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The Association of Age, Sex, and RT-PCR Results with the Lymphocyte and Neutrophil Counts in SARS-CoV-2 Infection: A Cross-sectional Analysis of 1450 Iranian Patients with COVID-19

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ABSTRACT

Containment of pandemic infections mainly depends on prompt identification of carriers, achievable through strict surveillance and truthful diagnostic testing. Although molecular identification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the gold standard method, its low sensitivity and long turnaround time are among major concerns.

In this retrospective single center study, we reviewed the results of the lymphocyte and neutrophil counts of 1450 Iranian patients with coronavirus disease 2019 (COVID-19) recruited at Baqiyatallah Hospital, Tehran, Iran.

Of 1450 patients, 439 cases (30.3%) were polymerase chain reaction (PCR) negative; further emphasizing that getting negative molecular testing is not as reliable as a positive result is. While the lymphocyte count in cases with less than 50 years old was $1.8\times10^3/\mu L$ (1.2-2.5), it was $1.47\times10^3/\mu L$ (0.84-2.16) in the older group ($p<0.001$). Also, men experienced lower lymphocytes as compared to women ($1.53\times10^3/\mu L$ vs $1.76\times10^3/\mu L$; $p=0.002$). Of particular interest, the lymphocyte count in the PCR-negative cases was $1.77\times10^3/\mu L$ (0.98-2.45) which was significantly higher than its count in their positive counterparts ($1.53\times10^3/\mu L$; $p=0.004$). Unlike lymphocytes, sex and PCR did not significantly affect the number of neutrophils. The odds ratio for neutrophilia in patients aged older than 50, either with a negative or a positive PCR, was 2.46 and 2.23, suggesting old age as the most significant associated factor.

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The number of lymphocytes along with increased neutrophil count may probably serve as simple, rapid, and economical biomarkers, and are seemingly appropriate items that should be taken into account in the identification of patients with COVID-19, especially those aged more than 50.

Keywords: COVID-19; Lymphocytes; Male; Neutrophils; SARS-CoV-2

INTRODUCTION

Despite all the scientific advances that humans have made over the years, nobody would have even imagined that the normal flow of life could stop or even slow down due to the emergence of a viral infection. The spark of all the events was struck from late 2019 when an outburst of pneumonia of unknown etiology in Wuhan, China, sooner or later impelled the World Health Organization (WHO) to announce a public health emergency of international concern on 30 January, and a pandemic on 11 March.[1] The coronavirus disease 2019 (COVID-19) is an ongoing pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), formerly known as the 2019nCoV.[2] Similar to other members of the Coronaviridae family, SARS-CoV-2 contains four structural proteins, including E (envelope), M (membrane), N (nucleocapsid), and S (spike) proteins. Notably, the spike protein allows the virus to attach to and fuse with the membrane of a target cell.[3,4] To be more in detail, following attachment of a SARS-CoV-2 virion that is 50–200 nanometers in diameter,[5] the cell's protease TMPRSS2 cuts open the spike protein to create a fusion peptide.[6] The membrane then encloses the virion to form an endosome which after exiting from this vesicle, releases RNA content into the cells and forces them to produce and disseminate copies of the virus, which infects more cells.[7]

Taking advantage of the fact that each infection may infect 1.4 to 3.9 new cases when no protective efforts are executed and no members of the community are immune,[8] one may conclude that early detection of COVID-19 carriers is critical not only to mitigate viral spread also to diminish disease progression. Albeit molecular testing of pharyngeal swab specimens is the gold standard method for the etiological detection of SARS-CoV-2, the existence of false-negative results missing 30% to 50% of infected cases denote a major limitation to the polymerase chain reaction (PCR)-based methods.[9,10] Besides, many countries with restricted assets are not equipped with sufficient laboratory and human resource capacity to perform massive molecular identification, further uncovering the urgent necessity for alternative tests to detect COVID-19 patients in a timely as well as simple manner. In a recent study, Liu et al. reported that the calculation of neutrophil-to-lymphocyte ratio (NLR) may serve as an independent risk factor to predict COVID-19 severity. To be more in detail, they found that an increase in each NLR unit was associated with an 8% increase in in-hospital mortality.[11] Given this, the present study was aimed to investigate whether abnormal values in lymphocyte and neutrophil counts could predict SARS-CoV-2 infection and evaluate if there is a correlation between alteration of these parameters with age, sex, and RT-PCR results in1450Iranian COVID-19 patients.

MATERIALS AND METHODS

Population and Procedures

We retrospectively reviewed 1450 patients with a diagnosis of COVID-19 from March to April 2020 recruited at Baqiyatallah Hospital, as a reference hospital for patients with SARS-CoV-2 infection in Tehran, Iran. This Single-Centre study was approved by Baqiyatallah University of the Medical Sciences Ethics Committee (IR.BMSU.REC.1398.434) and written informed consent was waived from patients. RT-PCR analysis and chest CT were requested for all the patients with clinical symptoms of cough, fever, dyspnea, and pleuritic chest pain as well as coarse crackles on auscultation. The sequences of the primers targeting the envelope gene of CoV were mentioned in Table 1. Conditions for the amplifications were 50°C for 15 min, 95°C for 3 min, followed by 45 cycles of 95°C for 15 s and 60°C for 30 s. All imaging features including pure ground-glass opacity (GGO), pure consolidation, mixed GGO, and consolidation, reversed halo, intralesional traction bronchiectasis, crazy-paving, intralesional vascular enlargement, linear opacities, lymph node enlargement, pleural effusion, and pericardial effusion were reviewed and evaluated by an expert radiologist. A thin-section CT

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Table 1. Nucleotide sequences of primers used for reverse transcription-polymerase chain reaction (RT-PCR) analysis

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
Envelope of CoV	ACTTCTTTTTCTTGCTTTCGT GGT	GCAGCAGTACGCACAC AATC

involvement score was assigned based on all abnormal areas involved. The number of affected lung lobes was also counted, and the location of the lesion was considered as peripheral if it was in the outer one-third of the lung; otherwise, it was considered as central. Other radiological patterns were also evaluated. Data on the lymphocyte and neutrophil counts, obtained from routinely drawn peripheral venous blood on admission, and the percentage of lymphopenia (lymphocytes<1.1×10^3/μL) and neutrophilia (neutrophils >6.3×10^3/μL) in the studied population were retrospectively extracted from patients' electronic medical records. All the patients with a positive CT scan, either with or without a positive RT-PCR, were included in this study. Notably, we excluded COVID-19 cases that did not have data on the lymphocyte and neutrophil counts on admission. Incomplete information concerning patients' clinical characteristics and inadequate data for the disease severity were the major limitations that we have faced with.

Statistical Analysis

The continuous variables were examined to determine the normality of the distribution using histograms, measures of skewness and kurtosis, and Kolmogorov–Smirnov test. The skewed distributed variables were described as the median and interquartile range. Categorical variables were summarized as frequencies (percentages). The non-normally distributed continuous variables were compared between binary and categorical variables using the Mann–Whitney U and Kruskal-Wallis tests respectively. Logistic regression models were applied to assess the associations of age group, sex, PCR, and their combinations with lymphopenia and neutrophilia. For each model, the odds ratio (OR) and the 95% confidence interval (CI) were calculated. All tests were two-sided, and a *p*-value of less than 0.05 was considered to indicate a statistically significant difference. All the statistical analyses were performed using the IBM SPSS version 24.0 (IBM Corp., Armonk, NY, U.S.A).

Role of the Funding Source

The funder of the study had no role in study design, data collection, data analysis, and interpretation, or writing of the manuscript. The corresponding authors had full access to all the data in this study and had final responsibility for the decision to submit for publication.

RESULTS

The Association between Lymphocyte Count and Age, Sex, and SARS-CoV-2 PCR results

Of 1450 COVID-19 patients with the mean age of 54.92 (±13.31), 963 (66.4%) were>50 years old and 979 (67.5%) were male. Notably, only 1011 (69.7%) were PCR positive, further emphasizing the fact that getting negative molecular testing is not usually as reliable as a positive result. Univariate analysis showed that the lymphocyte count differed between age categories, sex, PCR, and their combinations, and notified that cases older than 50 years and male sex have the lower lymphocyte count. As represented in Table 2, while the lymphocyte count in COVID-19 cases with less than 50 years old was 1.8×10^3/μL (1.2-2.5), it was 1.47×10^3/μL (0.84-2.16) in the older group (p<0.001). Also, men experienced a lower number of lymphocyte as compared to women (1.53×10^3/μL vs 1.76×10^3/μL; p=0.002). As mentioned, nearly 30% of the patients have negative PCR results which may be, at least partly, due to the lower copies of the virus reflecting less severity of the disease. Of particular interest, the lymphocyte count in the PCR-negative cases was 1.77×10^3/μL (0.98-2.45) which was significantly higher than its count in their positive counterparts (1.53×10^3/μL; p=0.004). Analysis of combinations of age, sex, and PCR further confirmed that the number of lymphocytes in male cases aged more than 50 years together with positive PCR results was significantly lower than the other classified groups (1.31×10^3/μL; p=0.000). The distribution patterns of the lymphocyte count concerning age, sex, PCR, and their combinations were represented in Figure 1. In a univariable logistic regression model, age (2.234; 95% CI: 1.732-2.881) and sex (1.354; 95% CI: 1.063-1.725)

were significantly associated with the lymphopenia (Table 3). Albeit PCR affects the percentage of lymphopenic COVID-19 patients with an odds ratio (OR) of 1.245, it was not statistically significant (p=0.08). Moreover, the odds ratios represented in Table 3 revealed that male cases with more than 50 years old age and positive PCR results have the greatest OR (2.88; 95% CI: 1.295-6.417) among all the classified groups.

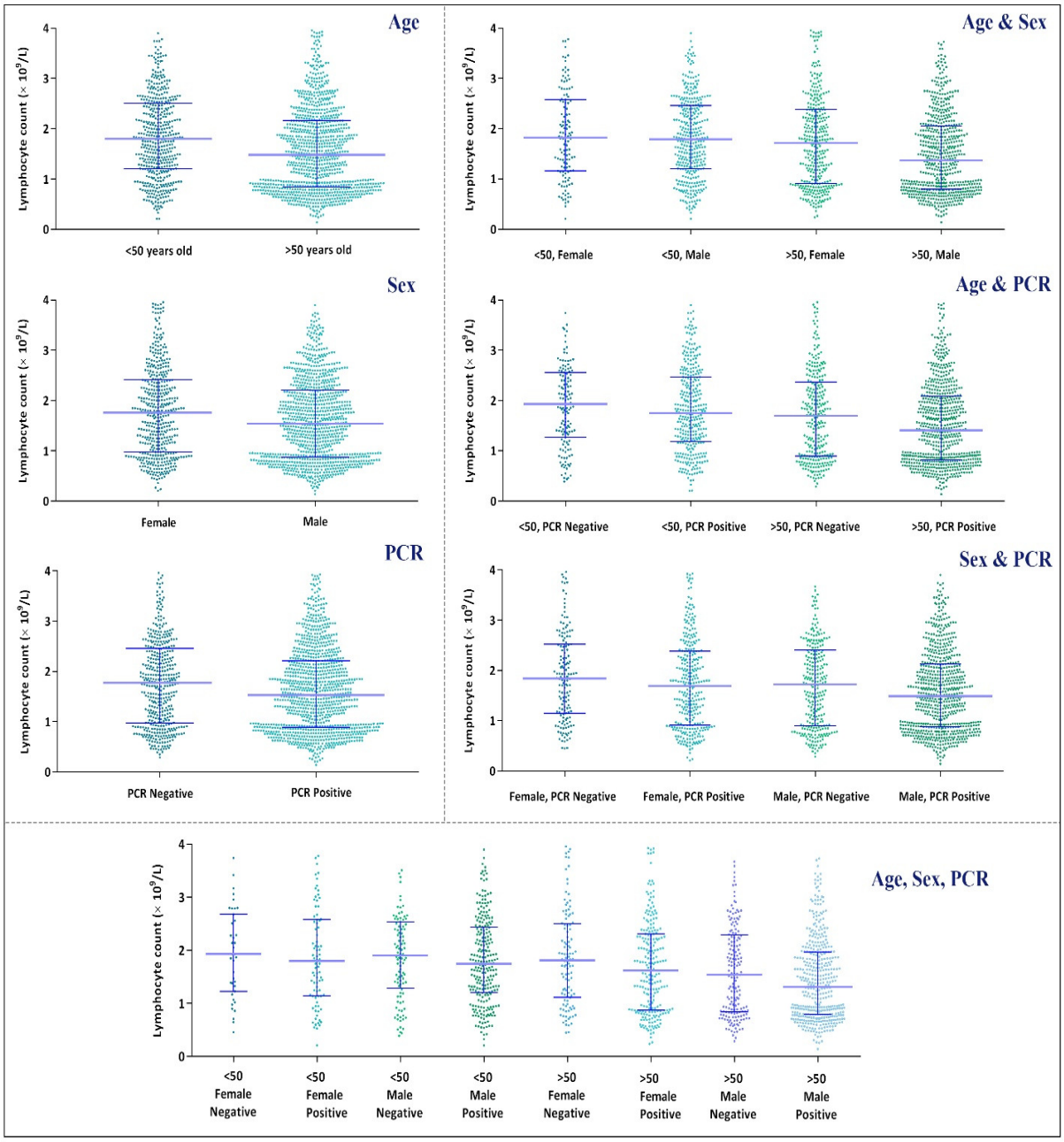

Figure 1. The distribution patterns of the lymphocyte count concerning age, sex, polymerase chain reaction (PCR), and their combinations

Table 2. Univariate analysis of the lymphocyte count between age categories, sex, polymerase chain reaction (PCR), and their combinations

	N (%)	Lymphocyte count ($\times 10^3/\mu L$) (Median, Q1, Q3)	*p*
Age (years)			
< 50	487 (33.6)	1.8 (1.2-2.5)	**≤0.001**
> 50	963 (66.4)	1.47 (0.84-2.16)	
Sex			
Female	471 (32.5)	1.76 (0.97-2.41)	**0.002**
Male	979 (67.5)	1.53 (0.88-2.2)	
PCR			
Negative	439 (30.3)	1.77 (0.98-2.45)	**0.004**
Positive	1011 (69.7)	1.53 (0.89-2.21)	
Age & Sex			
< 50, Female	127 (8.8)	1.82 (1.15-2.58)	**≤0.001**
< 50, Male	360 (24.8)	1.79 (1.2-2.46)	
> 50, Female	344 (23.7)	1.72 (0.91-2.38)	
> 50, Male	619 (42.7)	1.36 (0.79-2.05)	
Age & PCR			
< 50, Negative	144 (9.9)	1.92 (1.27-2.55)	**≤0.001**
< 50, Positive	343 (23.7)	1.74 (1.18-2.46)	
> 50, Negative	295 (20.3)	1.7 (0.89-2.37)	
> 50, Positive	668 (46.1)	1.41 (0.82-2.08)	
Sex & PCR			
Female, Negative	151 (10.4)	1.83 (1.14-2.51)	**≤0.001**
Female, Positive	320 (22.1)	1.68 (0.91-2.38)	
Male, Negative	288 (19.9)	1.72 (0.89-2.39)	
Male, Positive	691 (47.7)	1.48 (0.87-2.12)	
Age, Sex & PCR			
< 50, Female, Negative	39 (2.7)	1.92 (1.23-2.68)	**≤0.001**
< 50, Female, Positive	88 (6.1)	1.8 (1.14-2.58)	
< 50, Male, Negative	105 (7.2)	1.9 (1.28-2.53)	
< 50, Male, Positive	255 (17.6)	1.74 (1.2-2.43)	
> 50, Female, Negative	112 (7.7)	1.81 (1.11-2.49)	
> 50, Female, Positive	232 (16)	1.61 (0.87-2.31)	
> 50, Male, Negative	183 (12.6)	1.54 (0.83-2.28)	
> 50, Male, Positive	436 (30.1)	1.31 (0.79-1.96)	

The Association between Neutrophil Count and Age, Sex, and SARS-CoV-2 PCR Results

Unlike lymphocyte count which has been affected by age, sex, and PCR results, age older than 50 years was the only factor that significantly affected the number of neutrophils among COVID-19 patients. As represented in Table 4, the neutrophil count was $6.07\times10^3/\mu L$ in the older group as compared to its younger counterpart

(6.07×10^3/μL vs 4.96×10^3/μL; p=≤0.001). Although we found a higher number of neutrophils in males than females (5.8×10^3/μL vs 5.58×10^3/μL), there was statistically no significant difference (p=0.134). As summarized in Table 4, the results of SARS-CoV-2 PCR analysis had no significant effect on the neutrophil count, as well. The distribution patterns of the neutrophil count concerning age, sex, PCR, pairwise and triple combinations were represented in Figure 2.

The results of logistic regression were presented in Table 5. Analysis of the odds ratio for the number of neutrophils in COVID-19 cases revealed that the age older than 50 years was significantly associated with this factor. As represented in Table 5, OR for neutrophilia (neutrophil count >6.3×10^3/μL) was 2.25 (95% CI:

Table 3. Univariable logistic regression model of lymphopenia (lymphocytes <1.1) between age categories, sex, polymerase chain reaction (PCR), and their combinations

	Lymphopenia (<%)	OR (95% CI)	*p*
Age (Years)			
< 50	102/487 (20.9)	Reference	Reference
> 50	358/963 (37.2)	2.234 (1.732-2.881)	**≤0.001**
Sex			
Female	129/471 (27.4)	Reference	Reference
Male	331/979 (33.8)	1.354 (1.063-1.725)	**0.014**
PCR			
Negative	125/439 (28.5)	Reference	Reference
Positive	335/1011 (33.1)	1.245 (0.974-1.591)	0.080
Age & Sex			
< 50, Female	26/127 (20.5)	Reference	Reference
< 50, Male	76/360 (21.1)	1.040 (0.631-1.714)	0.879
> 50, Female	103/344 (29.9)	1.660 (1.018-2.707)	**0.042**
> 50, Male	255/619 (41.2)	2.721 (1.718-4.310)	**≤0.001**
Age & PCR			
< 50, Negative	29/144 (20.1)	Reference	Reference
< 50, Positive	73/343 (21.3)	1.072 (0.662-1.737)	0.777
> 50, Negative	96/295 (32.5)	1.913 (1.190-3.075)	**0.007**
> 50, Positive	262/668 (39.2)	2.559 (1.655-3.957)	**≤0.001**
Sex & PCR			
Female, Negative	35/151 (23.2)	Reference	Reference
Female, Positive	94/320 (29.4)	1.379 (0.881-2.158)	0.160
Male, Negative	90/288 (31.3)	1.506 (0.958-2.369)	**0.076**
Male, Positive	241/691 (34.9)	1.775 (1.179-2.672)	**0.006**
Age, Sex & PCR			
< 50, Female, Negative	8/39 (20.5)	Reference	Reference
< 50, Female, Positive	18/88 (20.5)	0.996 (0.392-2.536)	0.994
< 50, Male, Negative	21/105 (20)	0.969 (0.389-2.413)	0.946
< 50, Male, Positive	55/255 (21.6)	1.066 (0.463-2.450)	0.881
> 50, Female, Negative	27/112 (24.1)	1.231 (0.506-2.996)	0.647
> 50, Female, Positive	76/232 (32.8)	1.888 (0.828-4.304)	0.131
> 50, Male, Negative	69/183 (37.7)	2.345 (1.020-5.394)	**0.045**
> 50, Male, Positive	186/436 (42.7)	2.883 (1.295-6.417)	**0.009**

1.78-2.85, p<0.001) in cases aged over 50 years compared to those aged <50. In addition, the odds ratio of neutrophilia in women and men aged > 50 compared to their counterparts were 1.798 (95% CI: 1.157-2.796, p= 0.009) and 2.393 (95% CI: 1.577-3.630, p<0.001) respectively. Notably, OR for neutrophilia in patients aged older than 50 years, either with negative or positive PCR results, was 2.465 (95% CI: 1.59-3.80, p<0.001) and 2.23 (95% CI: 1.50-3.32, p<0.001), respectively; all suggesting that age older than 50 years was the most significant associated factor.

Table 4. Univariate analysis of the neutrophil count between age categories, sex, polymerase chain reaction (PCR), and their combinations

	N (%)	Neutrophil count ($\times 10^3/\mu L$) (Median, Q1-Q3)	*P*
Age (Years)			
< 50	487 (33.6)	4.96 (3.74-6.62)	**≤0.001**
> 50	963 (66.4)	6.07 (4.42-7.75)	
Sex			
Female	471 (32.5)	5.58 (4.04-7.18)	0.134
Male	979 (67.5)	5.8 (4.19-7.47)	
PCR			
Negative	439 (30.3)	5.81 (4.23-7.22)	0.691
Positive	1011 (69.7)	5.68 (4.13-7.42)	
Age & Sex			
< 50, Female	127 (8.8)	5.02 (3.72-6.73)	**≤0.001**
< 50, Male	360 (24.8)	4.91 (3.74-6.590	
> 50, Female	344 (23.7)	5.88 (4.29-7.44)	
> 50, Male	619 (42.7)	6.25 (4.57-7.86)	
Age & PCR			
< 50, Negative	144 (9.9)	4.90 (3.75-6.48)	**≤0.001**
< 50, Positive	343 (23.7)	4.97 (3.72-6.71)	
> 50, Negative	295 (20.3)	6.23 (4.49-7.73)	
> 50, Positive	668 (46.1)	6.04 (4.41-7.76)	
Sex & PCR			
Female, Negative	151 (10.4)	5.78 (4.13-7.17)	0.485
Female, Positive	320 (22.1)	5.51 (4.02-7.21)	
Male, Negative	288 (19.9)	5.82 (4.27-7.37)	
Male, Positive	691 (47.7)	5.8 (4.16-7.49)	
Age, Sex & PCR			
< 50, Female, Negative	39 (2.7)	4.87 (3.72-6.73)	**≤0.001**
< 50, Female, Positive	88 (6.1)	5.05 (3.73-6.74)	
< 50, Male, Negative	105 (7.2)	4.91 (3.77-6.44)	
< 50, Male, Positive	255 (17.6)	4.91 (3.71-6.7)	
> 50, Female, Negative	112 (7.7)	6.03 (4.34-7.34)	
> 50, Female, Positive	232 (16)	5.64 (4.27-7.69)	
> 50, Male, Negative	183 (12.6)	6.39 (4.69-7.97)	
> 50, Male, Positive	436 (30.1)	6.15 (4.51-7.83)	

The Association between Neutrophil-to-Lymphocyte Ratio and Age, Sex, and SARS-CoV-2 PCR Results

Several studies are reporting that the calculation of neutrophil-to-lymphocyte ratio (NLR) may allow clinicians to stratify COVID-19 severities on admission and guide early interventions to accelerate recovery.

To investigate whether there is a correlation between admission NLR and age, sex, and SARS-CoV-2 PCR results, we calculated this scoring tool in COVID-19 patients. Of particular interest, we found that the NLR was significantly associated with age and sex. As represented in Table 6, while the NLR in cases aged over 50 years was 3.48, it was 2.57 in the younger patients (p<0.001). Albeit the same finding was found concerning the correlation between sex and NLR (3.24 vs 2.80; p=0.001), we could find no significant association between the NLR and RT-PCR results. The resulting data also declared that male cases with more than 50 years old age and positive PCR results have the greatest NLR (3.95; 2.55-9.81) among all the classified groups (Table 6).

Table 5. Univariate logistic regression model of neutrophilia (neutrophils >6.3) between age categories, sex, polymerase chain reaction (PCR), and their combinations

	Neutrophilia (<%)	OR (95% CI)	*P*
Age (years)			
< 50	134/487 (27.5)	Reference	Reference
> 50	444/963 (46.1)	2.254 (1.780-2.853)	**≤0.001**
Sex			
Female	179/471 (38)	Reference	Reference
Male	399/979 (40.8)	1.122 (0.896-1.406)	0.316
PCR			
Negative	180/439 (41)	Reference	Reference
Positive	398/1011 (39.4)	0.934 (0.744-1.174)	0.559
Age & Sex			
< 50, Female	36/127 (28.3)	Reference	Reference
< 50, Male	98/360 (27.2)	0.946 (0.603-1.483)	0.807
> 50, Female	143/344 (41.6)	1.798 (1.157-2.796)	**0.009**
> 50, Male	301/619 (48.6)	2.393 (1.577-3.630)	**≤0.001**
Age & PCR			
< 50, Negative	39/144 (27.1)	Reference	Reference
< 50, Positive	95/343 (27.7)	1.031 (0.666-1.597)	0.890
> 50, Negative	141/295 (47.8)	2.465 (1.599-3.800)	**≤0.001**
> 50, Positive	303/668 (45.4)	2.235 (1.501-3.327)	**≤0.001**
Sex & PCR			
Female, Negative	62/151 (41.1)	Reference	Reference
Female, Positive	117/320 (36.6)	0.827 (0.557-1.229)	0.348
Male, Negative	118/288 (41)	0.996 (0.668-1.487)	0.986
Male, Positive	281/691 (40.7)	0.984 (0.688-1.407)	0.929
Age, Sex & PCR			
< 50, Female, Negative	12/39 (30.8)	Reference	Reference
< 50, Female, Positive	24/88 (27.3)	0.844 (0.369-1.928)	0.687
< 50, Male, Negative	27/105 (25.7)	0.779 (0.347-1.748)	0.545
< 50, Male, Positive	71/255 (27.8)	0.868 (0.417-1.807)	0.706
> 50, Female, Negative	50/112 (44.6)	1.815 (0.836-3.940)	0.132
> 50, Female, Positive	93/232 (40.1)	1.505 (0.726-3.120)	0.271
> 50, Male, Negative	91/183 (49.7)	2.226 (1.063-4.661)	**0.034**
> 50, Male, Positive	210/436 (48.2)	2.091 (1.033-4.233)	**0.040**

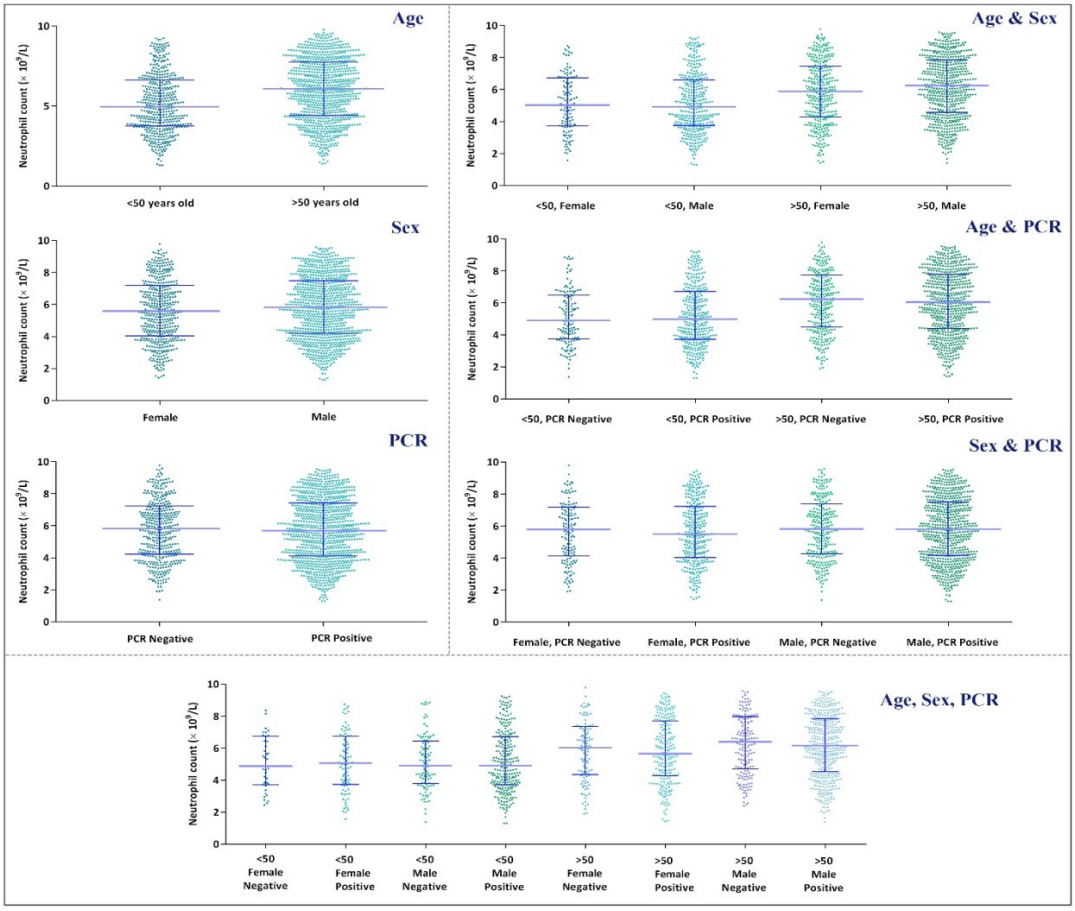

Figure 2. The distribution patterns of the neutrophil count concerning age, sex, polymerase chain reaction (PCR), and their combinations

DISCUSSION

At the time of writing this article (May 27, 2020), over 5,780,000 cases were confirmed all around the world with sorrowful statistics of more than 355,000 deaths (https://www.who.int/), recalling that SARS-CoV-2 still takes its toll. Albeit death statistics are different depending on the geographical setting, a recent Single-Centre study conducted on 2968 hospitalized COVID-19 cases revealed an overall case fatality rate (CFR) of 8.06% among hospitalized patients in Iran.[12] Containment of pandemic infections mainly depends on prompt identification of carriers,[13] achievable through strict surveillance and truthful diagnostic testing. Even though molecular identification of SARS-CoV-2 in the pharyngeal swab specimens using nucleic acid amplification test is the gold standard method, its low sensitivity in early infection, and the discomfort of the collection process together with its long turnaround time are among major concerns facing with this method.[9,10,14] In this retrospective Single-Centre study reviewing the results of the lymphocyte and neutrophil counts of 1450 Iranian COVID-19 patients, we found that 439 cases (30.3%) were PCR negative that is in agreement with a study reporting that only 59% (601/1014) of COVID-19 patients had positive RT-PCR results.[10] In a scramble to fix this challenge before it is too late, an urgent necessity to apply an alternative method is felt much more than before; one that would miss fewer cases while still being simple.

In a recent study, it has been reported that routine blood test analysis might be used as an alternative approach for identifying COVID-19 patients, especially in those countries which suffer from a large shortage of specialized laboratory.[15] Notably, our results showed

that lymphopenia (lymphocyte count $<1.1\times10^3/\mu L$) is a common finding among patients with SARS-CoV-2 infection. Our results were in agreement with several studies that reported the occurrence of lymphopenia in 9%,[16] 50%,[17] and even up to 73%[18] and 75%[19] of infected cases. It is worth mentioning that age and sex were significantly correlated with the percentage of lymphopenia in our study. The calculation of the odds ratio revealed that COVID-19 cases who were more than 50 years old experienced lymphopenia 2.23 times more than those aged less than 50 ($p<0.001$). Besides, the emergence of lymphopenia in the male sex was 1.35 times more than female ($p=0.014$). As it is quite clear from the results, age is the most significant factor affecting lymphocyte count in patients with SARS-CoV-2 infection. Notably, in a prediction model for diagnosis of COVID-19, Wynants et al. suggested age, body temperature, and clinical symptoms as the most reported predictors of the presence of COVID-19 in patients with the suspected disease.[20]

Contrary to the current belief that a decreased number of lymphocytes is seemingly an appropriate item that should be taken into account in the identification of COVID-19, there are conflicting results concerning the alteration in the neutrophil count. While previous reports indicated the probability of neutrophilia (neutrophil count $>6.3\times10^3/\mu L$) in 38%[21] and 20%[22] of infected cases, another study found completely different data suggesting that neutrophil count is lower in COVID-19 cases as compared to patients negative for the disease.[15] In the present study, we found that nearly 40% of patients —either with positive or negative PCR- had neutrophilia. However, when the percentage of increased neutrophils was analyzed for age, we found that the infected cases aged more than 50 experienced higher percentages than those with <50 years (46 vs 27.5; $p=\leq0.001$); proposing that patients with old age usually experience disease with more severity, which in turn, may lead to an increased rate of neutrophils release from bone marrow storage to the blood to more effectively battle with the virus.

A shred of evidence reported that the calculation of neutrophil-to-lymphocyte ratio (NLR) may be an appropriate approach to predict the severity of the disease in SARS-CoV-2 infection. This scoring tool may guide early interventions to accelerate recovery and shorten the course of the disease to alleviate the shortage of medical resources and reduce mortality.[23] Yang et al reported that the elevated NLR may contribute as an independent factor to reflect the progression of COVID-19 towards an unfavorable clinical outcome.[24] A meta-analysis of six studies also demonstrated that an increased NLR level may probably reflect an enhanced inflammatory process and may suggest a poor prognosis in patients with SARS-CoV-2 infection.[25] Also, the results of a recent study revealed that the incidence of critical illness in COVID-19 patients aged more than 50 was 9.1% (1/11) for patients having NLR < 3.13, while it was 50% (7/14) for those with NLR ≥ 3.13.[26] In agreement, we found that the ratio of neutrophil-to-lymphocyte was significantly higher in COVID-19 patients concerning age. While NLR was 2.57 in cases with less than 50 years old,it was 3.48 in patients aged more than 50. Notably, the same data was found when we compared the NLR between males and females; suggesting that men have a greater NLR than women (3.24 vs 2.80). Taken together, the present study suggests that a decreased number of lymphocytes along with increased neutrophil count may probably serve as simple, rapid, and economical biomarkers, and are seemingly appropriate items that should be taken into account in the identification of patients with COVID-19, especially those aged more than 50.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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