

Diagnostic Value of Monocyte, Eosinophil, Platelet-Lymphocyte Ratio And Monocyte Lymphocyte Ratio In Previously Healthy Covid-19 Patients

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Abstract

Aim: In this study we aimed to investigate diagnostic usefulness and optimal cut-off values of monocyte, mean platelet volume (MPV), eosinophil, neutrophil lymphocyte ratio (NLR), platelet lymphocyte ratio (PLR), monocyte lymphocyte ratio (MLR), red blood cell distribution width (RDW) in laboratory confirmed Covid-19 cases with and without pneumonia who were totally healthy before being infected with Covid-19. **Materials and Methods:** Data of a total 10000 patients who were admitted to hospital with complaints appropriate with Covid-19 in six months (March–August 2020) interval and underwent SARS-CoV-2 Polymerase Chain Reaction (PCR) investigated. Out of 10000 patients we included only previously healthy 174 patients with Covid-19 pneumonia, 144 SARS-CoV-2 positive but no pneumonia and SARS-CoV-2 PCR negative 156 healthy controls. The laboratory results and demographic findings were collected from patient files retrospectively before undergoing treatment for Covid-19. **Results:** We found wbc levels lower in Covid-19 patients with pneumonia ($p < 0.001$ vs healthy), as well as neutrophil ($p < 0.001$ vs healthy), Monocyte ($p < 0.001$ vs healthy and Covid-19 without pneumonia), Eosinophil ($p < 0.001$ vs healthy and Covid-19 without pneumonia) and Platelet count ($p < 0.001$ vs healthy). MLR remained higher in patients without pneumonia ($p < 0.001$ vs Covid-19 with pneumonia and healthy controls) as well as PLR ($p < 0.001$ versus Healthy). **Conclusion:** In the light of the obtained results, decrease in wbc, neutrophil, monocyte and eosinophil levels support the diagnosis of Covid-19 pneumonia on the other hand increase in MLR and PLR may indicate Covid-19 without pneumonia. **Keywords:** Covid-19 pneumonia, eosinophil, monocyte

Introduction

Coronavirus disease-2019 (COVID-19), caused by the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) is cause of illness and death of millions since December 2019. It is a highly contagious disease that spreads via contact and aerosol transmission. There are much more cases than reported because many are asymptomatic but infectious. Gold standart diagnostic tool is testing nasopharyngeal swab samples by real-time reverse-transcription polymerase chain reaction (PCR). But PCR is not available in every health-care institution due to its cost and working technique, elsemore getting results takes time. Computed tomography (CT) of the chest is indicated in those patients suspicious of pneumonia with a prior chest x-ray that is undiagnostic. CT is expensive, not available in every healthcare institution and patients are exposed to radiation. There is a need for fast diagnostic parameters to assist doctors in the diagnostic phase and to help differentiating patients with Covid-19 pneumonia than those without. Complete blood parameters are studied in many patients at admission in Covid-19 clinics. Biomarkers of inflammation derived from the peripheral blood, hemogram parameters such as white blood cell (WBC) count, neutrophil lymphocyte ratio

(NLR) and platelet lymphocyte ratio (PLR) have been investigated as independent predictors for prognosis of systematic inflammatory diseases ¹. Monocyte-macrophage activation markers are increased and correlate with other inflammatory markers in SARS-CoV-2 infection, in association to hospital admission. The red blood cell distribution width (RDW) is a measure of the variation of red blood cell volume. There are recent studies focusing on RDW ². Mean platelet volume (MPV) is one of the platelet function indices which reflects the platelet production rate and stimulation ³. Platelet distribution width (PDW) is a direct measure of platelet size variation and a marker of platelet activation. High levels of PDW have been associated with Covid-19 mortality ⁴. All above mentioned parameters are studied by routine complete blood count test that might be overlooked by clinicians. The major difficulty for physicians is to translate the recommended guidelines into clinical practice. In this study we aimed to determine wheather monocyte levels, eosinophil levels, MPV, PDW, RDW, NRL, MLR and PLR can be used as biomarkers in the early diagnosis of laboratory-confirmed COVID-19 patients as well as to explore the most useful diagnostic biomarkers and optimal cut-off values. Else more to investigate if these parameters can be used in differentiation of Covid-19 patients with pneumonia than those without and healthy controls. To the best of our knowledge this is the first study investigating above mentioned parameters in previously totally healthy Covid-19 patients.

Materials and methods

We investigated files of those patients admitted to Bursa City Hospital/Bursa/Turkey between March-August 2020 with complaints compatible with COVID-19. In a total 10000 patients retrospectively evaluated. Out of 10000 patients those who were found to be positive for Covid-19 and were completely healthy before the disease were included in the study. In a total 318 Covid-19 patients who met the specified criteria and 156 healthy controls those tested negatively and had no chronic diseases included. We divided Covid-19 patients into two groups, the main group consisted of those patients who had penumonia on computed tomography of the lungs, the second gruop consisted of those patients tested positively with PCR but had no penumonia on computed tomography of the chest. And the third group consisted of healthy subjects. The demographic and clinical data of all patients is shown on table 1. The three patient groups were compared by examining the hemogram parameters (WBC, neutrophil, lymphocyte, monocyte, eosinophil, platelets, PDW, MPV and RDW) obtained at the time of hospital admission before using any kind of medication. NLR, PLR and MLR values were calculated by dividing neutrophil, platelet and monocyte levels to lymphocyte count. Patients with chronic diseases, those using a medication and pregnant were exluded. After obtaining scientific research approval for the study from the Ministry of Health General Directorate of Health Services, the ethics committee approval was obtained from Bursa City Hospital Clinical Research Ethics Committee (Ethics Committee Approval No:2021-2/2). All the statistical analyses were carried out using SPSS 25.0 software. A Kolmogorov-Smirnov test was performed for the normality of the sample data, and the continuous variables were defined by the mean $\pm$ standard deviation, median (interquartile range %25–%75), while the categorical variables were expressed as frequency and percent. In order to compare the independent groups, One-way ANOVA Bonferroni for the parametric test assumptions, and a MannWhitney U test for non-parametric assumptions were used. The Roc analysis was performed for optimal cut-off values to predict SARS-CoV-2 positivity. We exploited Youden Index values to identify the optimal cut-off values. In addition, p value less than 0.05 was set as the statistical significance level.

Results

The median age turned out to be 42 (19-82) in the Covid-19 pneumonia, 33 (19-81) in Covid-19 patients without pneumonia and 34 (19-87) in the healthy group (Table 1). The median age was significantly higher in the pneumonia group than those without pneumonia ($p < 0.001$) and healthy controls ($p < 0.001$). Although the gender distribution in the Covid-19 pneumonia group was 43% (75) women and 57% (99) men, it was 45.8% (66) women and 54.2% (78) men in those without pneumonia. WBC, neutrophil, platelet and lymphocyte levels remained significantly low (Table 2) in patients with and without pneumonia compared to healthy subjects (both $p < 0.001$). When we compared WBC, neutrophil, lymphocyte and platelet levels of Covid-19 patients with pneumonia to those without we found no difference ($p = 0.482, 0.923, 0.164$ and 0.447 respectively). Covid-19 patients with pneumonia had the lowest monocyte levels compared to those

without pneumonia and healthy controls (Both $p < 0.001$) but we found no difference between Covid-19 patients without pneumonia and healthy controls ($p = 0.261$). Eosinophil levels were lower in patients with Covid-19 pneumonia compared to those without and healthy controls as well as in Covid-19 patients without pneumonia compared to healthy controls ($p < 0.001$ for three comparisons). There was no difference between MPV, RDW, PDW and NLR levels of all groups. MLR was higher in Covid-19 patients without pneumonia compared to those with pneumonia and healthy controls (both $p < 0.001$) yet no difference between patients with pneumonia versus healthy controls ($p = 0.045$). PLR was higher in Covid-19 patients without pneumonia compared to control group ($p < 0.001$) but no difference when compared to those with pneumonia ($p = 0.025$) and patients with pneumonia compared to healthy controls ($p = 0.013$). We found no difference between PLR of Covid-19 patients with and without pneumonia ($p = 0.025$). We analyzed the optimal cut-off values calculated by the ROC analysis, and the ROC curves are presented in Figure 1, Figure 2. When we compared Covid-19 patients with pneumonia to healthy controls the areas under the curve (AUC) of WBC, Neutrophil, Lymphocyte, Monocyte, Eosinophil and Platelet were found as 0.828 ($p < 0.0001$), 0.769 ($p < 0.001$), 0.727 ($p < 0.001$), 0.657 ($p < 0.0001$), 0.815 ($p < 0.0001$) and 0.688 ($p < 0.001$) respectively (Table 3). For MLR and PLR 0.574 ($p = 0.21$), 0.591 ($p = 0.05$) respectively (Table 3). When we compared Covid-19 patients without pneumonia to healthy controls AUC of WBC, Neutrophil, Lymphocyte, Eosinophil, Platelets, Monocyte, MLR and PLR were found as 0.826 ($p < 0.0001$), 0.775 ($p < 0.001$), 0.756 ($p < 0.001$), 0.724 ($p < 0.0001$), 0.684 ($p < 0.0001$), 0.535 ($p = 0.304$), 0.714 ($p < 0.001$) and 0.657 ($p < 0.001$) respectively (Table 4). Significant levels for comparison between Covid-19 patients with and without pneumonia were observed between monocyte levels (AUC 0.66 $P < 0.001$) and Eosinophil levels (AUC 0.612 $P < 0.001$).

Discussion

In suspicious cases of Covid-19 PCR is used in the diagnosis and is the gold standard, in some patients suspicious of having pneumonia may have to undergo a CT scanning. Yet PCR and CT both are not available in every healthcare institution. Therefore, there is a need for simpler and more practical methods to detect Covid-19 cases and else more Covid-19 patients with pneumonia which may have to be internated or carefully followed.

Our study demonstrated that neutrophil levels decrease in Covid-19 patients with and without pneumonia compared to healthy subjects ($p < 0.001$ for both comparisons). The data on neutrophil levels in Covid-19 are incomplete and have not been widely studied. The available data suggest that neutrophilia is an expression of the cytokine storm and hyperinflammatory state which have an important pathogenetic role in COVID-19 and related infections. In previous studies neutrophilia is reported in patients treated in intensive care unit ⁵. Yet neutrophil levels may be effected by many secondary diseases. In our study we included previously totally healthy patients at admission before using any kind of medicine. In a study Gumus et al. demonstrated low neutrophil levels in pediatric Covid-19 patients⁶. Similar to our study Xie et al. reported low levels of neutrophils in Covid-19 patients ⁷ yet their study contained patients who had chronic diseases like asthma, chronic obstructive pulmonary disease, coronary heart disease etc.

Lymphocytes play an important role in the maintenance of immune system function. After a viral infection, changes in total lymphocyte numbers varies with different virus types. Wang et al. reported decreased lymphocyte levels in patients with Covid-19 ⁸. Similar to their study we found low lymphocyte levels in patients with Covid-19 pneumonia. Eventhough low lymphocyte levels have been demonstrated previously our study demonstrated that lymphocyte levels decrease in previously healthy Covid-19 patients but no statistically difference between those with and without pneumonia.

Monocytes and macrophages are the most enriched immune cell types in the lungs of Covid-19 patients and appear to have a central role in the pathogenicity of the disease ⁹. Current data suggest a preponderant role for monocyte-macrophage activation in the development of immunopathology of Covid-19 patients ¹⁰. In our study we found low monocyte levels in Covid-19 patients with pneumonia compared to healthy controls ($p < 0.001$) and patients without pneumonia ($p < 0.001$). Zhang et al observed that during SARS-CoV-2 infection, there are morphological and inflammation-related phenotypic changes in peripheral blood monocytes that correlate with the patient's outcome ¹¹. Zhang et al. did not detect significant differences in the number

of monocytes between patients with Covid-19 and normal healthy individuals. In a study Kos et al. reported reduced rate of activated monocytes mainly observed in patients with severe Covid-19¹². Eventhough our study demonstrated that monocyte levels decrease in previously healthy Covid-19 pneumonia and in those without these results reflect begining of the disease before treatment. Levels may differ after progression of the disease and may be effected by drugs used in treatment.

Eosinophils are circulating and tissue-resident leukocytes that have potential of proinflammatory effects in some diseases. Eosinophils also have been shown to have various other functions like immunoregulation and antiviral activity. Yet their role in Covid-19 is not well known. Previosly it has been reported that Covid-19 patients had a decrease in circulating eosinophil counts, which was significantly more frequent than other types of pneumonia patients ⁷. Zhang et al. has previously reported eosinopenia in patients with acute respiratory deterioration during infection with SARS-CoV-2¹³. Zhanh et al. included 140 Covid-19 patients with comorbidities which may effect eosinophil levels yet similar to their study we also found low levels of circulating eosinophils in patients with Covid-19. Our study demonstrated that circulating eosinophil counts decrease in preivously totally healthy patients with Covid-19 and much more in Covid-19 patients with pneumonia. These results may indicate involvement of the lungs is associated with decrease in eosinophil levels. So a treatment strategy which increases eosinophil levels or targeting reasons of eosinopenia may help in dealing with Covid-19.

Covid-19 causes a spectrum of disease; some patients develop a severe proinflammatory state which can be associated with a unique coagulopathy and procoagulant endothelial phenotype. We found Covid-19 patients with pneumonia had the lowest platelet counts. Trombocytopenia is a big problem during Covid-19 because many patients need anticoagulant prophylaxy which implies that thrombocytopenia needs to be attached more importance in the follow-up of Covid-19 patients. The difficulty is to choose the aproprate anticoagulant while on the other side there is trombocytopenia.

Prveiosly it has been reported that increased MPV levels was associated with mortality in Covid-19 patients ¹⁴. In another study Ozcelik et al. compared Covid-19 patients with influenza pneumonia and reported low MPV levels in patients with Covid-19¹⁵. MPV levels also found significantly high in asymptomatic children infected with Covid-19 ⁶. In this study we found no difference in MPV levels between groups. MPV can be effected from many diseases and conditions and the teqnique used which may effect the results. In this study similar to MPV levels we didnt find statistically differences between PDW and RDW levels. In a study Henry et al. found a progressive increase of RDW with advancing Covid-19 severity ¹⁶. The discrepancy between two studies might be due to patients grops, Henry et al. included patients with comorbidities yet we aimed to see if RDW can be usefull in the early diagnosis of previously healthy Covid-19 patients.

In recent years, some ratios have been in use by researchers in the diagnosis and prognosis of many inflammatory conditions. Some of these are neutrophil/lymphocyte, platelet/lymphocyte, and monocyte/lymphocyte ratio. In a study Seyit et al. Reported high NLR in patients tested positive for SARS-CoV-2 compared to controls ¹⁷. In our study we found no differences between NLR of three groups. In a study from Wuhan/China Yang et al. reported elevated NLR significantly associated with illness severity ¹⁸. Difference between our study and the study conducted by Seyit et al. migt be due to patient characteristics or to the heterogenisity of Covid-19. Seyit et al found high levels of PLR in patients with Covid-19. In acoordance with their study we also found high levels of PLR in patients with Covid-19 pneumonia compared to healthy subjects (p <0.013) and Covid-19 patients without pneumonia compared to healthy controls (p <0.005). Our study demonstrated that PLR is high in Covid-19 patients but it might not be associated with disease severity at least at the begining stage of the disease. Yang et al. investigated 93 Covid-19 patients and found out that lyphocyte to monocyte ratio (LMR) levels of severe patients were significantly higher than those of non-severe patients.¹⁸. In our study we aimed to observe if MLR could be used in the diagnosis of Covid-19 patients at the admmission before undergoing treatment. We found highest MLR in patients without Covid-19 pneumonia versus patients with pneumonia and healthy controls (both p <0.001). More studies are needed to obtain more detailed information.

Limitations

Since we only included previously healthy individuals in our study, the results of the study do not provide information about those with additional diseases which stands as a limitation. We aimed to evaluate the above mentioned parameters the first hospital admission, thus results do not give an idea about the progressive stages of the disease and the prognosis.

Conclusion

In the light of the obtained results, decrease in wbc, neutrophil, monocyte and eosinophil levels support the diagnosis of Covid-19 pneumonia on the other hand increase in MLR and PLR may indicate Covid-19 without pneumonia. Deep fall in eosinophil and monocyte levels may be indicating a possible Covid-19 pneumonia.

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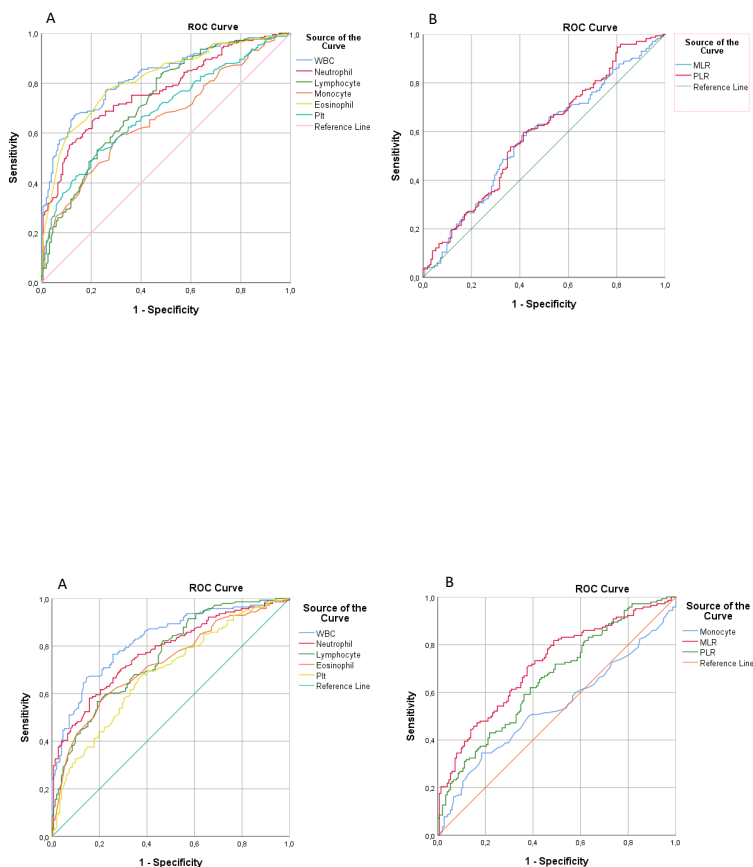
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Figure 1: The ROC curve of White Blood Cell Count, Neutrophil, Lymphocyte, Monocyte, Eosinophil, Platelets (Plt) (Figure 1-A), MLR and PLR (Figure 1-B). for discriminating between Covid-19 patients with pneumonia and healthy groups.

Figure 2: The ROC curve of White Blood Cell Count (WBC) Neutrophil, Lymphocyte, Eosinophil and Platelets for discriminating between Covid-19 patients without pneumonia and healthy group.



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4.Tables.docx available at <https://authorea.com/users/732502/articles/710827-diagnostic-value-of-monocyte-eosinophil-platelet-lymphocyte-ratio-and-monocyte-lymphocyte-ratio-in-previously-healthy-covid-19-patients>