer (Sysmex instrument xp-300). To measure white blood cell and differential count (neutrophil, lymphocyte, and mixed which include monocytes, eosinophil, and basophil) all parameters at percent and absolute.

**Ethical Approval and Statistical Analysis**

The Ethical Committee of the Academic Staff of Medical Laboratory Sciences at Al-Zaaim Al-Azhari University in Khartoum, Sudan approved this study. Informed verbal consent was taken from study subjects before participation in the study. Statistical Package for Social Sciences (SPSS) software version 26 was used, and P. values equal to or less than 0.05 were statistically significant.

**Results and Discussion**

787 Sudanese subjects were enrolled in this study, 487 patients diagnosed by real-time PCR, and 300 healthy volunteers as a control group. The age of patients ranged from 29 to 89 years (Mean± SD: 56.4± 14.02). The age sub was divided into two groups up to 50 and more than 50, four hundred and fifty-four patients were males and three hundred and thirty-three were females. Patients with COVID-19 were divided according to the severity of disease into three groups (patients in ICU, ER, mild), the complete blood count (CBC) worked for all patients, and control by a Sysmex instrument (XP-300). Comparison of differential count between case subgroup and control, show there was strongly clinically significant variation (P. value= 0.00); as shown in (Table 1).

The result showed clinically significant variation when comparing the differential parameter between the ICU subgroup and control (p. value 0.000), but not significant in absolute mixed cells (p. value 0.97) as shown in (Table 2), in addition, the result showed clinically significant variation when compared the differential parameter among ER and control with (p. value 0.000) as shown in (Table 3). The result showed clinically significant variation when compared mild subgroup and control group in neutrophil (%), lymphocyte (%), and absolute neutrophil, but not significant in TWBCs, absolute mixed cells, absolute lymphocyte and mixed cells (%) (P. value: 0.19, 0.77, 0.24 and 0.17 respectively) differential parameter in comparison with control with (p. value 0.000) as shown in (Table 4). The result showed clinically significant variation when comparing the differential parameter between ICU subgroup and mild (p. value 0.000), but not significant in absolute mixed cells (p. value 0.76) as shown in (Table 5), in addition, the result showed clinically significant variation when compared the differential parameter among ICU and ER with (p. value 0.000) as shown in (Table 6). There were significant differences in TWBCs and differential count between ER and mild subgroup (P. value less than 0.05); data illustrated in the Table 7.

**Table 1. Comparison of differential count between case subgroup and control**

| Parameters               | ICI n=273 | ER n=137 | Mild n=77 | Control n=300 | P. value |
|--------------------------|-----------|----------|-----------|---------------|----------|
| TWBC×10³ cell/µl         | 12.69     | 10.8     | 7.34      | 6.41          | 0.000    |
| Neutrophil (%)           | 85.4      | 72.9     | 58.0      | 49.32         | 0.000    |
| Lymphocyte (%)           | 9.30      | 17.89    | 33.3      | 40.30         | 0.000    |
| Mixed Cells (%)          | 5.58      | 8.21     | 9.8       | 10.78         | 0.000    |
| Absolute neutrophil×10³ cell/µl | 10.7     | 8.5      | 4.42      | 3.22          | 0.000    |
| Absolute Lymphocytes×10³ cell/µl | 0.99     | 1.60     | 2.32      | 2.5           | 0.000    |
| Absolute mixed ×10³ cell/µl | 0.6      | 0.94     | 0.65      | 0.69          | 0.047    |

One Way ANOVA Test used to calculate P. value
P. value less than 0.05 is considered significant
Mean are calculated.
The saga of the Coronavirus epidemic is still being written, and, fortunately, various decisions faced by health authorities had an important role in limiting the risk of the dissemination. Despite an abundance of clinical and epidemiological research on the virus and patients that extensively elucidated, the understanding of the clinical spectrum of COVID-19 infection continues to be a challenge. As of the virus's sustained mutation, the range of possible symptoms, the impact on the immune system, and the proposed vaccines in the research area, as well as viral effects on immune cells, which stimulates numeric and morphologic shifts in peripheral blood WBC that are well categorized and can help guide diagnostic workup to potential starting treatment strategies, and until just now, the risk variables that affect death have remains unclear. The present study was conducted in Khartoum state to aimed to determine the differential leukocyte count in SARS-CoV-2 among Sudanese patients and its correlation with the clinical course of the disease.

787 subjects participated in the study (487 patients suffering from COVID-19 and 300 healthy volunteers as the control group). The age of patients ranged from 29 to 89 years (Mean ± SD: 56.4 ± 14.02). The age sub was divided into two groups up to 50 and more than 50: (262) patients admitted into ICU group. The age sub range was from 29 to 89 years (Mean ± SD: 56.4 ± 14.02). The age sub was divided into two groups up to 50 and more than 50: (262) patients admitted into ICU unit had age above 50 years old, 85 patients their age more than 50 admitted to ER unit, but only (4) patients aged above 50 years old had mild symptoms of COVID-19. Four hundred and fifty-four patients were males and three hundred and thirty-three were females.

Table 2. Comparison of differential count between ICU patient group and control

| Parameters          | ICI n=273 | Control n=300 | P. value |
|---------------------|-----------|---------------|----------|
| TWBCx10³ cell/µl    | 12.69     | 6.41          | 0.000    |
| Neutrophil (%)      | 85.4      | 49.32         | 0.000    |
| Lymphocyte (%)      | 9.30      | 40.30         | 0.000    |
| Mixed Cells (%)     | 5.58      | 10.78         | 0.000    |
| Absolute neutrophilx10³ cell/µl | 10.7 | 3.22 | 0.000 |
| Absolute Lymphocytesx10³ cell/µl | 0.99 | 2.5 | 0.000 |
| Absolute mixed x10³ cell/µl | 0.6 | 0.69 | 0.047 |

Table 3. Comparison of differential count between ER patient subgroup and control

| Parameters          | ER n=137 | Control n=300 | P. value |
|---------------------|----------|---------------|----------|
| TWBCx10³ cell/µl    | 10.8     | 6.41          | 0.000    |
| Neutrophil (%)      | 72.9     | 49.32         | 0.000    |
| Lymphocyte (%)      | 17.89    | 40.30         | 0.000    |
| Mixed Cells (%)     | 8.21     | 10.78         | 0.000    |
| Absolute neutrophilx10³ cell/µl | 8.5 | 3.22 | 0.000 |
| Absolute Lymphocytesx10³ cell/µl | 1.60 | 2.5 | 0.000 |
| Absolute mixed x10³ cell/µl | 0.94 | 0.69 | 0.010 |

Table 4. Comparison of differential count between mild patient subgroup and control

| Parameters          | Mild n=77 | Control n=300 | P. value |
|---------------------|-----------|---------------|----------|
| TWBCx10³ cell/µl    | 7.34      | 6.41          | 80.19    |
| Neutrophil (%)      | 58.0      | 49.32         | 0.000    |
| Lymphocyte (%)      | 33.3      | 40.30         | 0.000    |
| Mixed Cells (%)     | 9.8       | 10.78         | 0.017    |
| Absolute neutrophilx10³ cell/µl | 4.42 | 3.22 | 80.04 |
| Absolute Lymphocytesx10³ cell/µl | 2.32 | 2.5 | 40.24 |
| Absolute mixed x10³ cell/µl | 0.65 | 0.69 | 60.77 |

Table 5. Comparison of differential count between ICU patient subgroup and Mild

| Parameters          | ICI n=273 | Mild n=77 | P. value |
|---------------------|-----------|-----------|----------|
| TWBCx10³ cell/µl    | 12.69     | 7.34      | 0.000    |
| Neutrophil (%)      | 85.4      | 58.0      | 0.000    |
| Lymphocyte (%)      | 9.30      | 33.3      | 0.000    |
| Mixed Cells (%)     | 5.58      | 9.8       | 0.000    |
| Absolute neutrophilx10³ cell/µl | 10.7 | 4.42 | 0.000 |
| Absolute Lymphocytesx10³ cell/µl | 0.99 | 2.32 | 0.000 |
| Absolute mixed x10³ cell/µl | 0.6 | 0.65 | 20.76 |

Table 6. Comparison of differential count between ICU patient sub group and ER

| Parameters          | ICI n=273 | ER n=137 | P. value |
|---------------------|-----------|----------|----------|
| TWBCx10³ cell/µl    | 12.69     | 10.8     | 20.000   |
| Neutrophil (%)      | 85.4      | 72.9     | 0.000    |
| Lymphocyte (%)      | 9.30      | 17.89    | 0.000    |
| Mixed Cells (%)     | 5.58      | 8.21     | 0.000    |
| Absolute neutrophilx10³ cell/µl | 10.7 | 8.5 | 0.000 |
| Absolute Lymphocytesx10³ cell/µl | 0.99 | 1.60 | 0.000 |
| Absolute mixed x10³ cell/µl | 0.6 | 0.94 | 40.01 |

Table 7. Comparison of differential count between ER patient subgroup and Mild

| Parameters          | ER n=137 | Mild n=77 | P. value |
|---------------------|----------|----------|----------|
| TWBCx10³ cell/µl    | 10.8     | 7.34     | 0.000    |
| Neutrophil (%)      | 72.9     | 58.0     | 0.000    |
| Lymphocyte (%)      | 17.89    | 33.3     | 0.000    |
| Mixed Cells (%)     | 8.21     | 9.8      | 0.000    |
| Absolute neutrophilx10³ cell/µl | 8.5 | 4.42 | 0.000 |
| Absolute Lymphocytesx10³ cell/µl | 1.60 | 2.32 | 0.000 |
| Absolute mixed x10³ cell/µl | 0.94 | 0.65 | 70.03 |

*Independent Test used to calculate P-value
*P-value less than 0.05 considered significant
*Mean is calculated.
The present study demonstrated that there was a strong significant variation between the case subgroup (mainly ICU) and control group in TWBCs, neutrophil (%), lymphocyte (%), mixed cells (%), absolute neutrophil, and absolute lymphocyte (P-value = 0.000), but insignificant in the absolute mixed cells (P-value = 0.97). This result is similar to a study carried out by Ding et al., in 2021 that showed significant association in neutrophil, lymphocyte, monocyte, and eosinophil when comparing patients with control and to some extent agrees with Anurag et al., 2020 study which showed that: neutrophil (%), lymphocyte (%), monocyte (%), eosinophil (%), neutrophil:monocyte ratio (NMR) and neutrophil-lymphocyte ratio (NLR) among mild, moderate and severe COVID-19 was statistically significant (P-value = 0.005). Lymphocyte monocyte ratio (LMR) and basophil (%) were statistically significant among the three groups [16].

A statistically significant difference was revealed when comparing ER patients with control in TWBCs, differential and absolute count (P-value = 0.000). This result does not agree with the study of Djakpo et al., in 2020: the study found no statistical difference between control and all patient groups for these five laboratory parameters: WBC (×10⁹/l) (P=0.09), neutrophil (P=0.7), lymphocyte (P=0.1), monocyte (P=0.8), eosinophil (P=0.8) and basophil (P=0.3); this variation may be a due low sample size of Djakpostudy [10].

Moreover; our findings demonstrated significant variation in neutrophil %, lymphocyte%, and absolute neutrophil (P-value = 0.000,0.000 and 0.04 respectively), but no clinically significant in the case of absolute mix, absolute lymphocyte, WBCS count, and mixed cell % (P. value=0.7, 0.2, and 0.19) respectively when compared mild subgroup and control group. Current findings are the same as to study done by Devajit Nath, et al. 2020 who noted a clinically significant association in lymphocyte % (P. value= 0.05) and no clinically significant in other CBC parameters, MONO, ESO) with p. value= 0.4, 0.06 respectively, when compared with control [17].

This study showed that there was clinically significant variation when comparing differential parameters between case subgroups (ICU, ER, and mild) (P-value < 0.05), but not significant in absolute mixed cells between ICU and mild (P-value = 0.76); this is resembling to study by Xu, X. et a., in 32 patients with COVID-19 which 50 % of patients showed decreased lymphocyte counts and 75% of patients showed decreased eosinophil counts and significantly higher levels of MONO% (P-value < 0.05) [18].

The immune system is critical for controlling and eliminating CoV 19 infections. Nonetheless, increasing evidence suggests that critically ill COVID-19 patients could be suffering from cytokine storm syndrome [19]. those COVID-19 patients who have dysfunctional immune responses may advance immune-pathological consequences. A better understanding of the interplay between coronaviruses and the hosts’ innate immune systems may highlight the potential and persistence of inflammatory response in the breath disease [20].

Neutrophils play an essential part in innate immune responses, whereas lymphocytes contribute to systemic inflammation. Thus, increased Neutrophil Lymphocyte Ratio (NLR) reflects a disparity in the inflammatory process and could be used to predict disease severity in infectious diseases like sepsis and bacteremia. The reliability of NLR in the identification of viral diseases has been demonstrated; as approved by numerous studies, NLR has been confirmed to be a more significant marker in COVID 10 patients [21-23]. In tandem with the increased clinical evidence on the predictive and prognostic potential of NLR, which has already increased due to disruption in neutrophil and lymphocytes count, hence elevated NLR should be implicated as predict for a serious course of the disease as well as mortality.

Ultimately, increased white blood cell and neutrophil counts with lymphopenia were revealed in the present study among COVID-19 patient populations, and it is uncertain whether which was before blood cell counts were related to the risk of serious COVID-19 infection or if the increase upon infection played a role in disease exacerbation. However, it has become clear that the percentage of these types of WBCs can be influenced by a variety of considerations, including age, race, gender, disease status, encountered comorbidities, and medications. Thus, differential leukocyte count should be considered as an independent predictor of clinical outcomes in SARS-CoV-2 patients.

CONCLUSION

The study demonstrated that leukocytosis, neutrophilia, and lymphopenia are associated with the severity of COVID-19 infection. Throughout the treatment of COVID-19, a significant increase in WBC count (10.5 10⁹ cells/ml) should be emphasized.

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