Original Article

Diagnostic and Prognostic Value of Routine Blood Tests in Patients with COVID-19 in Iran, Tehran

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Abstract

Background: Routine blood testing consists of Complete Blood Count (CBC) indices together with Comprehensive Metabolic Panel (CMP) which have significant roles in both diagnosis and prognosis of the novel coronavirus disease 2019 (COVID-19).

Methods: A total number of 942 COVID-19 patients and 400 healthy persons as the control group were enrolled in this study. All patients were admitted to a single center and were divided into two groups according to disease severity (severe or non-severe). Routine laboratory findings of peripheral blood sample were collected and then analyzed.

Results: Neutrophil-Lymphocyte Ratio (NLR) had the highest sensitivity and specificity value for COVID-19 diagnosis. Among patients with different severities of COVID-19, the amount of neutrophil, NLR, platelet, hemoglobin, Red cell Distribution Width (RDW) and total bilirubin was significantly different (p<0.01).

Conclusion: Some indices of complete blood count and comprehensive metabolic panel have diagnostic and prognostic roles in COVID-19 patients, which are helpful in early diagnosis, predicting severity and adverse outcomes of patients with COVID-19.

Keywords: Bilirubin, Blood cell count, Blood platelets, Coronavirus, COVID-19, Erythrocyte indices, Hematologic tests, Humans, Lymphocytes, Neutrophils

Introduction

Over 80 million people were infected by the contagious virus, COVID-19, and nearly 2 million deaths occurred all over the world (1). Person to person potential transmission modes of COVID-19 include airborne, droplet, oral and fecal transmission and contact with contaminated surfaces (2).

Many patients present with fever, chills and myalgia within first days and other symptoms may appear in the following days (3). COVID-19 is known to cause serious respiratory symptoms but it can exclusively affect other body systems like immune, neurological and hematopoietic system (4). COVID-19 causes mild to severe illness ranging from flu-like syndrome and gastroenteritis to more severe diseases such as Acute Respiratory Distress Syndrome (ARDS), metabolic acidosis and coagulopathy leading to multiple organ dysfunction and death. Disease mortality rate is about 1% and mainly due to acute respiratory failure, septic shock and failure of major organs (5,6). Previous studies suggest that diabetes mellitus, hypertension, and old age cause a significant increased risk of mortality among patients with COVID-19 (7).

There are also some potential long-lasting problems caused by COVID-19 Lung and heart injuries, vascular thrombosis, heart attack and brain stroke are the most frequent late complications (8).

The disease is confirmed by detection of SARS-CoV-2 RNA, through nasopharyngeal swab, by reverse transcription Polymerase Chain Reaction (PCR) which gives about 30% false negative results and in such cases, typical findings of chest CT scan may be helpful (6,9). As lungs are not always involved in this disease and both PCR and CT scan are expensive and not always available, clinicians may need other laboratory indices for early diagnosis of COVID-19.

On the other hand, both clinical and laboratory findings at the first visit may help the clinician to detect patients who may develop the severe form and need more intensive care. Blood cell indices and serum levels of C-Reactive Protein (CRP), Erythrocyte Sedimentation Rate (ESR), D-dimers, ferritin, cardiac troponin and IL-6 may reflect the severity of COVID-19 at the early stage (10,11).

Therefore, this study was designed to evaluate the diagnostic and prognostic value of Complete Blood Count (CBC) and metabolic indices as well as Liver Function Tests (LFT) in COVID-19 patients. In fact, an attempt was made to provide laboratory evidence for early diagnosis and treatment of patients and prediction of severe cases that required more intensive care.

Materials and Methods
Study design and participants

In this retrospective case-control study, nine hundred forty-two confirmed COVID patients (whether with PCR or typical findings of chest CT) from March 2020 to January 2021 and 400 age-sex matched healthy persons as the control group were enrolled in this study. All the patients were admitted to a single center (Amir Alam Hospital, Tehran, Iran) and divided into two groups according to the illness severity (Severe or non-severe). The detailed criteria for detecting severe patients were based on the national guidelines, with any of the following listed items: rapid progressive respiratory manifestations with a respiratory rate of ≥ 30 breaths per minute, the oxygen saturation (SpO_2) $\leq 90\%$ at rest, increase in alveolar-arterial oxygen gradient, PaO2/FiO$_2 \leq 300$ *mmHg* and $>50\%$ lung involvement (12). Routine laboratory findings of peripheral blood smear were collected from all patients and healthy group members and then analyzed.

Ethical approval for this study was obtained from the institutional ethics board of Tehran University of Medical Sciences (IR.TUMS.AMIRALAM. REC.1399.036) and participants signed informed written consent forms.

Statistical analysis

For data analysis, SPSS software v 22 (IBM, USA) was used and a p value <0.05 was considered statistically significant. Normally distributed data were analyzed by t-test and expressed as mean $\pm$ SD; non-normally distributed data were analyzed by Mann-Whitney U-test or the Wilcoxon signed-rank test and presented as medians with IQR (P25, P75). A Chi-square test was used to analyze categorical data. Qualitative data were reported as number (%).

Results

Nine hundred forty-two confirmed COVID-19 patients from March 2020 to January 2021 were enrolled in this retrospective case-control study

including 602 males (64%) and 340 females (36%) with mean age of 54.7±16.3. Healthy subjects including 260 males (65%) and 140 females (35%) with mean age of 52.15±9.74 were selected as the control group. Patients were divided into two groups according to the need for intensive care (severe or non-severe). Those with severe disease included 167 patients (17.7%) with mean age of 67.1±15.5 years. In non-severe group, there were 775 patients (82.3%) with mean age of 48.6±12.74 years.

There was no significant difference in terms of sex or age among two groups (p>0.05).

In whole blood evaluation, unlike White Blood Cell (WBC), Mean Corpuscular Volume (MCV), Red cell Distribution Width (RDW), the value of Neutrophil (Neut), Lymphocyte (Lymph), Hemoglobin (Hb), platelet, Neutrophil-Lymphocyte Ratio (NLR) and Platelet-Lymph Ratio (PLR) were significantly different (p<0.05) between COVID-19 patients and healthy cases, while NLR had the highest sensitivity and specificity for COVID-19 diagnosis. Alanine Transaminase (ALT), Aspartate Transaminase (AST), ESR and CRP levels were significantly higher in patients rather than healthy control group. Urea, creatinine (Cr),

Table 1. The diagnostic value of blood routine test and general biochemical results in baseline laboratory findings of all included patients and healthy controls

	COVID -19 group	Healthy group	p-value
WBC ($10^9/L$)	7.81± 1.74	6.80± 2.24	0.319
Neutrophil ($10^9/L$)	5.26 ± 1.43	4.219 ±0.92	<0.01
Lymphocyte ($10^9/L$)	1.69± 0.98	2.69 ± 1.10	<0.01
Neut/ Lymph	2.5±0.52	1.41±0.64	<0.01
Hemoglobin (*gr/dL*)	11.9 (10.8, 13.7)	13.7 (12.5, 15.6)	<0.01
MCV (*fL*)	83.60 ± 5.865	85±5.75	0.222
RDW (%)	13.29±0.91	13.78±1.13	0.178
PLT ($10^9/L$)	211.50 ± 70.16	249.90± 59.12	<0.01
PLT/Lymphocyte	201.44 ± 87.9	76.67± 68.44	<0.01
Urea (*mg/L*)	37±12.8	30±10.9	0.189
Cr (*mg/dl*)	1.16±0.5	1.07±0.41	0.117
AST (*U/L*)	38.5 ± 18.9	19.9 ± 7.5	<0.01
ALT (*U/L*)	40.4 ± 19.5	21.6 ± 10.7	<0.01
ALP (*IU/L*)	165.50± 70.6	173.40± 70.6	0.228
Bili-T (*mg/dL*)	0.7± 0.4	0.6± 0.3	0.341
Bili-D (*mg/dL*)	0.41 ± 0.2	0.36± 0.1	0.458
ESR (*mm/hr*)	54.78±24	12±4	<0.01
CRP (*mg/L*)	56.7±29.8	4±2	<0.01

Table 2. The prognostic value of baseline blood routine test and general biochemical results among patients with different severities of COVID-19

|	Non-severe	severe	p-value
WBC (10^9/L)	7.3 ± 1.3	8.2 ± 1.2	0.232
Neutrophil (10^9/L)	4.433 ± 1.44	6.6419 ± 0.26	<0.01
Lymphocyte (10^9/L)	1.96 ± 0.39	1.84± 0.64	0.429
Neut/ Lymph	2.39±0.52	3.81±0.64	<0.01
Hemoglobin (*gr/dL*)	12.9 ±2.1	11.00 ±2.3	<0.01
MCV (*fL*)	83.70 ± 2.7	83.55 ± 2.5	0.155
RDW (%)	13.01±0.41	14.19±0.54	<0.01
PLT (10^9/L)	221.00 ±40.41	169.00±46.40	<0.001
PLT/Lymphocyte	213 ± 169.7	159 ± 87.9	0.312
Urea (*mg/L*)	36.34 ±12.57	40.75±15.76	0.100
Cr (*mg/dl*)	1.14±0.55	1.24±0.48	0.386
AST (*U/L*)	37.56 ± 21.03	39.33 ± 12.90	0.841
ALT (*U/L*)	39.11 ± 24.54	41.02 ± 19.52	0.773
ALP (*IU/L*)	169.50 ± 46.6	173.40 ± 65.6	0.127
Bili-T (mg/dL)	1.04± 0.45	1.43 ± 0.56	<0.001
Bili-D (*mg/dL*)	0.40± 0.15	0.42± 0.22	0.452
ESR (*mm/hr*)	48.76 ±0.56	59.66 ±21.39	0.726
CRP (*mg/L*)	43.33 (±19.79)	54.00 (±24.74)	0.965
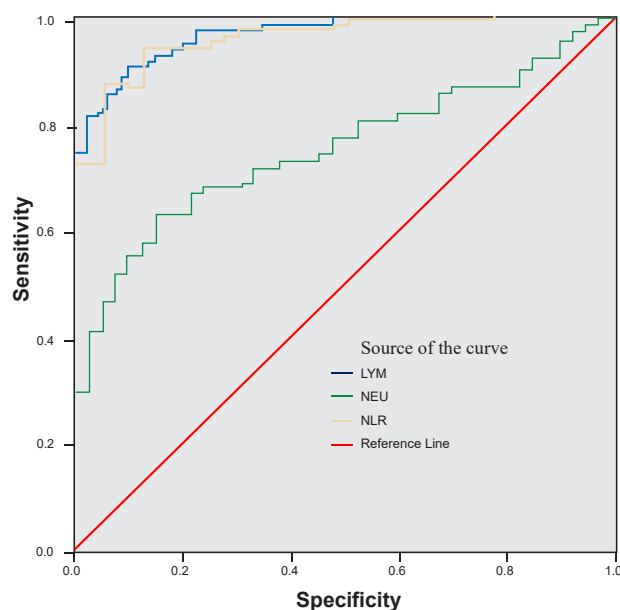
Figure1. ROC curve of lymphocyte (Lymph), neutrophil (Neu), and NLR for diagnosis of patients with COVID-19. Univariate logistic regression analysis was used to identify these patients.

bilirubin, and Alkaline Phosphatase (ALP) levels did not differ between two groups (p>0.1) (Table 1).

Among patients with different severity of COVID-19, the amount of Neut, NLR, PLT, Hb, RDW and total bilirubin was significantly different (p<0.01) (Table 2). To evaluate the diagnostic value of Lymph, Neut and NLR in patients with COVID-19, Receiver Operating Characteristic (ROC) curves were drawn. For diagnosis of the COVID-19, the area under ROC curve of Lymph, Neut and NLR was 0.97, 0.76 and 0.92, respectively. The preoperative Lymph value of $1.298 \times 10^9/L$ was the optimal cutoff value for predicting COVID-19 (Sensitivity=90.6%, specificity =92.4%), the preoperative Neut value of $4.41 \times 10^9/L$ was the optimal cutoff value for predicting COVID-19 (Sensitivity=63.2%, specificity=86.1%) and the preoperative NLR value of 2.009 was the optimal cutoff value for predicting COVID-19 (Sensitivity=93.4%, specificity=88.1%) (Figure 1). Univariate logistic regression analysis was used to identify COVID-19

patients. ROC curves of lymphocyte, neutrophil and NLR were used for auxiliary diagnosis of patients with COVID-19.

Discussion

This retrospective study of 942 patients with COVID-19 showed that the value of some blood sample indices like CBC, metabolic blood test and LFT were useful in diagnosis and prognosis of these patients and a cutoff value of $1.298 \times 10^9/L$ was validated for the lymphocyte as the most sensitive and specific blood test for early diagnosis (Sensitivity = 90.6%, specificity=92.4%).

Recently, some retrospective studies have identified that patients with COVID-19 had significantly higher AST, ALT and LDH levels and these indices had very good accuracy in predicting patients with COVID-19. The differences in HCT, Hb, MCH, MCHC, MCV, Neut, RBC and D-dimer were statistically significant between COVID-19 patients

and healthy group (13,14).

Increased Blood Urea Nitrogen (BUN), decreased thrombocytes and increased Interleukin 6 (IL-6) levels were all identified as risk factors for the severity of the disease. Peripheral blood lymphocyte was reduced in COVID-19 patients in comparison to non–COVID-19 patients. Low platelet count and lymphopenia are associated with severity and mortality rate of COVID-19 (15-17). CRP level was positively correlated with lung lesions at the early stage of COVID-19 and associated with COVID-19 severity (18) and the NLR measured at admission can be used to effectively predict the subsequent presence of serious clinical outcomes (19).

In this study, the value of Neut, Lymph, NLR, Hb, PLT and PLR was significantly different between COVID-19 patients and healthy group. AST, ALT, ESR and CRP levels were significantly higher in patients. MCV, RDW, urea, Cr and ALP levels showed no difference between COVID-19 patients and healthy group.

ROC curve analysis confirmed that Lymph, Neut and NLR could be used as indices for distinguishing COVID-19 patients from healthy cases. The cutoff values of Lymph and NLR were optimal for COVID-19 diagnosis. The sensitivity of Neut at the cutoff value was not high; therefore, if only Neut is used as the diagnostic index, it may lead to missed diagnosis of patients with COVID-19. More importantly, those indices can easily be obtained and should be preferentially evaluated for auxiliary diagnosis of patients with COVID-19. Laboratories can provide many biochemical indices for clinicians to assess the effect of treatment and patients' organ function. Some laboratory findings such as elevated D-dimer, CRP and LDH levels as well as severe lymphopenia were associated with diseases severity, mortality and poor outcomes in hospitalized patients. All in all, a considerable number of comorbidities and biomarkers are associated with severity and presentations of COVID-19 disease, affecting its morbidity and mortality rates (6,20).

Our investigation showed the amount of Neut, NLR, PLT, Hb, RDW and total bilirubin was significantly different among patients with various severities of COVID-19. Unlike these indices, total WBC, Lymph, PLR, MCV, renal and liver function tests (Except for total bilirubin) could not be used to predict disease deterioration and serious clinical outcomes.

The results indicate that multiple indices of analysis of peripheral blood cells are useful for differentiating patients with COVID-19 from healthy cases; for differentiating severity and mortality of COVID-19, clinicians should consider the fact that although these indices are also useful, but the obtained values are different and they should be interpreted with caution.

Limitations

The present study has some limitations. First of all, it describes the potential value of blood cells and biochemistry for risk stratification of admitted patients, so that these results may not be applied to those who are not hospitalized. Second, it was impossible to eliminate the impact of treatments before hospital admission on the outcome. For example, liver function tests may be affected by NSAID therapy. Third, other factors which may have potential association with disease severity, like past medical history or socioeconomic status were not investigated. In addition, disease mortality may affect the performance of blood tests in risk stratification of disease severity and studies in populations with various rates of COVID-19 mortality are warranted. Finally, this cohort study was conducted in Tehran/Iran and may not be representative of other populations.

Conclusion

In conclusion, some indices of complete blood count, comprehensive metabolic panel, and LFT have diagnostic and prognostic roles in detection of COVID-19. As these tests are available and cost-effective, they may have valuable role in predicting the disease severity and early diagnosis.

Conflict of Interest

We declare that we have no conflicts of interest.

Ethical Approval

This study was approved by the institutional ethics board of Tehran University of Medical Sciences (No: IR.TUMS.AMIRALAM.REC.1399.036) and participants signed informed written consent forms.

References

1. https://www.worldometers.info/coronavirus/

2. Mehraeen E, Salehi MA, Behnezhad F, Moghaddam HR, SeyedAlinaghi SA. Transmission Modes of COVID-19: A Systematic Review. Infect Disord Drug Targets 2020 Nov 15.

3. Ghiasvand F, Miandoab SZ, Harandi H, Golestan FS, SeyedAlinaghi SA. A patient with COVID-19 disease in a referral hospital in Iran: A typical case. Infect Disord Drug Targets 2020;20(4):559-62.

4. Sadr S, SeyedAlinaghi SA, Ghiasvand F, Hassan Nezhad M, Javadian N, Hossienzade R, et al. Isolated severe thrombocytopenia in a patient with COVID-19: A case report. IDCases 2020 May 29;21:e00820.

5. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497-506.

6. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med 2020;382(3):1199-207.

7. Mehraeen E, Karimi A, Barzegary A, Vahedi F, Afsahi AM, Dadras O, et al. Predictors of mortality in patients with COVID-19-a systematic review. Eur J Integr Med 2020 Dec;40:101226.

8. SeyedAlinaghi S, Afsahi AM, MohsseniPour M, Behnezhad F, Salehi MA, Barzegary A, et al. Late Complications of COVID-19; a systematic review of current evidence. Arch Acad Emerg Med 2021 Jan 20;9(1):e14.

9. Covino M, Sandroni C, Santoro M, Sabia L, Simeoni B, Bocci MG, et al. Predicting intensive care unit admission and death for COVID-19 patients in the emergency department using early warning scores. Resuscitation 2020;156:84-91.

10. Thirumalaisamy P. Velavan, Christian G. Meyer, Mild versus severe COVID-19: Laboratory markers. Int J Infect Dis 2020;95:304-7.

11. Yang Y, Yang M, Shen C, Wang F, Yuan J, Li Z, et al. Evaluating the accuracy of different respiratory specimens in the laboratory diagnosis and monitoring the viral shedding of 2019-nCoV infections. medRxiv 2020;1(3).

12. https://file.qums.ac.ir/repository/vch/Edu/1399/991227.Low_Covid-19_noskhe9.pdf

13. Mardani R, Ahmadi Vasmehjani A, Zali F, Gholami A, Mousavi Nasab SD, Kaghazian H, et al. Laboratory parameters in detection of COVID-19 patients with positive RT-PCR: a diagnostic accuracy study. Arch Acad Emerg Med 2020;8(1):e43.

14. Mo P, Xing Y, Xiao Y, Deng L, Zhao Q, Wang H, et al. Clinical characteristics of refractory COVID-19 pneumonia in Wuhan, China. Clin Infect Dis 2020;ciaa270.

15. Lv Z, Wang W, Qiao B, Cui X, Feng Y, Chen L, et al. The prognostic value of general laboratory testing in patients with COVID-19. J Clin Lab Anal 2021;35(2):e23668.

16. Jiang SQ, Huang QF, Xie WM, Lv C, Quan XQ. The association between severe COVID-19 and low platelet count: evidence from 31 observational studies involving 7613 participants. Br J Haematol 2020;190(1):e29-e33.

17. Mei J, Hu W, Chen Q, Li C, Chen Z, Fan Y, et al. Development and external validation of a COVID-19 mortality risk prediction algorithm: a multicentre retrospective cohort study. BMJ Open 2020;10(12):e044028.

18. Wang L. C-reactive protein levels in the early stage of COVID-19. Med Mal Infect 2020;50(4):332-4.

19. Zeng ZY, Feng SD, Chen GP, Wu JN. Predictive value of the neutrophil to lymphocyte ratio for disease deterioration and serious adverse outcomes in patients with COVID-19: a prospective cohort study. BMC Infect Dis 2021 Jan 18;21(1):80.

20. Dadras O, Shahrokhnia N, Boron S, Asadollahi-Amin A, SeyedAlinaghi SA. Factors associated with COVID-19 morbidity and mortality: A narrative review. J Iran Med Counc 2020;3(4):177-86.