

ORIGINAL ARTICLE

Evaluation of Routine Blood Tests for Diagnosis of Suspected Coronavirus Disease 2019

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SUMMARY

Background: Real-time reverse transcription polymerase chain reaction assay (RT-PCR) is the gold standard for diagnosis of coronavirus disease 2019 (COVID-19); however, it is not universally available and may have limitations in response times. The aim was to evaluate the routine blood tests for diagnosis of COVID-19, determining the diagnostic accuracy of blood biomarkers to differentiate between patients with and without COVID-19.

Methods: Clinical charts, nursing records, laboratory findings, and chest x-rays from adult patients with clinical suspicion of COVID-19 (fever, cough and/or dyspnea) at hospital admission were reviewed. Patients were classified into two groups according to RT-PCR COVID-19: positive (COVID-19) or negative (NON-COVID-19). Diagnostic accuracy was determined by analyzing receiver operating characteristic (ROC) curve, calculating the area under the ROC curve (AUC) and the cutoff value. In order to reduce the number of false positives, the cutoff value with a specificity of 80% was considered.

Results: Two hundred three patients (101 females, 102 males) with ages between 18 and 96 years (mean = 61.3) were studied. Ninety-four were COVID-19 and 109 were NON-COVID-19. Plasma ferritin level was the most accurate biomarker (AUC = 0.847 and 0.804 in women and men, respectively). The following diagnostic criteria for suspected COVID-19 were established with biomarker cutoff values to differentiate between COVID-19 and NON-COVID-19 patients: lymphocytes $\leq 1.0 \times 10^9/L$; eosinophils $\leq 0.02 \times 10^9/L$; ferritin $> 125\%$ of upper reference limit (URL); LDH $> 125\%$ of URL; hsCRP > 80 mg/L; and D-dimer > 1.2 mg/L. Sensitivity was 66%, 64%, 62%, 46%, 43%, and 33% for ferritin, eosinophils, LDH, hsCRP, lymphocytes, and D-dimer, respectively. Of those determined to be COVID-19 patients, 91% met one or more of the diagnostic criteria with these blood biomarkers, and of the NON-COVID-19 patients, 47% did not meet any diagnostic criteria.

Conclusions: Blood counts of lymphocytes and eosinophils, and plasma levels of D-dimer, LDH, hsCRP, and ferritin can be used to differentiate patients with and without COVID-19 and as a tool for diagnosis of suspected COVID-19 in adult patients at hospital admission.

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KEY WORDS

coronavirus disease 2019, lymphocytes, eosinophils, ferritin, lactate dehydrogenase, C-reactive protein, D-dimer

INTRODUCTION

Coronavirus disease 2019 (COVID-19) or the severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) emerged in December 2019 in Wuhan, Hubei Prov-

ince, China [1]. On January 30, 2020, the World Health Organization declared a Public Health Emergency, and on March 11, 2020, the pandemic [2]. COVID-19 can be asymptomatic, with a mild upper respiratory tract symptom or produce severe disease characterized by interstitial pneumonia and severe acute respiratory syndrome with multiple organ dysfunction and death [3,4]. Common clinical manifestations are fever and cough, which may be accompanied by severe symptoms such as respiratory distress or dyspnea [3-6]. Chest radiograph and computed tomography (CT) can show bilateral pulmonary ground-glass infiltrates, although these findings lack specificity, and many patients with COVID-19 present normal radiological study, especially in early stages of the disease [7,8]. Real-time reverse transcription polymerase chain reaction assay (RT-PCR) is the gold standard for diagnosis of COVID-19 in samples of nasopharyngeal exudate or respiratory tract; however, it is not universally available and may have limitations in response times [8]. A previous paper identifies some routine blood test as a potential diagnostic tool for COVID-19 [9]. Other studies have observed a decreased lymphocyte count (lymphopenia) and an increase of some plasma biochemical parameters (transaminases, lactate dehydrogenase (LDH), high sensitivity C-reactive protein (hsCRP), ferritin, and D-dimer) in COVID-19 patients [4-6,9-14]. However, there are no published studies that determine diagnostic accuracy and cutoff value of blood biomarkers to differentiate patients with and without COVID-19 at hospital admission. The aim of this study was to evaluate the following routine blood tests: red blood cells (RBC), hemoglobin, hematocrit, white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelets, D-dimer, creatinine, sodium, potassium, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), lactate dehydrogenase, creatine kinase (CK), high sensitivity C-reactive protein, and ferritin for diagnosis of COVID-19 in adult patients at hospital admission, determining the diagnostic accuracy and the cutoff value of these blood biomarkers to differentiate between patients with and without COVID-19.

MATERIALS AND METHODS

Study design and patients

This is a descriptive cross-sectional diagnostic study carried out at Virgen del Rocio University Hospital (Seville, Spain), and it adhered to the ethical recommendations of the Declaration of Helsinki [15]. The study was approved by the Research Ethics Committee of Virgen Macarena and Virgen del Rocio University Hospitals (0938-N-20). All participants signed an informed consent form.

White patients treated from March 26 to April 8, 2020 at Virgen del Rocio University Hospital were studied. The following inclusion criteria were used: patients

over the age of 18 years; of both genders; with clinical suspicion of COVID-19 for presenting at least one of the following symptoms: fever, cough and/or dyspnea; with COVID-19 RT-PCR in nasopharyngeal exudate sample and all routine blood tests of this study; and with blood and nasopharyngeal samples, were obtained on the same day of hospital admission. Exclusion criteria were: pregnant women; immunosuppressed patients; patients with chronic kidney disease, cirrhosis or other chronic diseases that could affect any of the blood biomarkers included in this study; hemolyzed blood samples; and patients with recent surgical interventions (less than 6 months). Included patients were classified into two groups according to the result of the COVID-19 RT-PCR: positive (COVID-19) or negative (NON-COVID-19).

Data collection

Clinical charts, nursing records, laboratory findings, and chest x-rays of adult patients with clinical suspicion of COVID-19 at hospital admission, who met all inclusion criteria and none of the exclusion, were reviewed. The following clinical data and complementary tests performed at hospital admission were collected: a) Demographic data: age and gender; b) Personal history: diabetes, hypertension, cardiovascular disease (ischemic heart disease, valve disease, cardiomyopathies and arrhythmias) and pulmonary disease (asthma, pneumonitis or chronic bronchitis); c) Signs and symptoms: fever, cough, dyspnea and symptomatology period; d) Chest x-ray: presence of bilateral pulmonary infiltrates; e) Laboratory tests: routine blood tests (RBC, hemoglobin, hematocrit, WBC, neutrophils, lymphocytes, monocytes, eosinophils basophils, platelets, D-dimer, creatinine, sodium, potassium, total bilirubin, ALT, AST, GGT, LDH, CK, hsCRP, and ferritin) and COVID-2019 RT-PCR in nasopharyngeal samples. Two researchers also independently reviewed the data collection forms to double check the data collected.

Sample analysis

Blood and nasopharyngeal samples from each patient were obtained at hospital admission. Blood sample by venipuncture in three tubes with anticoagulants: one with EDTA for plasma cell count using the Sysmex XN-2000 analyzer (Sysmex, Kobe, Japan); another with sodium citrate to determine the plasma concentration of D-dimer in Sysmex CS-5100 analyzer (Sysmex, Kobe, Japan) by immunoassay; and another with lithium heparin for plasma analysis of biochemical parameters in Hitachi Cobas c 702 modular analyzer (Roche Diagnostics, Rotkreuz, Switzerland): creatinine, total bilirubin, AST, ALT, GGT, LDH and CK by photometry, sodium, and potassium by potentiometry, and hsCRP and ferritin by particle enhanced immunoturbidimetric assay. Nasopharyngeal exudate with throat swabs in Copan eNATTM molecular medium was used for RT-PCR for COVID-2019. Viral RNAs were extracted from samples using the MagNA Pure Compact RNA Isolation Kit

(Roche Diagnostics, Rotkreuz, Switzerland) and quantitative reverse transcription polymerase chain reaction was performed using the primers and probes targeting the *ORF1ab* and *N* genes of COVID-19 as recommended by Centers for Disease Control and Prevention, using a commercial kit specific for COVID-19 detection (VIASURE CerTest BIOTEC).

Sample size and statistical analysis

Data were processed using MedCalc 13.0 (MedCalc Software, Ostend, Belgium). The diagnostic accuracy was determined by receiver operating characteristic (ROC) curves, calculating the area under the ROC curve (AUC). The sample size required for the comparison of the expected AUC with the null hypothesis AUC was calculated. The sample size considered the level of significance and the power of the test [16]. The expected AUC was 0.800 and the null hypothesis AUC was 0.500 (without discriminant power). A minimum of 37 patients with COVID-19 and another 37 without COVID-19 for a level of significance < 0.05 and a test power < 0.10 were required. Statistical analysis of blood biomarkers was performed independently by gender, as some laboratory tests have different reference values for men and women. Therefore, a minimum of 148 patients divided equally by gender and results of the COVID-19 RT-PCR were required. Quantitative variables were analyzed with the D'Agostino-Pearson test to determine whether they followed a normal (Gaussian) distribution or not. For the descriptive analysis, the frequencies of qualitative variables were used; range, arithmetic mean, and standard deviation (SD) for normally distributed quantitative; and range, median and interquartile range (IQR) for non-Gaussian variables. Groups were compared using chi-squared test for qualitative variables; the Student's *t*-test for normally distributed quantitative variables; and using the Mann-Whitney U test for non-Gaussian variables. Blood biomarkers with mean or median values outside of reference values in COVID-19 patients and with statistically significant differences between COVID-19 and NON-COVID-19 patients were selected. These biomarkers were analyzed by ROC curves, calculating the AUC, and the optimal cutoff value, sensitivity and specificity. The cutoff value was determined by gender, checking the difference between men and women. In order to reduce the number of false positives, the cutoff value with a specificity of 80% was considered.

RESULTS

Patients

Population of study comprised 203 patients with ages between 18 and 96 years (mean = 61.3), 101 were females and 102 males, 94 patients were COVID-19 (16 patients were admitted to the intensive care unit) and 109 were NON-COVID-19 (55 patients with respiratory tract infection, 14 congestive heart failure, 13 bacterial

pneumonias, 8 febrile syndrome, 7 asthmatic exacerbation, 7 chronic obstructive pulmonary disease exacerbation, and 5 febrile syndrome with urinary infection). All quantitative variables in this study followed a non-Gaussian distribution except for age. Table 1 shows the demographic data, personal history, signs and symptoms, and chest x-ray results in patients with and without COVID-19 at hospital admission. There were statistically significant differences between the two groups of patients with age, personal history of cardiovascular disease, fever presence, symptomatology period, and bilateral pulmonary infiltrate presence on chest x-ray.

Laboratory test

Table 2 shows the results of the laboratory tests by gender. There were statistically significant differences between the two groups of patients with the WBC, lymphocytes, monocytes, eosinophils, basophils, platelets, AST, ALT, D-dimer, LDH, hsCRP, and ferritin, both men and women. However, in patients with COVID-19, only the median values of lymphocyte and eosinophil counts were below the lower reference limit, and the median values of ferritin, LDH, hsCRP, and D-dimer were greater than the upper reference limit (URL). The rest of the blood biomarkers had medians within the reference values. Lymphocytes, eosinophils, D-dimer, LDH, hsCRP, and ferritin were selected as blood biomarkers for the diagnosis of suspected COVID-19 and were analyzed using ROC curves to differentiate patients with and without COVID-19 in men and women (Figure 1). In order to reduce the number of false positives, the cutoff value with a specificity of 80% was considered. Table 3 shows the AUCs, cutoff values and sensitivities obtained with these blood biomarkers. The cutoff value of ferritin was higher in men than in women, and the cutoff values of lymphocytes, eosinophils, D-dimer, LDH, and hsCRP were similar in both genders. The cutoff values of ferritin and LDH were approximately 1.25 times (125%) their URL, in both female and male patients. The following diagnostic criteria for suspected COVID-19 in adult patients at hospital admission have been established with cutoff values obtained:

1. Blood lymphocytes counts $\leq 1.0 \times 10^9/L$.
2. Blood eosinophils counts $\leq 0.02 \times 10^9/L$.
3. Plasma D-dimer level $> 1.2 \text{ mg/L}$.
4. Plasma LDH level $> 125\%$ of URL.
5. Plasma hsCRP level $> 80 \text{ mg/L}$.
6. Plasma ferritin level $> 125\%$ of URL.

Table 4 shows the sensitivity and specificity of these criteria to differentiate between patients with and without COVID-19 at hospital admission. The combination of these six criteria showed a sensitivity of 91% and a specificity of 47%.

Table 1. Demographic data, personal history, signs and symptoms, and chest x-ray results in patients with and without COVID-19 (n = 203).

Demographic data	NON-COVID-19 patients (n = 109)	COVID-19 patients (n = 94)	p-value
Age (years)	Range: 18 - 96	Range: 32 - 93	p = 0.0141 ^b
	Mean (SD): 58.5 (18.7)	Mean (SD): 64.6 (14.9)	
Gender (n)	Female: 57 (52.3%)	Female: 44 (46.8%)	p > 0.05 ^a
	Male: 52 (47.7%)	Male: 50 (53.2%)	
Personal history			
Diabetes mellitus (n)	Yes: 23 (21.1%)	Yes: 17 (18.1%)	p > 0.05 ^a
	No: 86 (78.9%)	No: 77 (81.9%)	
Hypertension (n)	Yes: 45 (41.3%)	Yes: 46 (48.9%)	p > 0.05 ^a
	No: 64 (58.7%)	No: 48 (51.1%)	
Cardiovascular diseases (n)	Yes: 23 (21.1%)	Yes: 8 (8.5%)	p = 0.0220 ^a
	No: 86 (78.9%)	No: 86 (91.5%)	
Pulmonary disease (n)	Yes: 30 (27.5%)	Yes: 21 (22.3%)	p > 0.05 ^a
	No: 79 (72.5%)	No: 73 (77.7%)	
Signs/symptoms at hospital admission			
Fever (n)	Yes: 62 (56.9%)	Yes: 72 (76.6%)	p = 0.0050 ^a
	No: 47 (43.1%)	No: 22 (23.4%)	
Cough (n)	Yes: 88 (80.7%)	Yes: 73 (77.7%)	p > 0.05 ^a
	No: 21 (19.3%)	No: 21 (22.3%)	
Dyspnea (n)	Yes: 70 (64.2%)	Yes: 50 (53.2%)	p > 0.05 ^a
	No: 39 (35.8%)	No: 44 (46.8%)	
Symptomatology period (days)	Range: 1 - 30	Range: 1 - 30	p = 0.0141 ^c
	Median (IQR): 5 (4.3)	Median (IQR): 7 (6)	
Chest x-ray at hospital admission			
Bilateral pulmonary infiltrates (n)	Yes: 15 (13.8%)	Yes: 56 (59.6%)	p < 0.0001 ^a
	No: 94 (86.2%)	No: 38 (40.4%)	

^a Chi-square test; ^b Student's *t*-test; ^c Mann-Whitney U test.
SD - standard deviation, IQR - interquartile range.

DISCUSSION

Patients

According to published studies, mean or median age of patients with COVID-19 is variable (47 to 62 years); hypertension is the most frequent comorbidity in COVID-19 patients; and fever and cough are the most common clinical manifestations [3-6,9-14]. In this study, mean age of COVID-19 patients was 64 years, half of COVID-19 patients were hypertensive, 77% had fever and cough, and 50% had dyspnea (Table 1). Chest x-ray and CT can have a high sensitivity for the diagnosis of COVID-19, detecting 75 - 100% of the COVID-19 patients [4,6,7,12,13]. In this study, chest x-ray showed bilateral pulmonary infiltrates in 60% of COVID-19 patients (Table 1).

Laboratory tests

Lymphocytes, eosinophils, D-dimer, LDH, hsCRP, and ferritin were the blood biomarkers with an abnormal median value in COVID-19 patients and with statistically significant differences between COVID-19 and NON-COVID-19 patients.

Blood lymphocyte count

Lymphopenia is very frequent in COVID-19 patients at hospital admission. In previous studies, the median values of lymphocyte count were between 0.8 and 1.1 x 10⁹/L [4-6,9-14]. In this study, the median value was 1.1 x 10⁹/L in both men and women (Table 2). AUC was higher in women than in men. The cutoff value in both genders was close to 1 x 10⁹/L (Table 3).

Table 2. Laboratory tests in patients with and without COVID-19 (n = 203).

Laboratory test (units) (reference values)	Female patients (n = 101)			Male patients (n = 102)		
	NON-COVID-19 (n = 57)	COVID-19 (n = 44)		NON-COVID-19 (n = 52)	COVID-19 (n = 50)	
	Median (IQR) Range		p-value *	Median (IQR) Range		p-value *
RBC (x 10 ¹² /L) ♀(3.9 - 5.1); ♂(4.3 - 5.6)	4.5 (0.6) 3.1 - 5.4	4.4 (0.8) 3.1 - 7.2	p > 0.05	4.9 (0.9) 2.9 - 5.8	5 (0.6) 2.4 - 5.9	p > 0.05
Haemoglobin (g/L) ♀(120 - 146.8); ♂(137.4 - 164.7)	133 (20.3) 87 - 160	130 (23.5) 94 - 179	p > 0.05	145.5 (28.5) 96 - 196	145.5 (15) 75 - 178	p > 0.05
Haematocrit (%) ♀(36 - 45 %); ♂(40 - 50)	41 (6) 29 - 49	40 (6) 30 - 60	p > 0.05	45 (7) 31 - 60	45 (5) 25 - 53	p > 0.05
WBC (x 10 ⁹ /L) (3.9 - 9.5)	8.9 (4.2) 4.2 - 26.2	6.5 (4.4) 3.5 - 15.7	p = 0.0004	8.8 (5.2) 3 - 41.4	6.5 (4.8) 3.3 - 124.6	p = 0.0006
Neutrophils (x 10 ⁹ /L) (1.5 - 5.7)	5.4 (3.5) 2.2 - 24.6	4.7 (4) 1.7 - 13.9	p > 0.05	5.5 (5.9) 1.8 - 39.2	4.9 (4.2) 1.6 - 17.7	p = 0.0285
Neutrophils (%) (37.1 - 68.4)	66.7 (13.9) 39.5 - 93.9	74 (19.4) 7 - 92.9	p > 0.05	68.6 (22.3) 42.5 - 94.9	73.5 (19.3) 4.6 - 95.1	p > 0.05
Lymphocytes (x 10 ⁹ /L) (1.3 - 3.4)	1.7 (1.1) 0.5 - 4.7	1.1 (0.6) 0.4 - 2.6	p < 0.0001	1.6 (1.5) 0.2 - 4	1.1 (0.8) 0.3 - 100.9	p = 0.0095
Lymphocytes (%) (21 - 50)	23.3 (15) 3.5 - 49.2	17.7 (13.1) 5.7 - 46.1	p > 0.05	21.1 (19.6) 1.6 - 44.8	16.9 (17.1) 1.9 - 81	p > 0.05
Monocytes (x 10 ⁹ /L) (0.31 - 0.92)	0.6 (0.3) 0.2 - 1.5	0.4 (0.3) 0.1 - 1	p = 0.0001	0.6 (0.4) 0.2 - 1.5	0.4 (0.4) 0.1 - 17.7	p = 0.0012
Monocytes (%) (5.1 - 11.2)	7.6 (3) 2.2 - 15.1	6.8 (4.5) 1 - 13.9	p > 0.05	7.7 (4.4) 1.3 - 17.8	7.8 (4.1) 1.8 - 20.6	p > 0.05
Eosinophils (x 10 ⁹ /L) (0.03 - 0.39)	0.09 (0.1) 0 - 0.7	0.02 (0.06) 0 - 1.1	p < 0.0001	0.1 (0.2) 0 - 0.8	0.02 (0.05) 0 - 0.8	p < 0.0001
Eosinophils (%) (0.4 - 6.6)	1.1 (1.7) 0 - 6.6	0.3 (0.7) 0 - 27	p < 0.0001	1.3 (2.3) 0 - 5.1	0.2 (0.7) 0 - 6.3	p < 0.0001
Basophils (x 10 ⁹ /L) (0.01 - 0.09)	0.04 (0.03) 0.01 - 0.2	0.02 (0.03) 0 - 0.1	p < 0.0001	0.04 (0.03) 0 - 7	0.01 (0.02) 0.01 - 0.2	p < 0.0001
Basophils (%) (0.2 - 1.3)	0.4 (0.4) 0.1 - 1.9	0.3 (0.4) 0 - 2.1	p = 0.0035	0.4 (0.4) 0 - 1.4	0.2 (0.2) 0.1 - 1.3	p = 0.0002
Platelets (x 10 ⁹ /L) ♀(153 - 368); ♂(149 - 303)	268 (88.8) 143 - 613	202 (94.5) 111 - 440	p < 0.0001	244 (101) 93 - 526	207 (103) 105 - 509	p = 0.0133
MPV (x 10 ⁻¹⁵ L) (9.7 - 13.2)	10.4 (1.1) 9.1 - 13.7	10.7 (1.3) 9.3 - 13.3	p = 0.0242	10.7 (1.1) 9.3 - 13.3	10.7 (1.5) 9.4 - 13.6	p > 0.05
D - dimer (mg/L) (< 0.1)	0.5 (0.7) 0.2 - 3.7	0.9 (1.1) 0.2 - 10.5	p = 0.0034	0.5 (0.8) 0.2 - 20	0.7 (0.9) 0.2 - 103	p = 0.0327
Creatinine (µmol/L) ♀(44.2 - 79.6); ♂(61.9 - 106.1)	68.1 (18.1) 44.2 - 178.6	75.1 (65) 38.9 - 233.4	p > 0.05	81.8 (22.1) 58.3 - 263.4	81.3 (25.6) 56.6 - 670.1	p > 0.05

Table 2. Laboratory tests in patients with and without COVID-19 (n = 203) (continued).

Laboratory test (units) (reference values)	Female patients (n = 101)			Male patients (n = 102)		
	NON-COVID-19 (n = 57)	COVID-19 (n = 44)		NON-COVID-19 (n = 52)	COVID-19 (n = 50)	
	Median (IQR) Range		p-value *	Median (IQR) Range		p-value *
Sodium (mmol/L) (136 - 145)	140 (3.3) 129 - 165	140 (4) 127 - 165	p > 0.05	140 (4) 130 - 151	139.5 (5) 125 - 157	p > 0.05
Potassium (mmol/L) (3.5 - 5.1)	4.1 (0.6) 3.2 - 6.7	4 (0.7) 3 - 5.9	p > 0.05	4 (0.5) 2.7 - 5.7	4 (0.6) 2.6 - 6.2	p > 0.05
Total bilirubin (µmol/L) (0 - 20.5)	6.7 (4.5) 3.4 - 21.2	6.9 (3.6) 2.2 - 16.4	p > 0.05	8.2 (6.4) 2.2 - 86.2	9.4 (5.5) 3.8 - 21.6	p > 0.05
AST (U/L) ♀(10 - 35 U/L); ♂(10 - 50)	21 (10.5) 10 - 241	26.5 (24) 12 - 190	p = 0.0003	21.5 (14.5) 8 - 236	33.5 (36) 13 - 475	p < 0.0001
ALT (U/L) ♀(10 - 35); ♂(10 - 50)	19 (15) 6 - 206	23 (18) 12 - 195	p = 0.0347	21 (18) 8 - 122	33.5 (30) 6 - 423	p = 0.0014
GGT (U/L) ♀(1 - 40); ♂(1 - 60)	20 (21) 5 - 671	37 (63.5) 7 - 207	p = 0.006	32 (33.5) 10 - 672	38.5 (44) 13 - 337	p > 0.05
LDH (U/L) ♀(135 - 214); ♂(135 - 225)	225 (85.3) 129 - 631	290.5 (157.5) 152 - 765	p < 0.0001	224 (62) 159 - 588	325 (223) 157 - 821	p < 0.0001
CK (U/L) ♀(1 - 170); ♂(1 - 190)	68 (41.8) 23 - 252	80.5 (62.5) 26 - 559	p > 0.05	97.5 (76.5) 30 - 1,202	92 (121) 28 - 2,459	p > 0.05
hsCRP (mg/L) (< 5)	7 (41.1) 0.3 - 424.7	54.5 (127.8) 0.8 - 334.2	p < 0.0001	17 (65.6) 0.2 - 287.1	76 (121.3) 5.5 - 340.3	p < 0.0001
Ferritin (µg/L) ♀(13 - 155); ♂(30 - 400)	87 (110) 11 - 695	245 (348) 28 - 2,223	p < 0.0001	201 (247) 13 - 1,572	813 (1,106) 94 - 11,255	p < 0.0001
COVID-19 RT-PCR	NEGATIVE	POSITIVE		NEGATIVE	POSITIVE	

* - Mann - Whitney U test, IQR - interquartile range.

Table 3. AUC, cutoff and sensitivity of blood biomarkers to differentiate between patients with and without COVID-19.

Blood biomarker	Gender	AUC (CI 95%)	Cutoff *	Sensitivity (CI 95%)
Lymphocytes	♀	0.752 (0.656 - 0.833)	≤ 1.09 x 10 ⁹ /L	54.5% (38.9 - 69.6)
	♂	0.641 (0.540 - 0.734)	≤ 0.97 x 10 ⁹ /L	44.0% (30.0 - 58.7)
Eosinophils	♀	0.767 (0.672 - 0.845)	≤ 0.02 x 10 ⁹ /L	61.4% (45.5 - 75.6)
	♂	0.798 (0.707 - 0.871)	≤ 0.02 x 10 ⁹ /L	66.0% (51.2 - 78.8)
D-dimer	♀	0.662 (0.561 - 0.753)	> 1.20 mg/L	38.6% (24.4 - 54.5)
	♂	0.619 (0.517 - 0.713)	> 1.16 mg/L	30.0% (17.9 - 44.6)
LDH	♀	0.734 (0.637 - 0.817)	> 274 U/L	59.1% (43.3 - 73.7)
	♂	0.764 (0.670 - 0.843)	> 278 U/L	64.0% (49.2 - 77.1)
hsCRP	♀	0.744 (0.647 - 0.825)	> 82.8 mg/L	40.9% (26.3 - 56.7)
	♂	0.737 (0.641 - 0.842)	> 80.5 mg/L	48.0% (33.7 - 62.6)
Ferritin	♀	0.847 (0.761 - 0.911)	> 197 µg/L	68.2% (52.4 - 81.4)
	♂	0.804 (0.714 - 0.876)	> 498 µg/L	64.0% (49.2 - 77.1)

AUC - area under the ROC curve, CI - confidence interval. * - Cutoff with specificity of 80% (CI 95%: 72.1 - 87.7).

Table 4. Sensitivity and specificity of COVID-19 criteria with blood biomarkers at hospital admission.

COVID-19 criteria	Sensitivity (CI 95%)	Specificity (CI 95%)
Ferritin > 125% of URL	66% (56.6 - 76.4)	85% (77.3 - 91.4)
Eosinophils $\leq 0.02 \times 10^9/L$	64% (54.4 - 74.5)	79% (71.1 - 86.9)
LDH >125% of URL	62% (51.1 - 71.5)	77% (67.0 - 83.8)
hsCRP >80 mg/L	46% (36.4 - 57.4)	81% (72.1 - 87.7)
Lymphocytes $\leq 1.0 \times 10^9/L$	43% (32.4 - 53.2)	81% (72.1 - 87.7)
D-dimer > 1.2 mg/L	33% (23.6 - 43.4)	79% (71.1 - 86.9)
One or more COVID-19 criteria	91% (83.9 - 96.2)	47% (38.1 - 57.5)

URL - upper reference limit, CI - confidence interval.

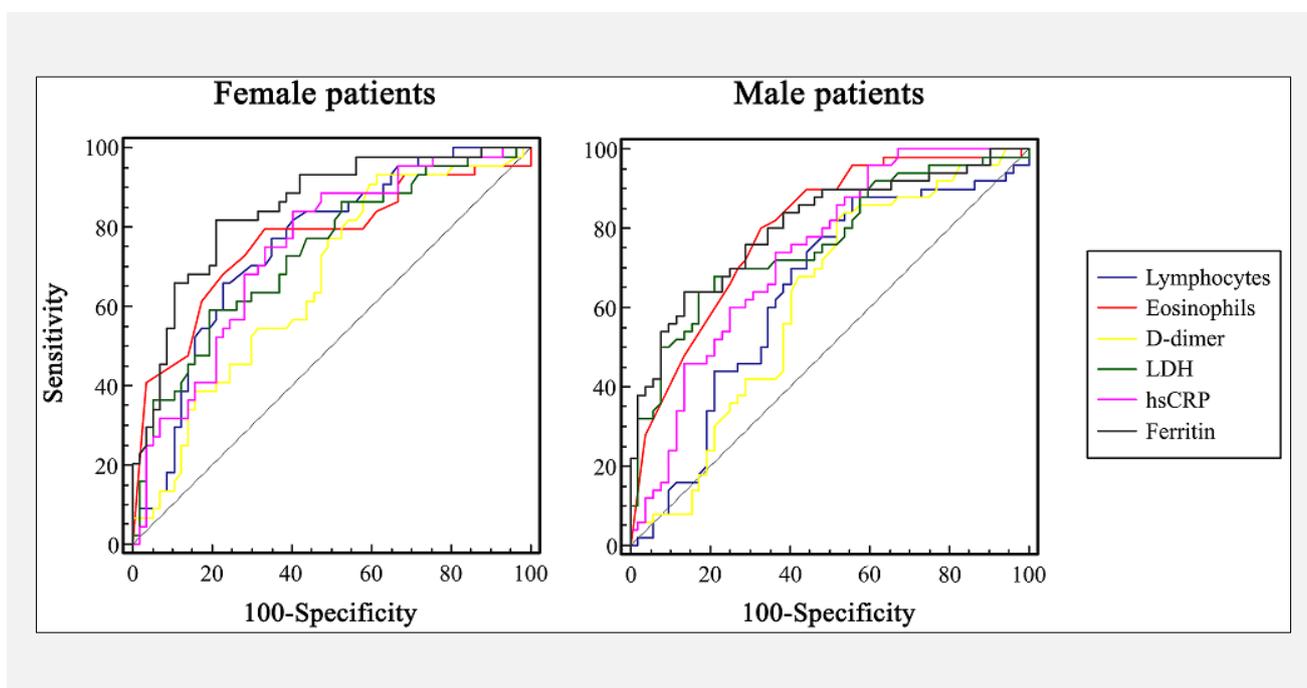


Figure 1. ROC curves of blood biomarkers to differentiate between patients with and without COVID-19.

Blood eosinophil count

Very few studies have evaluated the eosinophil count in COVID-19 patients. The median value obtained in two previous studies was $0.02 \times 10^9/L$ [9,10], in agreement with median in both genders obtained in this study (Table 2). AUCs were similar in women and men. The cut-off was $\leq 0.02 \times 10^9/L$, showing a sensitivity higher than 60% in both genders (Table 3).

Plasma D-dimer level

In clinical practice, the reference value of D-dimer ($< 0.1 \text{ mg/L}$) is not usually useful, and instead the thrombotic disease cutoff ($< 0.5 \text{ mg/L}$) is used. In most

published studies, median of D-dimer was higher than 0.5 mg/L [4,11-13]; however, some studies report a median lower than this value in COVID-19 patients [5,6]. Zhou F et al. [4] report a median value of 0.8 mg/L and the study by Chen T et al. [12] reports a median value was 1.1 mg/L in COVID-19 patients. In this study, plasma median D-dimer was 0.9 mg/L in women and 0.7 mg/L in men (Table 2). D-dimer was the biomarker with the lowest accuracy for diagnosis of COVID-19, both in men and women. The cutoff value was close to 1.2 mg/L with a low sensitivity in both genders (Table 3).

Plasma LDH level

Previous studies report a median value of plasma LDH levels in COVID-19 patients higher than reference values [4,6,9,12-14]. In some studies, median value was higher than 300 U/L [4,9,12,14]. In this study, the median value of plasma LDH levels was 290 U/L in women and 325 U/L in men (Table 2). The cutoff value of LDH was similar in both genders, about 1.25 times the URL with a sensitivity of 59% in women and 64% in men (Table 3).

Plasma hsCRP level

In the studies by Ferrari et al. [9] and Chen T et al. [12], the median values of plasma hsCRP levels in COVID-19 patients were 87.1 and 53.4 mg/L, respectively. In this study, the median value was 54.5 mg/L in women and 76.0 mg/L in men (Table 2). AUC and cutoff value were similar in both genders. The cutoff value was close to 80 mg/L with a sensitivity of less than 50% (Table 3).

Plasma ferritin level

In the papers by Zhou F et al. [4] and Chen T et al. [12], the median values of plasma ferritin levels in COVID-19 patients were 722 and 670 $\mu\text{g/L}$, respectively. In this study, the median values were very different according to gender, 245 $\mu\text{g/L}$ in women and 813 $\mu\text{g/L}$ in men (Table 2). Plasma ferritin was the most accurate biomarker, AUCs were 0.847 and 0.804 in women and men, respectively. The cutoff value of ferritin was higher in men (498 $\mu\text{g/L}$) than in women (197 $\mu\text{g/L}$). The cutoff values were approximately 1.25 times their URL, with sensitivity of 68% in women and 64% in men (Table 3).

Diagnostic criteria with blood biomarkers

Diagnostic criteria for suspicion of COVID-19 at hospital admission were established with blood counts of lymphocytes and eosinophils, and plasma levels of D-dimer, LDH, hsCRP, and ferritin according to the cutoff values obtained with the ROC curves. The diagnostic criterion with blood lymphocyte count was $\leq 1.0 \times 10^9/\text{L}$, and blood eosinophil count criterion was $\leq 0.02 \times 10^9/\text{L}$ exhibiting a sensitivity of 43% and 64%, respectively (Table 4). In some papers, 62 to 65% of COVID-19 patients had blood lymphocyte counts $\leq 1.0 \times 10^9/\text{L}$ [12-14]. The diagnostic criterion with plasma D-dimer levels was $> 1.2 \text{ mg/L}$ and showed a sensitivity of 33% (Table 4). In the study by Zhou et al. [4], D-dimer levels $> 1.0 \text{ mg/L}$ in 42% of COVID-19 patients were observed, and in the paper by Zhang G et al. [11], D-dimer levels $> 1.0 \text{ mg/L}$ were reported in 66% of COVID-19 patients. Plasma LDH level $> 125\%$ of URL (in this study: $> 268 \text{ U/L}$ for women and $> 281 \text{ U/L}$ for men) was proposed as COVID-19 criteria with sensitivity of 62% (Table 4). In some published studies, 67 to 78% of COVID-19 patients had plasma LDH levels $> 245 \text{ U/L}$ [4,11,13,14], and in the study by Chen T et al. [12], 42% of COVID-19 patients had LDH levels $> 350 \text{ U/L}$.

The plasma hsCRP concentration $> 80 \text{ mg/L}$ showed a sensitivity of 46% (Table 4). In the paper by Zhang G et al. [11], 43% of COVID-19 patients presented plasma hsCRP levels $> 90 \text{ mg/L}$, and in the study by Chen T et al. [12], 33% of COVID-19 patients had hsCRP levels $> 100 \text{ mg/L}$. COVID-19 criterion proposed with plasma ferritin was $> 125\%$ of URL (in this study: $> 194 \mu\text{g/L}$ for women and $> 500 \mu\text{g/L}$ for men), with a sensitivity of 66% (Table 4). In the paper by Zhou F et al. [4], they did not distinguish between genders and reported ferritin levels $> 300 \mu\text{g/L}$ in 80% of COVID-19 patients. Patients can be classified into two groups according to combination of these COVID-19 criteria with blood biomarkers:

- Patients with possible COVID-19: meet at least one of the criteria.
- Patients with low probability of COVID-19: do not meet any of the criteria.

In this study, 47% of patients without COVID-19 had not met any of the COVID-19 criteria with blood biomarkers and could be classified as patients with low probability of COVID-19, which could be very useful to prioritize the COVID-19 RT-PCR test, especially when test is not universally available. In addition, the 91% of patients with COVID-19 were detected with these criteria (Table 4).

These blood biomarkers have the advantage that they are frequently used in medical practice and are available in all hospitals and can also be determined quickly and easily on automated analyzers. However, this study has some limitations, decreased blood lymphocyte and eosinophil counts may be due to other diseases, and plasma D-dimer, LDH, hsCRP and ferritin concentrations may be elevated in many inflammatory diseases. Furthermore, the study was conducted in a pandemic situation with a very high prevalence of COVID-19. For all these reasons, the COVID-19 criteria with the blood biomarkers cannot be assessed independently, but in conjunction with other complementary tests and clinical data.

CONCLUSION

Blood counts of lymphocytes and eosinophils, and plasma levels of D-dimer, LDH, hsCRP, and ferritin can be used to differentiate patients with and without COVID-19 and as a tool for diagnosis of suspected COVID-19. Ferritin was the best biomarker for diagnosis of suspected COVID-19. Blood counts of lymphocytes $\leq 1.0 \times 10^9/\text{L}$ and eosinophils $\leq 0.02 \times 10^9/\text{L}$ and plasma levels of ferritin $> 125\%$ of URL, LDH $> 125\%$ of URL, hsCRP $> 80 \text{ mg/L}$, and D-dimer $> 1.2 \text{ mg/L}$ can be used as diagnostic criteria of suspected COVID-19 in adult patients at hospital admission.

Author Contributions:

Design of study (JDS), investigation and data collection (JDS, DNJ, ELB), statistical analysis (JDS), validation and discussion of results (JDS, DNJ, ELB), write the draft manuscript (JDS), review and approval of the final manuscript (JDS, DNJ, ELB).

Declaration of Interest:

There is no actual or potential conflict of interest for any author in relation with the results presented in the manuscript.

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