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ORIGINAL ARTICLE

The Importance of Neutrophil/Lymphocyte and Lymphocyte/Monocyte Ratios in The Diagnosis of Influenza in Children

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SUMMARY

Background: **We aimed to analyze the neutrophil/lymphocyte ratio (NLR) and lymphocyte/monocyte ratio (LMR) to investigate their value in supporting the diagnosis of influenza in cases with influenza-like symptoms.**
Methods: **A total of 5,693 pediatric patients who applied to the Pediatric Clinic between January 2015 and December 2018 were included in the study. Complete blood count and influenza rapid antigen tests were evaluated at the time of admission.**
Results: **The mean LMR was significantly lower in patients with influenza A than non-influenza A patients ($p < 0.001$). LMR was also significantly lower in those with influenza A or B compared to those in the influenza-negative group ($p < 0.001$). There was no significant difference ($p = 0.83$) in terms of the mean LMR between influenza B positive and negative patients. The mean NLR was significantly higher in influenza A positive patients in comparison with influenza A negative patients ($p < 0.001$), and it was significantly lower in influenza B positive patients than in influenza B negative patients ($p < 0.001$).**
Conclusions: **We concluded that LMR and NLR, which had been rarely examined in the literature, had important value in the diagnosis of influenza. However, these values alone were far from being sufficient for the definitive diagnosis of influenza.**
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KEY WORDS

influenza, child, neutrophil/lymphocyte, lymphocyte/monocyte

INTRODUCTION

Influenza is a viral infectious disease commonly seen all over the world. There are half a million cases reported annually in the USA and nearly 200 thousand of them are hospitalized. While influenza can be observed as mild cases, it can course with high morbidity and mortality rates, particularly in those classified as high-risk groups. In pediatric patients, influenza usually presents with symptoms such as fever, cough, myalgia, and nasal discharge, but it can also cause serious conditions such as respiratory failure and encephalitis [1-4].

Recent studies have reported that neutrophil/lymphocyte ratio (NLR) may be a new indicator for the detection of inflammation that develops after chronic obstructive pulmonary disease and coronary artery disease. Furthermore, it has been stated that it can provide information about the prognosis of many malignant diseases. It has been indicated that lymphopenia is usually observed in influenza cases and that lymphocyte, monocyte, and neutrophil values, and the ratios between them will give information about prognosis in influenza cases. However, the number of studies investigating whether these ratios have a diagnostic significance is very limited [5-8].

In our study, we aimed to analyze differences in hematological parameters, especially NLR and lymphocyte/monocyte ratio (LMR) in pediatric patients who presented with influenza-like illness (ILI) and to investigate whether they have a value in the diagnosis of influenza.

MATERIALS AND METHODS

This study was approved by the local ethics committee and planned retrospectively. The data of the patients were analyzed by scanning the hospital records.

Pediatric patients (n = 5,693) admitted to the pediatric emergency service and outpatient clinics of our tertiary hospital with ILI between January 2015 and December 2018 were included in the study. Influenza rapid antigen test (Quickvue Influenza A, B Quidel, USA) was used for the nasopharyngeal swab samples taken from the children. All patients' complete blood count and influenza rapid antigen test results were evaluated. NLR and LMR were then calculated and analyzed statistically.

Definitions

ILI was considered a combination of fever (38°C and above) with two or more signs and symptoms such as cough, cold, pharyngitis, fatigue, shortness of breath, myalgia, and headache which occurred in the last seven days.

ILI cases were proven to be an influenza infection when a positive result was detected by rapid antigen test of the nasopharyngeal swab samples.

Statistical analysis

All statistical analyses were performed using SPSS 25.0 software (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). Descriptive data were presented as numbers and percentages. Comparisons between the groups in terms of categorical variables were performed by Pearson's chi-square test and Fisher's exact test. Whether continuous variables were normally distributed was confirmed by the Kolmogorov-Smirnov test. Differences between the groups in terms of continuous variables were analyzed by Student's *t*-test. The capacity of hematological parameters to predict the presence of influenza in patients was analyzed by using the receiver operating characteristic (ROC) curve analysis. The results were evaluated in a 95% confidence interval, and $p < 0.05$ values were considered significant. Bonferroni correction was performed when necessary.

RESULTS

Of the 5,693 patients, 2,623 (46.1%) were female and 3,070 (53.9%) were male. Their average age was 4.98 ± 3.21 years (2 - 16 years). According to the rapid test results, 1,548 (27.2%) of the patients had influenza A, and 359 (6.3%) had influenza B. Of all patients, 1,907 (33.5%) were positive for influenza (A or B).

It was observed that the mean LMR was significantly lower in patients who were positive for influenza A compared to those who were negative ($p < 0.001$). LMR was also found to be significantly lower in those with influenza A or B compared to the influenza negative group ($p < 0.001$). However, there was no significant difference ($p = 0.83$) in terms of the mean LMR between influenza B positive and negative patients. The mean NLR was significantly higher in patients who were positive for influenza A compared to those that were negative ($p < 0.001$), and it was significantly lower in patients with positive influenza B than negative patients ($p < 0.001$). There was no significant difference seen in terms of the NLR ($p = 0.023$) between influenza A or B positive patients and influenza negative patients (Table 1, 2, 3).

Mean leukocyte count ($p < 0.001$), neutrophil count ($p < 0.001$), lymphocyte count ($p < 0.001$), monocyte count ($p < 0.001$), and platelet count ($p < 0.001$) were found to be significantly lower in patients with positive influenza A and B in comparison with the negative group (Table 1, 2).

Mean leukocyte count ($p < 0.001$), neutrophil count ($p < 0.001$), lymphocyte count ($p < 0.001$) values were found to be significantly lower in influenza A or B positive patients in comparison to the patients who were negative for both influenza A and B (Table 3).

In the ROC analyses, the sensitivity rate of the NLR in the diagnosis of influenza A for the value of 2.035 was 48%, and the specificity rate was 46.4% (AUC: 0.543; $p < 0.001$; LB: 0.526; UB: 0.560; CI 95%) (Figure 1). The sensitivity rate of the NLR in the diagnosis of influenza B for the value of 1.675 was 50.1%, and the specificity rate was 55.5% (AUC: 0.453; $p = 0.003$; LB: 0.424; UB: 0.482; CI 95%) (Figure 2). The sensitivity rate of the LMR in the diagnosis of influenza A for the value of 2.055 was 50.6%, and the specificity rate was 62.8% (AUC: 0.421; $p < 0.001$; LB: 0.404; UB: 0.437; CI 95%). The sensitivity rate of the LMR in the diagnosis of influenza B for the value of 2.485 was 49.9%, and the specificity rate was 47.4% (AUC: 0.519; $p = 0.228$; LB: 0.488; UB: 0.550; CI 95%).

As a result of the analysis of CRP and all other hematological parameters in the ROC analyses, the threshold values could not exceed a sensitivity of 60% and a specificity of 70% even in the data found to be most signifi-

Table 1. Evaluation with CRP and hematological parameters for influenza A.

	Influenza A positive (mean ± SD)	Influenza A negative (mean ± SD)	p
LMR	2.63 ± 2.07	3.11 ± 2.75	< 0.001
NLR	3.46 ± 4.27	2.96 ± 3.51	< 0.001
Leukocyte (10^3/mL)	8.66 ± 3.71	11.19 ± 6.52	< 0.001
Neutrophil (10^9/L)	5.12 ± 3.19	6.41 ± 4.69	< 0.001
Lymphocyte (10^9/L)	2.42 ± 1.57	3.32 ± 2.13	< 0.001
Monocyte (10^9/L)	1.05 ± 0.52	1.26 ± 0.98	< 0.001
Platelet (10^3/mL)	269.40 ± 89.68	301.86 ± 111.36	< 0.001
CRP	15.81 ± 58.10	23.00 ± 37.21	< 0.001

CRP - C-reactive protein, LMR - lymphocyte/monocyte ratio, NLR - neutrophil/lymphocyte ratio, SD - standard deviation.

Table 2. Evaluation with CRP and hematological parameters for influenza B.

	Influenza B positive (mean ± SD)	Influenza B negative (mean ± SD)	p
LMR	2.95 ± 1.86	2.98 ± 2.64	0.83
NLR	2.38 ± 2.31	3.15 ± 3.81	< 0.001
Leukocyte (10^3/mL)	7.50 ± 3.42	10.70 ± 6.07	< 0.001
Neutrophil (10^9/L)	4.08 ± 2.66	6.19 ± 4.43	< 0.001
Lymphocyte (10^9/L)	2.41 ± 1.40	3.12 ± 2.06	< 0.001
Monocyte (10^9/L)	0.95 ± 0.49	1.22 ± 0.90	< 0.001
Platelet (10^3/mL)	246.17 ± 78.19	296.14 ± 107.79	< 0.001
CRP	10.97 ± 14.48	21.70 ± 45.29	< 0.001

CRP - C-reactive protein, LMR - lymphocyte/monocyte ratio, NLR - neutrophil/lymphocyte ratio, SD - standard deviation.

Table 3. Evaluation with CRP and hematological parameters for influenza A or B.

	Positive (mean ± SD)	Negative (mean ± SD)	p
LMR	2.7 ± 2.04	3.12 ± 2.81	< 0.001
NLR	3.25 ± 3.99	3.02 ± 3.59	0.23
Leukocyte (10^3/mL)	8.44 ± 3.68	11.53 ± 6.62	< 0.001
Neutrophil (10^9/L)	4.92 ± 3.12	6.62 ± 4.77	< 0.001
Lymphocyte (10^9/L)	2.42 ± 1.54	3.41 ± 2.17	< 0.001
Monocyte (10^9/L)	1.03 ± 0.51	1.29 ± 1.01	< 0.001
Platelet (10^3/mL)	264.87 ± 87.99	307.02 ± 112.53	< 0.001
CRP	14.90 ± 52.83	24.07 ± 38.37	< 0.001

CRP - C-reactive protein, LMR - lymphocyte/monocyte ratio, NLR - neutrophil/lymphocyte ratio, SD - standard deviation.

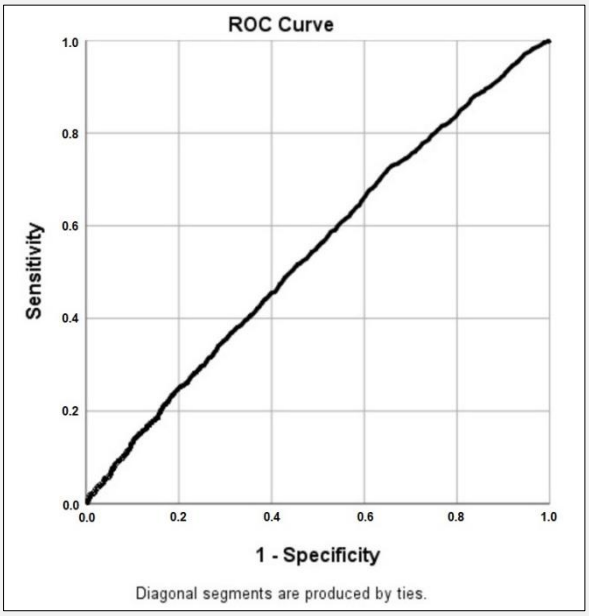

Figure 1. In the ROC analysis, the sensitivity rate of the neutrophil/lymphocyte ratio for the value of 2.035 in the diagnosis of influenza A was 48%, and the specificity rate was 46.4% (AUC: 0.543; p < 0.001; LB: 0.526; UB: 0.560; CI 95%).

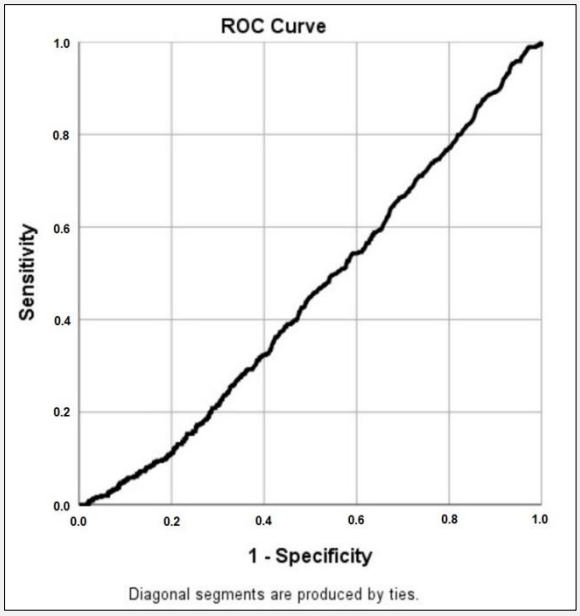

Figure 2. In the ROC analysis, the sensitivity rate of the neutrophil/lymphocyte ratio for the value of 1.675 in the diagnosis of influenza B was 50.1%, and the specificity rate was 55.5% (AUC: 0.453; p = 0.003; LB: 0.424; UB: 0.482; CI 95%).

cant. For example, for CRP, the sensitivity rate of the threshold value of 10.45 for the diagnosis of influenza was 48.0%, and the specificity value was 61.2% (AUC: 0.562; $p < 0.001$; LB: 0.546; UB: 0.578; CI 95%). According to these results, a reliable threshold value could not be determined in influenza A or B positive cases for any of the hematologic parameters including CRP.

DISCUSSION

Influenza can easily be confused with other bacterial or viral upper respiratory tract infections. Although the course of influenza is mostly mild, it may have a more severe course in some risk groups and children and may cause morbidity and mortality. Therefore, an accurate and rapid diagnosis of influenza is critical. Rapid tests used for influenza are not available in all health centers. This situation has led researchers to investigate whether there are other predictive indicators for the diagnosis of influenza. In our study, blood values in the complete blood count used in almost all health centers was investigated for the potential of carrying a predictive value for influenza [1-6].

Lymphopenia was reported to be an expected condition in seasonal influenza cases [5,9]. It was reported that there were distinct lymphopenia, neutropenia, and leukopenia in hospitalized children with influenza and similar infections and stated that these indicators had prognostic value in patients with ILI [7]. In a previous study, it was reported that leukopenia and neutropenia were present in patients hospitalized due to influenza [10]. It was indicated that lymphopenia was found in children with both influenza A and B [11]. In another study it was reported that besides lymphopenia, monocytosis accompanied by low or normal leukocyte values was also present in influenza patients [5]. In this analysis, the researchers stated that the LMR was significantly lower in influenza cases, and this indicator could be used instead of the rapid test in the diagnosis of influenza. In our study, besides the lymphocyte level, a significant decrease was also observed in the monocyte levels of influenza positive cases ($p < 0.001$). Furthermore, the mean LMR was significantly lower in influenza patients ($p < 0.001$). Nevertheless, a threshold value with a high level of reliability was not detected in the ROC analysis. In a study by Merekoulias et al. [5] and our study, the LMR decreased significantly in influenza cases. Our study evaluating more than 5,000 cases adds a more reliable value to the mentioned study which included a smaller population of only 58 cases. Nonetheless, although the LMR decreases significantly in influenza cases, this decrease is not sufficient to determine a threshold value. Consequently, it does not seem to replace the rapid test used in the diagnosis of influenza. The NLR is a simple indicator that can be obtained only from the patient's complete blood count values easily and at a low cost like the LMR. The NLR has been reported to contribute to the determination of the diagnosis and prognosis of many diseases such as malignancies, coronary artery disease, and bacteremia. Furthermore, it provides information in terms of inflammation in some respiratory diseases, especially in COPD [8,12, 13]. It was reported that the NLR provides information about mortality in pneumonia [14]. Kurtipek et al. [8] and Chiang et al. [15] defined threshold values for the NLR in terms of COPD diagnosis. In a previous study, it was found that the NLR was significantly high in hospitalized pediatric patients with viral upper respiratory tract infections including influenza [7]. These researchers indicated that the NLR might be a prognostic marker for ILI. In another study, it was reported that the NLR could be used for diagnostic screening in swine influenza cases [6]. In another study included patients with respiratory tract infections which included influenza and non-influenza, it was found that the sensitivity of NLR for influenza detection was higher than that of neutrophil and lymphocyte among the systemic inflammatory markers [16]. In our study, the NLR was found to be significantly high in influenza A patients ($p < 0.001$). It was also found to be significantly lower in influenza B patients compared to the negative patients ($p < 0.001$). However, a reliable threshold value for the diagnosis of influenza could not be determined in the ROC analysis. It was reported that CRP levels might have value in the diagnosis and prognosis of influenza in many studies [17-20]. In our study, CRP, leukocyte, and PLT levels were found significantly lower in influenza-positive patients than in influenza-negative patients ($p < 0.001$). According to these data, it can be expected that in patients with an ILI these parameters may increase in bacterial or viral diseases including influenza. Having these parameters at lower limit values may support the diagnosis of influenza. However, in the ROC analyses, reliable threshold values for CRP and for other hematological parameters could not be found for the diagnosis of influenza. Even though CRP and other blood values support influenza in influenza-like illness, these parameters cannot be directly relied on for the definitive diagnosis of influenza.

Based on the ROC analysis results of our study, no threshold value with an acceptable sensitivity or specificity was obtained in any of the hematological parameters.

Although our data included a large number of patients, there were some limitations in our study. For example, to reach a large number of cases, reference techniques such as polymerase chain reaction were unfortunately not performed; therefore, only rapid test results were evaluated in the diagnosis of influenza. Moreover, due to the retrospective nature of the study it was impossible to perform additional testing. However, the data that were analyzed in the study were sufficient enough to reach the desired result.

A completely healthy control group was not used in our study. The reason for this was to investigate whether there is a parameter that can be used in the differential diagnosis of influenza and ILI. All ILI patients were in-

cluded in the study so that our study was not a classic repetition of other studies in the literature. In this study, influenza-positive patients were used as the study group and influenza-negative patients as the control group. In this way, the differences between influenza and other diseases in pediatric patients presenting with upper respiratory tract infection were examined. It is thought that the values obtained using this method will provide healthier data to clinicians in the diagnosis of children with a pre-diagnosis of influenza. Our study included the results of influenza rapid test and hematological parameters of a large number of pediatric patients. In this study, we presented the diagnostic value of LMR and NLR which has rarely been examined in the literature. These values were found to be significant in the diagnosis of influenza, but were insufficient for the definitive diagnosis of influenza. Nevertheless, we believe that our data may contribute to pediatricians and researchers in developing the necessary material and methods to efficiently diagnose and therefore effectively treat influenza.

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Declaration of Interest:
The authors declare no competing interest.

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