Received: 3 May 2021 | Revised: 2 September 2021 | Accepted: 20 September 2021

DOI: 10.1111/ijlh.13728

ISLH International Journal of Laboratory Hematology WILEY

ORIGINAL ARTICLE

Diagnostic value of white blood cell parameters for COVID-19: Is there a role for HFLC and IG?

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Abstract

Introduction: As the Coronavirus disease 2019 (COVID-19) pandemic is still ongoing with patients overwhelming healthcare facilities, we aimed to investigate the ability of white blood cell count (WBC) and their subsets, high fluorescence lymphocyte cells (HFLC), immature granulocyte count (IG), and C-reactive protein (CRP) to aid diagnosis of COVID-19 during the triage process and as indicators of disease progression to serious and critical condition.

Methods: We collected clinical and laboratory data of patients, suspected COVID-19 cases, admitted at the emergency department of University General Hospital of Ioannina (Ioannina, Greece). We selected 197 negative and 368 positive cases, confirmed by polymerase chain reaction test for severe acute respiratory syndrome coronavirus 2. COVID-19 cases were classified into mild, serious, and critical disease. Receiver operating characteristic curve and binary logistic regression analysis were utilized for assessing the diagnosing ability of biomarkers.

Results: WBC, neutrophil count (NEUT), and HFLC can discriminate efficiently negative cases from mild and serious COVID-19, whereas eosinopenia and basopenia are early indicators of the disease. The combined WBC-HFLC marker is the best diagnostic marker for both mild (sensitivity: 90.6% and specificity: 64.1%) and serious (sensitivity: 90.3% and specificity: 73.4%) disease. CRP and Lymphocyte count are early indicators of progression to serious disease whereas WBC, NEUT, IG, and neutrophil-to-lymphocyte ratio are the best indicators of critical disease.

Conclusion: Lymphopenia is not useful in screening patients with COVID-19. HFLC is a good diagnostic marker for mild and serious disease either as a single marker or combined with WBC whereas IG is a good indicator of progression to critical disease.

KEYWORDS

biomarkers, COVID-19, diagnosis, leukocytes, statistic

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first discovered in December 2019 in Wuhan, China. Since then, the world has been caught up in one of the deadliest pandemics in history with SARS-CoV-2 having spread to over 210 countries, resulting in more than 150 million confirmed cases and more than 3 million deaths as of 3 May 2021.[1] Furthermore, the increased transmissibility of SARS-CoV-2 variants has raised the concern for the high admittance rate to the emergency department and the resulting load on public health systems.[2]

Under these circumstances, patients with clinical signs of infection (eg, fever, cough etc) presenting at the emergency department

of a hospital are treated as suspected COVID-19 patients. Diagnosis of COVID-19 patients usually relies on RT-PCR real-time polymerase chain reaction (RT-PCR) tests, which can be time-consuming. On the other hand, the availability of rapid PCR testing may be restricted. Given the possible consequences from the delayed diagnosis and quarantine of SARS-CoV-2-positive patients, triage at the emergency department has become a formidable task.

The complete blood count (CBC) may offer valuable information, indicative of a possible SARS-CoV-2 infection, thus assisting clinicians in making decisions at the time of admission. Several studies have demonstrated the decrease in leukocytes and their subpopulations in COVID-19 patients compared to healthy individuals and non-COVID-19 patients with other infectious diseases. Therefore, decrease in neutrophil count, lymphopenia, and eosinopenia are the most common markers suggested for the identification of COVID-19 patients. However, most of the reports regarding diagnostic value of hematologic parameters for COVID-19 refer mainly to comparison between the control group and COVID-19 patients[3-6] whereas few studies have evaluated their performance as diagnostic markers in the emergency room.[7-9]

On the other hand, there is a great load of data for the utilization of hematologic parameters for the diagnosis of progression to serious or severe disease and their performance as prognostic markers in COVID-19 patients. The most common hematologic parameters derived from CBC with evidenced prognostic value include the following: neutrophil count,[10] lymphocyte count,[11,12] neutrophil-to-lymphocyte ratio (NLR),[13,14] and platelet-to-lymphocyte ratio (PLR).[14,15]

We sought to determine the performance of parameters of white blood cells and their subpopulations as well as their combinations for the diagnosis of COVID-19 and as indicators of disease progression to serious and critical condition. Since the blood cell parameters may depend on the stage of the disease, we classified patients according to their clinical condition into three groups: patients with mild, serious, and critical disease. In this way, we anticipated to discover early diagnostic markers for COVID-19 disease with the potential to optimize the triage process as well as early indicators of disease progression in order to aid clinicians in the management of patients in need of close monitoring for developing serious and/or critical condition. In addition to the parameters of CBC, we also examined other known markers used as indicators of systematic inflammatory response such as NLR, PLR, and lymphocyte-to-monocyte ratio (LMR)[14] as well as high fluorescence lymphocyte cells (HFLC) and Immature Granulocyte count (IG).

HFLC are lymphoplasmacytoid cells or plasma cells present in the blood of patients as a response of the innate immunity to infectious disease.[16] Their detection is based on their characteristical high fluorescence intensity and their count is reported by modern automated hematology analyzers as part of the full blood count. HFLC are elevated in COVID-19 patients and are further increased in severe disease.[17] On the other hand, immature granulocytes in the peripheral blood can occur in response to infection, inflammation, or other cause of bone marrow stimulation. Both HFLC and IG have been thoroughly investigated as potential markers of sepsis.[18,19] C-reactive protein (CRP), a well-known inflammation and disease progression marker, was also included in the study.[7] In this respect, we explored their potential role in the diagnosis of COVID-19 as standalone markers and in combination with other parameters of the CBC.

2 | MATERIALS AND METHODS

2.1 | Patients

This is a retrospective case-control study conducted from 14 March 2020 to 6 March 2021, with data collected from patients admitted at the emergency department of University General Hospital of Ioannina (Ioannina, Epirus, Greece). Due to the low prevalence of COVID-19 disease in our country from March to October 2020, we had to extend the time of data collection to March 2021 in order to include as many COVID-19 patients as possible. All patients who presented at the emergency department with fever and/or respiratory symptoms were suspected for COVID-19 infection, and their nasopharyngeal swab specimens were tested for SARS-CoV-2 with real-time polymerase chain reaction (RT-PCR). 197 patients who tested negative in RT-PCR were selected as the control group (negative cases). Negative cases discharged to home were considered as mild negative cases (control 1, n = 103) while negative cases admitted to general ward were classified as serious negative cases (control 2, n = 94).

The clinical evaluation and management of SARS-CoV-2-positive patients were performed according to the Guidelines of the National Institute of Public Health of Greece.[21] COVID-19 patients were classified according to their clinical condition as evaluated at the emergency department into three groups defined as following: mild disease: discharged to home, serious disease: hospitalized in general ward and severe/critical disease: admitted to intensive care unit (ICU). Patients initially admitted to general ward and later transferred to ICU (n = 23) were included in the critical group; CBC on admission to ICU was used in this case.

Only adult patients were included in the study whereas patients with conditions associated with abnormal blood cell counts as hematological malignancies, metastatic bone marrow infiltration by malignancy, receiving chemotherapy or immunosuppressive therapy (n = 13) were excluded from the study. We also excluded patients without CBC on admission (n = 3) as well as patients transferred from another ward (n = 7). A total of 368 COVID-19 cases were included in the study and were classified as having mild (n = 96), serious (n = 215), and critical (n = 57) disease (Figure 1).

2.2 | Data collection and management

Demographic data and clinical symptoms and signs were obtained from electronic medical records. The complete blood count and the extended parameters HFLC and IG on day of admission were

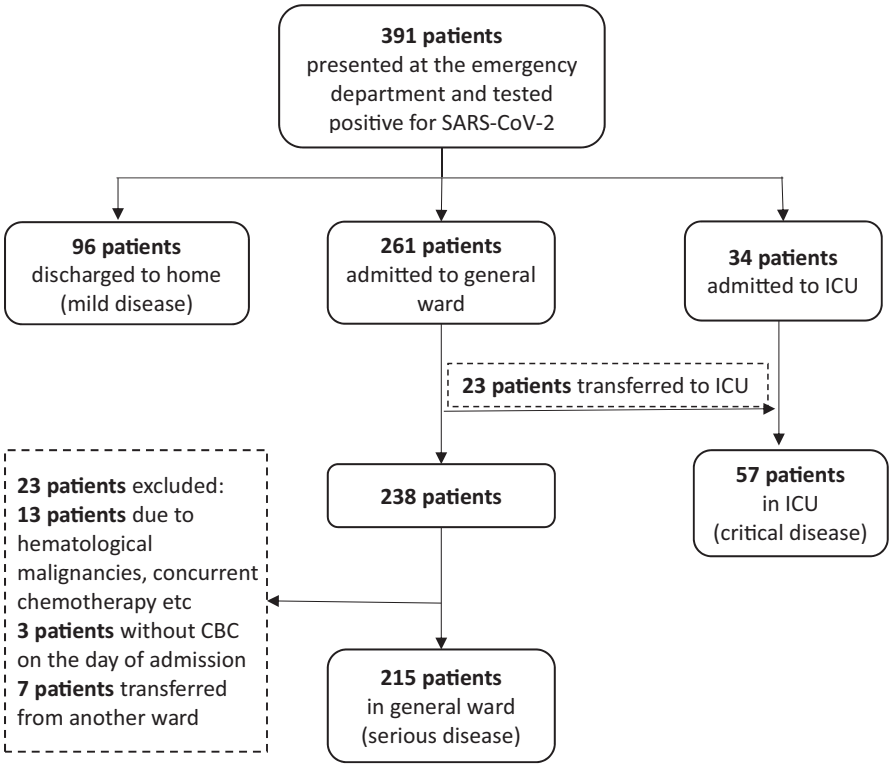

FIGURE 1 Patient flow chart

measured on Sysmex XN-3100 (Sysmex, Japan). CRP measurements were obtained from the hospital Laboratory Information System (LIS). RT-PCR test was performed on Xpert Xpress SARS-COV-2 (Cepheid AB).

2.3 | Study design and statistical analysis

The study of CBC parameters as diagnostic markers of COVID-19 comprised of two parts. In the first part, their ability to discriminate between negative and positive cases was tested separately for mild (mild negative cases vs mild positive cases) and serious (serious negative cases vs serious positive cases) disease. In the second part, biomarkers were tested as potentials indicators of COVID-19 disease progression. For this purpose, their ability to discriminate between mild and serious (mild positive cases vs serious positive cases) as well as between serious and critical disease (serious positive cases vs critical positive cases) was examined.

Continuous variables were expressed as medians and interquartile ranges whereas categorical variables were expressed as the counts and percentages in each category. Non-parametric Mann-Whitney test was used for testing the significance between two groups. Receiver operating characteristic (ROC) curve analysis was applied for the selection of parameters with high diagnostic performance. The area under the curve (AUC) was used as a measure of performance, and parameters with AUC>0.7 were selected for the multivariable analysis. Keeping in mind the impact of false negatives in the case of COVID-19, the selection of best cutoff values was based initially on Youden index and with a focus on maximizing sensitivity.

Enter binary logistic regression analysis was conducted to determine the influence of the parameters on the outcome and in developing pairwise combinations for different parameters. The comparison of AUC between pairwise combinations and individual parameters indicated whether there was improvement in the discriminatory power. Furthermore, Nagelkerke R and Akaike information criteria (AIC) were used for the assessment of the goodness of fit of all pairwise combinations, with lower AICs indicating better model fit. Hosmer and Lemeshow test was used for the calibration of the method.

MedCalc Statistical Software version 19.2.6 (MedCalc Software Ltd,; https://www.medcalc.org; 2020) was used for ROC curve analysis and comparison of ROC curves (z-statistic). Logistic regression analysis, Pearson correlation, and calculation of variation inflation factors (VIF) were conducted using SPSS 23.0 (IBM Corp). A 2-tailed *P* value <.05 was considered as statistically significant. Graphs were plotted using GraphPad Prism 6.00 (GraphPad Software).

3 | RESULTS

3.1 | Patients

The basic demographic data and laboratory findings of all groups of patients are summarized in Table 1 (Comorbidities are given in Table S1). Patients with mild disease have significantly lower WBC

TABLE 1 Basic demographic characteristics and blood biomarkers on admission of patients with and without COVID-19

	Negative cases		Positive cases		
	Mild	Serious	Mild disease	Serious Disease	Critical disease
Patients, n	103	94	96	215	57
Male, n (%)	54 (52.4%)	49 (52.1%)	47 (49.0%)	122 (56.7%)	47 (82.4%)
Female, n (%)	49 (47.6%)	45 (47.9%)	49 (51.0%)	93 (43.3%)	10 (17.6%)
Age, Years, median (IQR)	45 (33-61)	76 (52-86)	50 (37-61)	66 (54-81)	68 (60-86)
White blood cells (10^9/L) findings[a,b]					
WBC (Ref: 5.47-9.72)[c]	9.03 (6.97-11.69)	9.46 (7.43-12.51)	5.72 (4.58-7.23)	5.76 (4.58-7.64)	12.54 (8.62-16.03)
Normal or decreased WBC (≤9.72)	66 (64.1%)	49 (52.1%)	89 (92.7%)	191 (88.8%)	20 (35.1%)
NEUT (Ref: 2.32-5.65)	6.14 (4.33-8.48)	8.00 (5.29-10.66)	3.70 (2.54-4.99)	3.93 (2.80-6.04)	11.11 (7.57-15.09)
Decreased NEUT (<2.32)	2 (1.9%)	1 (1.1%)	16 (16.7%)	30 (14.0%)	1 (1.8%)
LYMPH (Ref: 1.41-3.36)	1.79 (1.33-2.39)	1.06 (0.60-1.40)	1.43 (1.14-1.92)	0.99 (0.73-1.50)	0.61 (0.44-0.94)
Decreased LYMPH (<1.41)	28 (27.2%)	71 (75.5%)	43 (44.8%)	154 (71.6%)	52 (91.2%)
MONO (Ref: 0.29-0.80)	0.58 (0.43-0.81)	0.54 (0.35-0.70)	0.45 (0.30-0.60)	0.20 (0.10-0.20)	0.30 (0.20-0.60)
Decreased MONO (<0.29)	7 (6.8%)	16 (17.0%)	15 (15.6%)	56 (26.0%)	20 (35.1%)
EOS (Ref: 0.03-0.47)	0.07 (0.03-0.14)	0.03 (0-0.08)	0.02 (0.01-0.02)	0 (0-0.03)	0 (0-0.03)
Decreased EOS (<0.03)	20 (19.4%)	45 (47.9%)	55 (57.3%)	156 (72.6%)	42 (73.7%)
BASO (Ref: 0.01-0.10)	0.03 (0.02-0.05)	0.02 (0.01-0.04)	0.02 (0.01-0.02)	0.01 (0.01-0.02)	0.02 (0.01-0.04)
Decreased BASO (<0.01)	1 (1.0%)	6 (6.4%)	4 (4.2%)	19 (8.8%)	6 (10.5%)
IG (Ref: 0.01-0.08)	0.02 (0.01-0.04)	0.04 (0.02-0.08)	0.02 (0.01-0.02)	0.02 (0.02-0.05)	0.11 (0.08-0.24)
Increased IG (>0.08)	4 (3.9%)	23 (24.5%)	2 (2.08%)	20 (9.3%)	43 (75.4%)
HFLC (Ref: 0.01-0.03)	0.01 (0-0.01)	0 (0-0.01)	0.02 (0.01-0.04)	0.02 (0.01-0.04)	0.04 (0.03-0.07)
Increased HFLC (>0.03)	6 (5.8%)	1 (1.1%)	30 (31.2%)	68 (31.6%)	32 (56.1%)
CRP (Ref: 0-6)	13 (4-62)	78 (21-176)	10 (5-22)	44 (13-93)	125 (65-201)
Increased CRP (>6)	68 (66.0%)	87 (93.5%)	61 (63.5%)	188 (87.4%)	51 (89.5%)
NLR	3.6 (2.1-5.7)	8.0 (4.8-14.4)	2.4 (1.7-3.4)	4.0 (2.2-7.1)	16.7 (10.5-26.6)
LMR	2.7 (1.8-4.9)	2.0 (1.3-3.1)	3.6 (2.3-4.6)	3.1 (2.0-4.4)	1.9 (1.4-3.2)
PLR	130.1 (98.17-190.5)	227.6 (136.7-325.0)	140.5 (114.0-175.9)	187.1 (133.3-291.5)	417.0 (241.9-720.2)

Abbreviations: BASO, Basophil count; CRP, C-reactive protein; EOS, Eosinophil count; HFLC, High Fluorescence Lymphocyte Cells; IG, Immature Granulocyte count; LMR, Lymphocyte-to-Monocyte Ratio; LYMPH, Lymphocyte count; MONO, Monocyte count; NEUT, Neutrophil count; NLR, Neutrophil-to-Lymphocyte Ratio; PLR, Platelet to Lymphocyte Ratio; WBC, White Blood Cell count.

[a]Data presented as n (%) and median (IQR).

[b]Units are mg/L for CRP and 10^9/L for the rest of the parameters.

[c]Reference ranges as reported for Sysmex XN [20].

(P <.0001), NEUT (P <.0001), LYMPH (P =.001), MONO (P <.0001), EOS (P <.0001), BASO (P <.0001), and NLR (P <.0001) compared with negative control. On the other hand, there is no significant difference for CRP (P =.1278), LMR (P = 00 549), and PLR (P =.3768) between negative group and mild disease. In the case of serious disease, significantly lower values are observed for WBC (P <.0001), NEUT (P <.0001), MONO (P =.0001), EOS (P <.0001), BASO (P <.0001), NLR (P <.0001), LMR (P <.0001), and CRP (P =.0001) but not for LYMPH (P =.7884) and PLR (P =.1166).

Progression of disease from mild to serious is accompanied by significant decrease of LYM (P <.0001), MONO (P =.0007), EOS (P <.0001), BASO (P =.0038), and increase in NEUT (P =.0346), NLR (P <.0001), PLR (P <.0001), and CRP (P <.0001). Also, progression from serious to critical disease results in significant increase in WBC (P <.0001), NEUT (P <.0001), BASO (P =.0012), NLR (P <.0001), PLR (P <.0001), and CRP (P <.0001) whereas LYMPH (P <.0001) and LMR (P <.0001) are significantly reduced.

Both mild and serious disease patients have significantly lower IG count compared with the negative groups. However, significant increase in IG occurs when serious disease progresses to critical (P <.0001). HFLC is significantly higher for both mild and serious disease compared with the negative group. Progression of serious to critical disease results also in significant rise of HFLC.

3.2 | Hematologic parameters as diagnostic markers of COVID-19

Initially, we performed ROC curve analysis in order to select the best diagnostic markers among CBC parameters and CRP for mild disease (Table S2). The parameters with AUC>0.7 (WBC, NEUT, BASO, HFLC, and EOS) were included in the multivariable analysis, and the odds ratio (OR) for the odds of having mild COVID-19 disease was calculated by conducting logistic regression (Table 2). Because of the strong correlation between WBC and NEUT (Pearson r = 0.959, P <.0001) and the high VIF values for WBC and NEUT due to collinearity, the best full model could not include both WBC and NEUT. Therefore, the best fitting logistic model indicated that WBC and HFLC were independently associated with mild COVID-19 disease. Due to the low values observed for EOS, BASO, and HFLC, adjusted ORs have been calculated in order for the one-unit change of the predictor to be meaningful (see Table 2). Hence, an increase in 0.01 ($\times 10^9$/L) in the value of HFLC corresponds to 1.655 increase in odds of having mild COVID-19 disease.

The best performing logistic models of pairwise combinations of CBC parameters for the diagnosis of mild COVID-19 disease are summarized in Table 3. Significant difference for the combinations WBC-HFLC and WBC-EOS was evidenced by comparison of ROC curves to all standalone blood biomarkers. Also, the significant difference between WBC-HFLC and WBC-EOS (P =.0240, z = 2.257) indicates that the combination WBC-HFLC constitutes the best of all biomarkers.

In the case of serious disease, the best performing markers (AUC > 0.7) were WBC, NEUT, HFLC, and NLR. WBC had strong correlation with NEUT (Pearson r = 0.982, P <.0001) producing strong collinearity effects; thus, WBC was once more selected for the multivariable model. WBC, HFLC, and NLR are all independent predictors of serious COVID-19 disease (Table 2). Pairwise combinations of biomarkers were evaluated by logistic regression, and the results of the best performing pairs of CBC parameters are listed in Table 3. As revealed from the comparison of ROC curves, WBC-HFLC and NEUT-HFLC are the best combinations showing significant difference from all single markers.

TABLE 2 Multivariable logistic regression analysis for the diagnosis of mild and serious COVID-19 disease

Mild disease			
Variables[a]	***P* value**	**Odds ratio (OR)**	**95% CI**
WBC	<.0001	0.638[b]	0.531-0.767
EOS	.632	0.899[c]	0.582-1.388
BASO	.110	0.832[d]	0.665-1.042
HFLC	<.0001	1.655[d]	1.344-2.036
Serious disease			
WBC	<.0001	0.673[b]	0.595-0.761
NLR	.189	1.025	0.988-1.062
HFLC	<.0001	2.729[d]	2.002-3.721

[a]See Table 1 for abbreviations.

[b]OR is calculated as the change in odds of having COVID-19 upon 1 unit ($\times 10^9$/L) increase.

[c]Adjusted OR calculated as the change in odds of having COVID-19 upon 0.1 unit ($\times 10^9$/L) increase.

[d]Adjusted OR calculated as the change in odds of having COVID-19 upon 0.01 unit ($\times 10^9$/L) increase.

3.3 | Hematologic parameters as indicators of COVID-19 disease progression

The univariable analysis indicated LYMPH and CRP as good indicators of progression from mild to serious COVID-19 disease (Table S3). Furthermore, multivariable analysis revealed that both markers are independent indicators of progression of mild to serious disease (Table 4). The logistic model of their combination failed the goodness of fit test (Hosmer and Lemeshow test <0.05).

ROC curve analysis highlighted several parameters for the indication of progression from serious to critical illness (Table S3). Therefore, NLR (AUC: 0.911), IG (AUC: 0.890), NEUT (AUC: 0.884), and WBC (AUC: 0.854) presented excellent performance whereas PLR (AUC: 0.806), CRP (AUC: 0.743), and LYMPH (AUC: 0.742) were also good indicators of critical disease. Due to collinearity effects, WBC and NEUT could not be included simultaneously in the full logistic model. Hence, logistic regression of the full model revealed that mostly WBC is an independent factor for progression to critical COVID-19 disease while LYMPH and PLR displayed borderline significance (Table 4). Respectively, the equivalent full logistic model including NEUT instead of WBC exhibited similar results with NEUT being also an independent variable. Logistic regression of pairwise combination of blood biomarkers yielded several combined markers with high performance: NEUT-PLR (AUC: 0.924), WBC-PLR (AUC: 0.923), HFLC-NLR (AUC: 0.918), NEUT-NLR (AUC: 0.912), WBC-NLR (AUC: 0.912), NEUT-LYM (AUC: 0.910), WBC-LYM (AUC: 0.910), NEUT-CRP (AUC: 0.898), NEUT-IG (0.890), NEUT-HFLC (AUC: 0.887), and WBC-CRP (AUC: 0.888). Interestingly, none of these combinations has statistically significant difference from the best performing single markers NLR, IG, WBC, and NEUT. Furthermore, comparison of ROC curves reveals that the AUC of the best performing parameter, NLR, does not differ significantly from the closely following parameters IG (P =.3331, z = 0.968), WBC (P =.0630, z = 1.859), and NEUT (P =.28902, z = 1.080). Consequently, standalone CBC parameters can be utilized sufficiently as diagnostic markers of progression from serious to critical disease and the combination of blood biomarkers does not contribute anything to their diagnostic value.

4 | DISCUSSION

Several studies have illustrated the utility of routine blood tests performed upon admittance of patients to the hospital for the

TABLE 3 Best performing pairwise combinations of CBC parameters for the diagnosis of mild and serious COVID-19 disease

Combination	Sensitivity (%)	Specificity (%)	Nagelkerke R	AIC	AUC (95% CI)
Mild disease					
WBC (≤7.8), HFLC (>0.01)	90.6	64.1	0.517	182.5	0.869 (0.814-0.912)
NEUT (≤5.67), HFLC (>0.01)	89.6	60.2	0.476	192.4	0.848 (0.790-0.895)
WBC (≤8.44), EOS (<0.05)	89.6	66.0	0.403	210.1	0.827 (0.768-0.877)
WBC (<8.88), BASO (≤0.02)	88.5	60.2	0.396	208.7	0.825 (0.765-0.875)
NEUT (<6.67), BASO (≤0.02)	80.5	60.2	0.383	211.4	0.821 (0.760-0.871)
Serious disease					
WBC (≤8.51), HFLC (>0.01)	90.3	73.4	0.575	219.8	0.914 (0.876-0.943)
NEUT (≤6.97), HFLC (>0.01)	90.3	70.2	0.560	226.7	0.908 (0.870-0.938)
NLR (≤14.84), HFLC (>0.01)	86.4	61.7	0.410	275.4	0.856 (0.811-0.894)

Note: WBC, NEUT, EOS, BASO, HFLC, and NLR: See Table 1 for abbreviations. Cutoff values (10^9/L) are shown in parenthesis.
Abbreviations: AIC, Akaike Information Criteria; AUC, Area Under the Curve.

diagnosis of COVID-19. Most of them have underlined the importance of lymphopenia and eosinopenia present upon admittance of patients with COVID-19.[5,7,22,23] In our study, no significant difference in the lymphocyte count was observed between the negative group and serious COVID-19 disease. With an AUC of 0.635 in the case of mild disease and 0.510 for serious disease, lymphocyte count constitutes a diagnostic biomarker of low efficacy. The observed difference in the lymphocyte subset compared with other studies can be attributed to the different study design. In most cases, patients with mild symptoms are not included or not examined separately whereas the control group in some cases is comprised of healthy individuals and not patients with infectious disease. Furthermore, lymphopenia is often associated with other causes such as congenital immunodeficiency disorders, malnutrition, alcohol abuse, medications, malignancies, systemic autoimmune diseases, and (bacterial or viral) infections resulting in increased risk of hospitalization with infection.[24] Hence, this fact may account for the higher frequency of patients with lymphopenia in the groups with serious condition and in need for hospitalization, thus explaining the absence of significant difference in lymphocytes between negative and positive serious cases. On the other hand, in the case of mild disease, lymphopenia is more pronounced for the positive group, characterizing the early stages of COVID-19 disease in contrast to other infectious diseases.

On the other hand, decrease in the eosinophil count is prominent for the mild disease group, present in 57.3% of patients compared with 19.4% of patients of the negative group and even more frequent in patients with serious disease reaching 72.6%. ROC curve analysis revealed medium performance for the eosinophil count (AUC: 0.659) compared with other blood biomarkers for the diagnosis of COVID-19 serious disease.

The diagnostic ability of leukocyte and neutrophil count for COVID-19 has been highlighted in several different studies.[6,7,9,25] Indeed, WBC and NEUT were significantly lower in mild and serious disease compared with the negative group and were both independent determinants of COVID-19 disease. WBC and NEUT, both displayed high efficiency in the diagnosis of mild and serious COVID-19 disease either as single or in combination with other parameters of the CBC.

NLR has been proposed as a possible sufficient diagnostic marker for the COVID-19 disease.[6,9,14] In our study, NLR is a diagnostic marker of medium performance (AUC: 0.656 for mild disease and 0.719 for serious disease).

The performance of basophil count as an indicator of COVID-19 disease is surprisingly high. The basophil count depletion is observed early in the course of COVID-19 disease following the trend in decrease of all white blood cell subsets. Based on the fact that basophil count is generally low, even in healthy individuals, concerns were raised about its variability not being specific to a certain pathological condition.[3] Consequently, it was assumed that basophils may not be implicated in the COVID-19 pathogenesis and diagnosis. However, recent findings have suggested that basophils have an immune regulatory function both in innate and adaptive immune response.[26-28] By comparing mostly mild cases of COVID-19 patients with other pulmonary infection patients, J. Dai et al found that among other CBC parameters, basophil count and proportion were the most discriminant biomarkers.[29] On the other hand, a protective role of high basophil count against developing severe disease was recently proposed and a causal association between basophil count and the risk of COVID-19 and susceptibility was evidenced whereas the same association was not confirmed for lymphocytes and eosinophils.[30] In light of these findings, it is not surprising that basophil depletion may serve as an early marker for the diagnosis of COVID-19. Our observations are indicative of high potency for basophil count as a diagnostic marker of mild COVID-19 disease and its combination with WBC results in a combined marker with sensitivity 88.5% and specificity 60.2%.

HFLC count is found to be elevated in COVID-19 patients[17] and is further increased upon progression of disease, especially in the second week of illness concurring with the presence in serum of anti-SARS-CoV-2-specific antibodies.[31] The increase in HFLC also correlates with worsening of clinical condition, especially in the case

TABLE 4 Age- and gender-adjusted odds ratios of CBC parameters and CRP for the diagnosis of COVID-19 progression in the case of mild and serious disease

Mild disease			
Variables[a]	***P* value**	**Odds ratio**	**95% CI**
LYMPH	.006	0.522[b]	0.327-0.832
CRP	<.0001	1.021[c]	1.011-1.031
Serious disease			
WBC	<.0001	1.417[b]	1.189-1.688
LYMPH	.032	0.103[b]	0.013-0.819
IG	.700	1.009[d]	0.966-1.053
HFLC	.080	1.101[d]	0.988-1.228
CRP	.107	1.004[c]	0.999-1.009
NLR	.087	0.954	0.904-1.007
PLR	.033	1.003	1.000-1.005

[a]See Table 1 for abbreviations.

[b]OR is calculated as the change in odds of having serious COVID-19 upon 1 unit ($\times 10^9$/L) increase.

[c]OR is calculated as the change in odds of having serious COVID-19 upon 1 unit (mg/L) increase.

[d]Adjusted OR calculated as the change in odds of having serious COVID-19 upon 0.01 unit ($\times 10^9$/L) increase.

of cytokine storm syndrome.[32] In our study, HFLC count is significantly increased and is independently associated with mild and serious COVID-19 disease. The most important finding is that HFLC can be utilized for the diagnosis of both conditions either as a single marker or in combination with WBC, a superior marker compared with all single and other combined markers (Tables S2 and S3). As a marker of progression to critical disease, HFLC count is of moderate efficacy (AUC: 0.710).

Immature granulocyte count has been proposed as a predictor of sepsis[19,33] and a marker of acute respiratory distress syndrome.[34] Furthermore, increase in neutrophil precursors is highly associated with severe COVID-19.[35,36] Our results indicate low IG count in all COVID-19 patients and medium performance as a diagnostic marker (Table S2). Interestingly, IG can be a very useful indicator of critical disease (AUC: 0.890, sensitivity: 86%, specificity: 83% at cutoff: >0.05), having no statistical difference from the other three excellent markers NLR, WBC, and NEUT.

The most significant markers proposed in literature as predictors of COVID-19 disease severity are CRP,[37,38] white blood count,[10,37,38] neutrophil count,[10,37] lymphocyte count,[12,37] NLR,[13,14] and PLR.[14,15] Our findings are in good agreement with previous studies. CRP and LYMPH are independently associated with progression from mild to serious disease, and they can both be used efficiently as indicators of serious disease. For critical disease, the AUC we found for NLR is 0.911 which is comparable with 0.90 reported in a meta-analysis conducted by Li et al[13] and 0.841 reported by Yang et al[14] Furthermore, there was good correlation for the AUC found for CRP (0.743) and PLR (0.806) compared with 0.714 and 0.784, respectively, found by Yang et al[14] Similarly, we concluded that NLR is a superior marker compared with PLR.[14] The added value from our observations in the area of diagnostic markers of COVID-19 disease severity is the addition of two more good predictors of severe disease, IG, and HFLC, and the comparative evaluation of biomarker performance. Consequently, NLR, WBC, NEUT, and IG are the most efficient indicators of critical disease, followed by PLR, LYMPH, CRP, and HFLC.

Our study has some limitations. First of all, it is a retrospective study conducted in a single clinical center. More valuable information could be gained from a multi-center study. Second, due to time limitations, the size for mild and critical disease groups is much smaller compared with the negative and serious disease groups. Finally, decisions about the clinical condition of patients are based on expert's opinion which may introduce some bias in the classification of patients.

Conclusively, it is apparent from our study that lymphopenia is not an efficient marker for the discrimination of COVID-19 patients from negative cases. On the other hand, eosinophil and basophil depletion are good indicators of COVID-19 at the early stages of the disease. HFLC is a potent marker for the diagnosis of mild and serious COVID-19 either as a single marker or combined with leukocyte count whereas IG shows excellent performance as an indicator of COVID-19 disease progression from serious to critical condition.

CONFLICT OF INTEREST

The authors have no competing interests.

ETHICAL APPROVAL

Due to the retrospective and non-interventional nature of the study, informed consent was not required. The study was approved by the review board of University General Hospital of Ioannina (registration number: 10/26-05-2021).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Myari A, Papapetrou E, Tsaousi C. Diagnostic value of white blood cell parameters for COVID-19: Is there a role for HFLC and IG? *Int J Lab Hematol*. 2022;44:104–111. https://doi.org/10.1111/ijlh.13728