Routine blood parameters can detect early influenza virus infection in children

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Research article

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Abstract

Purpose

We aimed to explore the value of routine blood parameters, such as the lymphocyte (LYM) count, platelet (PLT) count, lymphocyte-to-monocyte ratio (LMR), neutrophil-to-lymphocyte ratio (NLR), LYM*PLT and mean platelet volume-to-platelet ratio (MPV/PLT), are widely used to predict the prognosis of infectious diseases, for predicting influenza virus infection in children.

Methods

We conducted a single-center, retrospective, observational study on fever with influenza-like symptom in pediatric outpatients in different age groups and evaluated the predictive value of various routine blood parameters within 48 hours of the onset of fever after influenza virus infection.

Results

The LYM count, PLT count, LMR and LYM*PLT were lower, and the NLR and MPV/PLT were higher in the infected children. The LYM count, LMR and LYM*PLT in the infected group were lower in the 1- to 6-year-old group, and the LMR and LYM*PLT in the infected group were lower in the > 6-year-old group. In the 1- to 6-year-old group, the cutoff value of the LMR for predicting influenza A virus infection was 3.75, the sensitivity was 81.87%, the specificity was 84.31%, and the AUC was 0.886; the cutoff value of the LMR for predicting influenza B virus infection was 3.71, the sensitivity was 73.58%, the specificity was 84.31%, and the AUC was 0.843. In the > 6-year-old group, the cutoff value of the LMR for predicting influenza A virus infection was 3.05, the sensitivity was 89.27%, the specificity was 89.61%, and the AUC was 0.949; the cutoff value of the LMR for predicting influenza B virus infection was 2.88, the sensitivity was 83.19%, the specificity was 92.21%, and the AUC was 0.924.

Conclusions

Routine blood tests are simple, inexpensive and easy to perform, and they are useful for predicting influenza virus infection in children. The LMR had the strongest predictive value for influenza virus infection in children older than 1 year, especially influenza A virus infection.

Introduction

Influenza is an acute respiratory infectious disease caused by influenza viruses. There are 1 billion patients with seasonal influenza each year worldwide, among whom 3 to 5 million have severe cases and as many as 500,000 die [1]. Although most children recover spontaneously from infection, morbidity and mortality are higher in children with underlying diseases who are younger than 5 years, especially those younger than 2 years [2]. However, previously healthy children are also at risk. In the USA, the admission rate of non-high-risk children due to influenza was estimated to be 9 per 10000 children younger than 5 years old [3]. According to the WHO, in the past 11 influenza epidemic seasons, the annual infection rate of children was as high as approximately 50% [4]. Complications of influenza, including pneumonia, myocarditis, septic shock and multiple organ dysfunction, are
the main causes of death in children [5]. Early (within 48 hours after infection) use of anti-influenza drugs can significantly relieve symptoms, shorten the course of disease, and reduce complications. Therefore, the early and rapid diagnosis of influenza and the early use of anti-influenza drugs are essential to improve the prognosis of influenza in children.

The diagnosis of influenza depends on the detection of influenza virus nucleic acid in the respiratory tract, the isolation of influenza virus or the detection of a level of serum-specific antibodies that is at least 4 times the normal level. Common detection methods include virus isolation and culture, RT-PCR and serological detection, all of which have advantages and disadvantages. Virus isolation and culture, RT-PCR and serological detection are time-consuming and difficult and are not suitable for outpatient screening. Viral antigen detection, such as rapid influenza detection, is rapid and simple, with good specificity; however, the sensitivity is low, and it is prone to false negative results [6].

Routine blood tests are the first choice in pediatric fever clinics. They are easy to perform and inexpensive. In recent years, studies have found that the lymphocyte-to-monocyte ratio (LMR), neutrophil-to-lymphocyte ratio (NLR), mean platelet volume-to-platelet ratio (MPV/PLT) and lymphocytes multiplied by platelets (LYM*PLT) can be used as new inflammatory markers to predict the prognosis of infectious diseases [7-9], tumors [10, 11] and cardiovascular diseases [12, 13]; these markers have been widely studied in clinical practice.

There have been some studies on routine blood index values and influenza A infection in adults, but there have been few studies in children. In this study, we collected early routine blood test results from children suspected of having influenza and further explored the value of the LMR, NLR, LYM*PLT, MPV/PLT, lymphocyte (LYM) count and platelet (PLT) count for the prediction of influenza in children.

**Materials And Methods**

**Patients**

Children with fever and influenza-like symptoms who were 0.2 to 14 years old and presented at the First Affiliated Hospital of Wenzhou Medical University from January 2018 to February 2020 were included in this study. Influenza-like symptoms were defined as follows: fever (temperature \( \geq 38^\circ \text{C} \)), cough or sore throat [14]. The exclusion criteria were as follows: (1) systemic chronic diseases, such as diseases of the blood, heart, lung, liver and kidney; (2) immunodeficiency, due to a tumor, HIV infection, or the long-term use of oral hormones or immunosuppressive agents; (3) severe or critical illness; (4) bacterial infections, such as sepsis and suppurative tonsillitis; and (5) Epstein-Barr virus (EBV) infection. This study was approved by the Ethics Committee for Clinical Research (ECCR) of the First Affiliated Hospital of Wenzhou Medical University (No. 2020-065), and all the parents signed the informed consent form.

**Detection of Routine Blood Parameters**

Finger prick blood samples taken from children with suspected influenza were subjected to routine blood tests within 48 h of the onset of fever. A routine analyzer (XN-350, SYSMEX, Japan) was used for detection. The LYM count, monocyte (MON) count, PLT count and mean platelet volume (MPV) were recorded. Additionally, other hematological parameters were calculated: the LMR is the ratio of lymphocytes to monocytes, the NLR is the ratio
of neutrophils to lymphocytes, the MPV/PLT is the MPV divided by PLT count, and the LYM*PLT is the lymphocytes multiplied by the platelets.

Detection of Influenza Virus Nucleic Acid by RT-PCR

A fully automatic nucleic acid extractor and the associated reagents (Shanghai ZJ Bio-Tech Co., Ltd) were used to extract all nucleic acid from pharyngeal swabs. Throat swab specimens obtained for the purpose of influenza virus nucleic acid determination were subjected to RT-PCR, and influenza A and B virus nucleic acid detection kits were used (Z-RR-0097-02, Shanghai ZJ Bio-Tech Co., Ltd). The amplification system used a final volume of 25 µl, consisting of 19 µl of a mixture of influenza A and B virus nucleic acid fluorescent probes, 1 µl of enzymes, and 5 µl of the sample. The amplification conditions were as follows: 45°C reverse transcription for 10 min, predenaturation at 95°C for 15 min, denaturation at 95°C for 15 s, annealing elongation and fluorescence detection at 60°C for 60 s for 45 cycles. All amplification reactions were performed with an ABI7500 quantitative PCR instrument (Applied Biosystems, Inc. USA).

Statistical Analysis

SPSS 23.0 (SPSS Inc., Chicago, IL, USA) was used for data analyses. The measurement data are expressed as the means ± standard deviations (x±s). The measurement data were tested by one-way variance tests. The pairwise comparisons of the mean were performed by the LSD method. The data were tested with the chi-square test. Receiver operator characteristic curve (ROC) analysis was used to evaluate the diagnostic value of the LYM count, PLT count, LMR, NLR, LYM*PLT and MPV/PLT for influenza A and B virus infection. Statistical significance was indicated by P < 0.05.

Results

Patient Characteristics

A diagnosis of influenza infection was made based on the occurrence of influenza-like symptoms with a positive RT-PCR for influenza A or B [15]. In this study, a total of 388 children with influenza A virus infection (A+ group), 169 children with influenza B virus infection (B+ group), 198 children with influenza-like symptoms who were negative for both influenza A and B viruses (A-B- group) and 259 healthy children (H group) who underwent physical examinations at the same time were included. According to age, all children were divided into three groups: the <1-year-old group, the 1- to 6-year-old group and the >6-year-old group. In the <1-year-old group, there were 18 patients in the A+ group (mean age 0.7±0.2), 3 patients in the B+ group (mean age 0.6±0.2), 19 patients in the A-B- group (age 0.7±0.1), and 38 patients in the H group (mean age 0.6±0.2). In the 1- to 6-year-old group, there were 193 patients in the A+ group (mean age 3.6±1.4), 53 patients in the B+ group (mean age 3.9±1.2), 102 patients in the A-B- group (mean age 3.4±1.2), and 118 patients in the H group (mean age 3.6±1.5). In the >6-year-old group, there were 177 patients in the A+ group (mean age 9.4±2.4), 113 patients in the B+ group (mean age 8.8±2.2), 77 patients in the A-B- group (mean age 9.3±2.6), and 105 patients in the H group (mean age 9.6±2.3). There were no statistically significant differences in the age and sex distributions between groups within the three age groups (P>0.05) (Table 1).

Differences in Routine Blood Parameters in the Three Age Groups
The red blood cell count (RBC) and hemoglobin (Hb) level in the A+ group, B+ group, A-B- group and H group were not significantly different in the groups of patients <1 year old, 1-6 years old and >6 years old. In all three age groups, compared with the H group, the A+ group, B+ group, and A-B- group had lower LYM counts, PLT counts, LMRs and LYM*PLT values, and higher NLRs and MPV/PLT values (Figure 1). In the <1-year-old group, there were no significant differences in the LYM count, PLT count, LMR, NLR, LYM*PLT and MPV/PLT between the A+ group and A-B- group. There were only 3 patients in the B+ group, so no statistical analysis was performed. In the 1- to 6-year-old group, the LYM count, PLT count, LMR, LYM*PLT and MPV/PLT were significantly different between the A+ group and the A-B- group; the PLT count, LMR and LYM*PLT were significantly different between the B+ group and the A-B- group (Figure 1). In the >6-year-old group, the LYM count, LMR, NLR and LYM*PLT were significantly different between the A+ group and the A-B- group; the PLT count, LMR and LYM*PLT were significantly different between the B+ group and the A-B- group (Figure 1).

Value of the LYM Count, PLT Count, LMR, NLR, LYM*PLT, and MPV/PLT for the Prediction of Influenza

1- to 6-year-old A+ group

The variable that best predicted positivity for influenza virus A based on the area under the curve (AUC) was the LMR. When the A-B- group was taken as a reference, the cutoff value was 3.75, the AUC was 0.886, and the sensitivity and specificity were 81.87% and 84.31%, respectively. When the H group was used as the reference, the maximum AUC was obtained with the LYM*PLT (followed by the LMR); the cutoff value of the LYM*PLT was 680.48, the AUC was 0.958, and the sensitivity and specificity were 90.67% and 89.66%, respectively (Figure 2).

1- to 6-year-old B+ group

The variable that best predicted positivity for influenza virus B based on the AUC was the LMR. When the A-B- group was taken as a reference, the cutoff value was 3.71, the AUC was 0.843, and the sensitivity and specificity were 73.58% and 84.31%, respectively. When the H group was used as the reference, the cutoff value for the LMR was 4.47, the AUC was 0.918, and the sensitivity and specificity were 86.79% and 89.66%, respectively (Figure 3).

>6-year-old A+ group

The maximum AUC was obtained with the LMR. When the A-B- group was taken as a reference, the cutoff value was 3.05, the AUC was 0.949, and the sensitivity and specificity were 89.27% and 89.61%, respectively. When the H group was a reference, the cutoff value of the LMR was 3.09, the AUC was 0.975, and the sensitivity and specificity were 90.40% and 95.24%, respectively (Figure 4).

>6-year-old B+ group

The maximum AUC was obtained with the LMR. When the A-B- group was taken as a reference, the cutoff value was 2.88, the AUC was 0.924, and the sensitivity and specificity were 83.19% and 92.21%, respectively. When the H group was a reference, the cutoff value of the LMR was 3.48, the AUC was 0.954, and the sensitivity and specificity were 90.27% and 92.38%, respectively (Figure 5).

Discussion
According to the nucleocapsid protein and the matrix protein antigen, influenza viruses can be divided into three types: A, B and C. There is no cross-immunity among these types. The type with the greatest antigen variability is influenza A, which often causes regional outbreaks and epidemics and could even cause a worldwide pandemic. Over the past 100 years, influenza A outbreaks occur seasonally, and there have been several global pandemics. The most serious was the Spanish H1N1 influenza pandemic in 1918, which killed 50 million people [16, 17].

Influenza B has weak antigen variability, and it often causes moderate epidemics or local outbreaks. It is rare for influenza C to infect humans [18]. The clinical symptoms of influenza virus infections in children are similar to those of infections with other respiratory pathogens and are nonspecific; these include a high fever, chills, muscle aches, sore throat, cough, and runny nose. Gastrointestinal symptoms such as vomiting, abdominal pain, and diarrhea are relatively common in children infected with influenza B [15]. Patients with mild symptoms can recover within a short time, and patients with severe symptoms rapidly develop dyspnea accompanied by refractory hypoxemia and can eventually develop acute respiratory distress syndrome, septic shock, heart failure, acute necrotizing encephalopathy, and multiple organ dysfunction, which are life-threatening and even fatal conditions [19]. Therefore, it is important to seek a rapid and simple index for the early diagnosis of influenza infection in children.

The diagnosis of influenza mainly depends on the detection of viral nucleic acids and antibodies. Virus isolation and culture used to be the “gold standard” for the diagnosis of influenza, but it is time-consuming and expensive, has high technical requirements and hard to perform. RT-PCR is the most effective nucleic acid detection technology for influenza, with a sensitivity and specificity as high as 98.5% and 100% [20], respectively. RT-PCR has now replaced as the “gold standard” for the diagnosis of influenza. Although RT-PCR takes less time than virus isolation and culture, it is still expensive and takes several hours, making it difficult to use as a routine means of screening for influenza in pediatric fever clinics. Serological testing requires two serum samples from both the acute phase and the convalescent phase. Convalescent blood samples should be collected 2-4 weeks after the onset of the disease. If the antibody level is more than 4 times higher in the convalescent phase than in the acute phase, the patient can be diagnosed with influenza. Obviously, this is not suitable for influenza screening in outpatients. Influenza virus antigen detection, such as rapid influenza diagnostic tests, can be performed within 30 minutes, but the sensitivity is only 40-70% [6]. Routine blood tests are the most common tests in pediatric fever clinics and can be used as the primary means of identifying bacterial and viral infections. In recent years, researchers have performed an in-depth analysis of various routine blood parameters, these parameters have been found to be useful for the early diagnosis and prognostic assessment of other diseases [7-13].

Influenza strains infect respiratory epithelial cells and attachment of the virus to cells via sialic acid receptors enables uptake of the virus into the cells, followed by recognition of the virus via pattern recognition receptors (PRRs). PRRs trigger cytokine responses and the induction of protective immunity, but they might also contribute to immune pathology [21]. Lymphocytes are the main immune cells involved in the elimination of viruses. In conventional viral infections, the proportion of lymphocytes in the circulation is usually increased. Previous studies have suggested that there is a significant decrease in LYM counts in patients infected with influenza A [9, 22, 23], but there have been few studies on LYM counts in patients with influenza B. Nichols et al. [24] found that LYM can induce self-apoptosis by regulating the expression of FasL on the cell surface and the release of soluble FasL after influenza infection, leading to a decrease in the LYM count. In this study, it was found that the LYM count in children with influenza A or B infection was significantly lower than that in children from 1-6 years old with influenza-like symptoms who tested negative for influenza A and B viruses, and there was no significant
difference in the LYM count between children with influenza A and influenza B infections. In children >6 years old, the LYM count in those infected with influenza A was significantly lower than that in those not infected with influenza viruses. The LYM count was not significantly different between children infected with influenza virus B and those who were not infected with either influenza A or B viruses. Lewis et al [25] suggested that lymphopenia is mainly due to a reduction in T cells and, to a lesser extent, B cells and is of short duration.

Leukocytes such as neutrophils and monocytes provide anti-influenza host protection by releasing preformed cytokines, and the granule contents help hosts eliminate the threat posed by replicating viruses. Coskun O et al. [26] suggest that monocytosis may be considered a surrogate marker for infection with influenza A virus. In this study, there was no statistically significant difference in MON count in children older than 1 year who were infected with either influenza A or B viruses and those who were not infected with influenza viruses. The LMR is the ratio of the LYM count to the MON count, and a LMR<2 has been used as a surrogate marker for influenza A infection [27]. This study suggested that the LMR is the best index for the prediction of influenza virus infection. At the same time, the study showed that the AUCs for the prediction of influenza A and influenza B infections were higher in children who were >6 years old than in children who were 1-6 years old, suggesting that the diagnostic value of the LMR for influenza is greater in children over 6 years old than in children under 6 years old. In addition, the results showed that the AUC of the LMR for the prediction of influenza A infection was higher than that for influenza B in the >1-year-old group, indicating that the predictive value of the LMR was greater for influenza A than for influenza B.

Prior studies demonstrated that PLT can regulate host immunities and complement responses in the initial intrinsic defense against influenza virus infection [28] and explored the different mechanisms by which virus infection can interfere with PLT production and might trigger PLT destruction [29], thus decreasing the PLT count. In this study, the PLT count in the influenza A group was significantly lower than that in the group of children who were negative for both influenza A and influenza B among those 1- to 6 years old, which was consistent with the results in the study by Fei et al. [9]. However, there was no significant difference between the group infected with influenza B and the group of children who were negative for both influenza A and influenza B. Among the children older than 6 years, the PLT count was not statistically significant between the group infected with influenza A, the group infected with influenza B and the group of children who were negative for both influenza A and influenza B.

Our study suggests that the PLT count has predictive value for only children who are 1- to 6 years old and are infected with influenza A; furthermore, even in that group, its predictive value is low, with an AUC of 0.615 and sensitivity and specificity of 58.03% and 63.73%, respectively. In recent years, researchers have attempted to combine the PLT count with other indicators, such as LYM*PLT and MPV/PLT, for disease prediction [30]. Fei et al. reported [9] that the LYM*PLT and MPV/PLT had better predictive value for children under 6 years old who were infected with influenza A, and the predictive value was higher than that of the LMR. However, our study showed that the LMR had the highest predictive value for influenza infection, followed by the LYM*PLT, PLT count, and MPV/PLT among children over the age of 1 year.

Recently, studies have shown that the NLR is positively associated with systemic inflammation [7], acute pancreatitis [31], liver disease [32] and rheumatic diseases [33]. The NLR was found to have a high sensitivity for the detection of influenza virus infection [34]. In this study, there was no significant difference in the NLR among the group infected with influenza A, the group infected with influenza B and group of children who were negative for both influenza A and influenza B among those who were 1-6 years old. In children who were >6 years old, the NLR was significantly higher in the group infected with influenza A than those who were negative for both
influenza A and influenza B. However, there was no significant difference between the group infected with influenza B and the group of children who were negative for both influenza A and influenza B among those >6 years old. Our study suggested that the NLR has predictive value only for children over 6 years old who are infected with influenza A, with an AUC of 0.657 and a sensitivity and specificity of 58.19% and 70.13%, respectively. In addition, it was found in this study that there was no significant difference in the LYM count, PLT count, LMR, NLR, LYM*PLT and MPV/PLT between the group infected with influenza A and the group of children who were negative for influenza A and influenza B among those <1 year old. Due to the small sample size, no statistical analysis was performed to compare the influenza B group with the other groups among children <1 year old. This suggested that the routine blood parameters had scant predictive value for influenza in the <1-year-old age group. We believe that this may be related to immune function and the development of blood cells in children, and this finding needs further investigation.

Conclusion

This study showed that the LMR was significantly lower in children older than 1 year who had influenza, especially children older than 6 years infected with influenza A, compared to children without influenza. The LMR can be used as an early predictor of influenza A infection in children older than 6 years, with an AUC of 0.949, a sensitivity of 89.27% and a specificity of 89.61%.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee for Clinical Research (ECCR) of the First Affiliated Hospital of Wenzhou Medical University (No. 2020-065), and all the parents signed the informed consent form.

Consent for publication

All authors informed consent for publication.

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Authors' contributions

Ronghe Zhu: study design, managed the experiments, analyzed the results, was involved in manuscript preparation.

Qiu Wang: data collection, analyzed the results.

Cuie Chen: analyzed the results.

Xixi Zhang: analyzed the results, and was involved in manuscript preparation.

Chaosheng Lu: was involved in data collection and analysis.

Yuanyuan Sun: data collection.
Conflicts of interest

The authors have no conflicts of interest to disclose.

References

1. López-Labrador FX, Natividad-Sancho A, Pisareva M, et al. Genetic characterization of influenza viruses from influenza-related hospital admissions in the St. Petersburg and Valencia sites of the Global Influenza Hospital Surveillance Network during the 2013/14 influenza season. J Clin Virol. 2016;84:32–8.

2. Lisa A, Grohskopf E, Alyanak, Karen R, Broder, et al. Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices - United States, 2019-20 Influenza Season. MMWR Recomm Rep. 2019;68(3):1–21.

3. Astride Jules, Carlos G, Grijalva Y, Zhu, et al. Influenza-related hospitalization and ED visits in children less than 5 years: 2000–2011. Pediatrics. 2015;135(1):e66–74.

4. World Health Organization. Up to 65 000 people died of respiratory diseases linked to seasonal FLU each year [EB/OL]. [2017-12-14].

5. Sevliya Öcal Demir S, Atıcı EK, Kadayifci, et al. Influenza A (H1N1)-associated severe complications; hemolytic uremic syndrome, myocarditis, acute necrotizing encephalopathy. J Infect Dev Ctries. 2019;13(1):83–6.

6. Kristina Simeonsson and Zack Moore. Prevention and control of influenza: no easy task. N C Med J. 2013;74(5):425, 427 – 33.

7. Clark D, Russell A, Parajuli, Hugo J, Gale, et al. The utility of peripheral blood leucocyte ratios as biomarkers in infectious diseases: A systematic review and meta-analysis. J Infect. 2019;78(5):339–48.

8. Oh GH, Chung SP, Park YS, et al. Mean Platelet Volume to Platelet Count Ratio as a Promising Predictor of Early Mortality in Severe Sepsis. Shock. 2017;47(3):323–30.

9. Fei Y, Zhang H, Zhang C. The application of lymphocyte*platelet and mean platelet volume/platelet ratio in influenza A infection in children. J Clin Lab Anal. 2019;33(9):e22995.

10. Lalosevic MS, Markovic AP, Stankovic S, et al. Combined Diagnostic Efficacy of Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), and Mean Platelet Volume (MPV) as Biomarkers of Systemic Inflammation in the Diagnosis of Colorectal Cancer. Dis Markers. 2019;2019:6036979.

11. Li K-J, Xia X-F. Meng Su, et al. Predictive value of lymphocyte-to-monocyte ratio (LMR) and neutrophil-to-lymphocyte ratio (NLR) in patients with oesophageal cancer undergoing concurrent chemoradiotherapy. BMC Cancer. 2019;19(1):1004.

12. Shu Gong X, Gao F, Xu, et al. Association of lymphocyte to monocyte ratio with severity of coronary artery disease. Med (Baltim). 2018;97(43):e12813.

13. Kose N, Akin F, Yildirim T, et al. The association between the lymphocyte-to-monocyte ratio and coronary artery disease severity in patients with stable coronary artery disease. Eur Rev Med Pharmacol Sci. 2019;23(6):2570–5.

14. WHO. Human infection with pandemic(H1N1) 2009 virus: updated interim WHO guidance on global surveillance. Geneva: World Health Organisation; 2009.

15. Catharine Paules K, Subbarao. Influenza Lancet. 2017;390(10095):697–708.
16. Van Kerkhove MD, Mumford E, Mounts AW, et al. Highly pathogenic avian influenza (H5N1): pathways of exposure at the animal-human interface, a systematic review. PLoS One. 2011;6(1):e14582.

17. Biggerstaff M, Cauchemez S, Reed C, et al. Estimates of the reproduction number for seasonal, pandemic, and zoonotic influenza: a systematic review of the literature. BMC Infect Dis. 2014;14:480.

18. Nesmith N, Williams JV, Johnson M, et al. Sensitive Diagnostics Confirm That Influenza C is an Uncommon Cause of Medically Attended Respiratory Illness in Adults. Clin Infect Dis. 2017;65(6):1037–9.

19. Anna M, Bramley J, Bresee. Lyn Finelli. Pediatric influenza. Pediatr Nurs. 2009;35(6):335–45.

20. Cho CH, Woo MK, Kim JY, et al. Evaluation of five rapid diagnostic kits for influenza A/B virus. J Virol Methods. 2013;187(1):51–6.

21. Kirsty R, Short J, Kasper, Stijn van der Aa, et al. Influenza virus damages the alveolar barrier by disrupting epithelial cell tight junctions. Eur Respir J. 2016;47(3):954 – 66.

22. Cunha BA, Syed U, Strollo S. Non-specific laboratory test indicators of severity in hospitalized adults with swine influenza (H1N1) pneumonia. Eur J Clin Microbiol Infect Dis. 2010;29(12):1583–8.

23. Suat, Biçer, Hülya Ercan Sariçoban, Ahmet Oğuzhan Özen, et al. Experience of influenza A H1N1 in a paediatric emergency unit. Infez Med. 2015; 23(2):125 – 33.

24. Nichols JE, Niles JA, Roberts NJ Jr. Human lymphocyte apoptosis after exposure to influenza A virus. J Virol. 2001;75(13):5921–9.

25. Lewis DE, Gilbert BE, Knight V. Influenza virus infection induces functional alterations in peripheral blood lymphocytes. J Immunol. 1986;137(12):3777–81.

26. Coskun O, Avci IY, Sener K, et al. Relative lymphopenia and monocyteosis may be considered as a surrogate marker of pandemic influenza a (H1N1). J Clin Virol. 2010;47(4):388–9.

27. Cunha BA, Connolly JJ, Irshad N. The clinical usefulness of lymphocyte:monocyte ratios in differentiating influenza from viral non-influenza-like illnesses in hospitalized adults during the 2015 influenza A (H3N2) epidemic: the uniqueness of HPIV-3 mimicking influenza A. Eur J Clin Microbiol Infect Dis. 2016;35(1):155–58.

28. Milka Koupenova, Heather A, Corkrey O, Vitseva, et al. The role of platelets in mediating a response to human influenza infection. Nat Commun. 2019;10(1):1780.

29. Alice Assinger. Platelets and infection - an emerging role of platelets in viral infection. Front Immunol. 2014;5:649.

30. Hiroya Iida M, Kaibori KM, Iida, et al. Ratio of mean platelet volume to platelet count is a potential surrogate marker predicting liver cirrhosis. World J Hepatol. 2018;10(1):82–7.

31. Jeon TJ, Ji Young Park. Clinical significance of the neutrophil-lymphocyte ratio as an early predictive marker for adverse outcomes in patients with acute pancreatitis. World J Gastroenterol. 2017;23(21):3883–9.

32. Zeng F, EnQiang C, Yao DL, et al. Neutrophil-lymphocyte ratio predicts short term mortality in patients with hepatitis B virus-related acute-on-chronic liver failure treated with an artificial liver support system. PLoS One. 2017;12(4):e0175332.

33. Weiming Yang X, Wang W, ZhangYang, et al. Neutrophil-lymphocyte ratio and platelet-lymphocyte ratio are 2 new inflammatory markers associated with pulmonary involvement and disease activity in patients with dermatomyositis. Clin Chim Acta. 2017;465:11–6.
Zhang Y, Zou P, Zhang HG, et al. Neutrophil-lymphocyte ratio as an early new marker in AIV-H7N9-infected patients: a retrospective study. Ther Clin Risk Manag. 2019;15:911–9.

Tables

Table 1. The baseline characteristics of patients.

|                  | A+ group (n=388) | B+ group (n=169) | A-/B- group (n=198) | H group (n=259) | χ²/F | P-value |
|------------------|------------------|------------------|---------------------|-----------------|------|---------|
| <1 year old      |                  |                  |                     |                 |      |         |
| Males            | 11               | 1                | 12                  | 21              | 1.136| .768    |
| Females          | 7                | 2                | 7                   | 17              |      |         |
| Mean age(y)      | 0.7±0.2          | 0.6±0.2          | 0.7±0.1             | 0.6±0.2         | 0.749| .526    |
| 1-6 years old    |                  |                  |                     |                 |      |         |
| Males            | 98               | 24               | 49                  | 62              | 1.218| .749    |
| Females          | 95               | 29               | 53                  | 54              |      |         |
| Mean age(y)      | 3.6±1.4          | 3.9±1.2          | 3.4±1.2             | 3.6±1.5         | 1.204| .308    |
| >6 years old     |                  |                  |                     |                 |      |         |
| Males            | 104              | 53               | 33                  | 57              | 2.415| .060    |
| Females          | 73               | 60               | 44                  | 48              |      |         |
| Mean age(y)      | 9.4±2.4          | 8.8±2.2          | 9.3±2.6             | 9.6±2.3         | 2.541| .056    |

A+ group, influenza A virus infection; B+ group, influenza B virus infection; A-/B- group, influenza-like symptoms who were negative for both influenza A and B viruses; H group, healthy children. There were no statistically significant differences in the age and sex distributions between groups within the three age groups, P>0.05.

Table 2. Hematological parameters of the four groups in the <1-year-old group.
### Table 3. Hematological parameters of the four groups in the 1- to 6-year-old group.

| Parameters | A+ group       | B+ group       | A-/B- group    | H group        | F     | P-value |
|------------|----------------|----------------|----------------|----------------|-------|---------|
| WBC\*10^9/L | 7.25±2.62      | 7.62±2.81      | 6.49±1.89      | 9.14±2.07      | 7.194 | .000    |
| NEU%       | 45.26±13.36    | 34.00±16.70    | 37.53±16.13    | 19.74±8.22     | 19.858| .000    |
| LYM%       | 36.72±12.79    | 51.00±19.08    | 45.58±17.76    | 68.05±8.34     | 30.831| .000    |
| LYM\*10^9/L | 2.72±1.49      | 3.98±2.63      | 2.88±1.36      | 6.28±1.84      | 26.735| .000    |
| MON%       | 16.44±4.88     | 14.00±2.00     | 14.21±3.91     | 7.18±2.68      | 33.712| .000    |
| MON\*10^9/L | 1.18±0.53      | 1.02±0.26      | 0.89±0.29      | 0.65±0.25      | 7.432 | .008    |
| NLR        | 1.57±1.18      | 0.84±0.74      | 1.14±1.03      | 0.31±0.18      | 9.585 | .005    |
| LMR        | 2.48±1.40      | 3.80±1.86      | 2.77±1.45      | 10.86±5.23     | 25.814| .000    |
| RBC\*10^{12}/L | 4.50±0.51     | 4.56±0.26      | 4.56±0.32      | 4.44±0.80      | .168  | .917    |
| Hb g/dl    | 12.05±0.10     | 11.93±0.64     | 12.10±0.73     | 12.03±0.74     | .062  | .980    |
| PLT\*10^9/L | 284.56±66.36   | 287.33±85.85   | 264.47±60.26   | 366.39±91.08   | 8.875 | .000    |
| LYM*PLT    | 781.16±508.13  | 1040.84±461.46 | 741.64±339.72  | 2379.39±1134.19| 20.396| .000    |
| MPV/PLT    | 0.037±0.013    | 0.036±0.014    | 0.038±0.009    | 0.028±0.008    | 6.313 | .001    |

Abbreviations:

WBC, white blood cell count; LYM, lymphocyte; MON, monocyte; NLR, neutrophil-to-lymphocyte; LMR, lymphocyte-to-monocyte, RBC, red blood cell count; Hb, hemoglobin; PLT, platelet; LYM*PLT, lymphocyte* platelet; MPV/PLT, mean platelet volume/platelet ratio.
| Parameters | A+ group | B+ group | A-/B- group | H group | F    | P-value |
|-----------|---------|---------|-------------|---------|------|---------|
| WBC*10^9/L | 6.55±2.33 | 6.35±2.50 | 7.77±2.94 | 7.94±1.77 | 14.973 | .000    |
| NEU%    | 59.44±15.72 | 56.69±14.53 | 57.69±14.60 | 38.33±11.82 | 72.189 | .000    |
| LYM%    | 28.43±14.16 | 30.94±12.60 | 31.13±13.35 | 50.85±12.38 | 74.42  | .000    |
| LYM*10^9/L | 1.76±1.01  | 1.84±0.81  | 2.28±1.10  | 4.01±1.28  | 91.858 | .000    |
| MON%    | 11.28±4.22 | 11.26±0.48 | 10.12±0.34 | 7.25±0.20  | 54.637 | .000    |
| MON*10^9/L | 0.72±0.36  | 0.71±0.51  | 0.76±0.34  | 0.56±0.18  | 14.458 | .000    |
| NLR  | 3.11±2.65  | 2.48±1.85  | 2.52±1.75  | 0.92±0.83  | 58.988 | .000    |
| LMR  | 2.84±2.11  | 3.16±1.65  | 6.61±3.36  | 7.60±2.91  | 103.302 | .000    |
| RBC*10^{12}/L | 4.58±0.34 | 4.59±0.33 | 4.56±0.31 | 4.62±0.28 | 0.731  | .534    |
| Hb g/dL | 12.59±0.81 | 12.59±0.81 | 12.47±0.73 | 12.63±0.67 | 1.031  | .379    |
| PLT*10^9/L  | 216.99±65.96 | 225.57±64.79 | 239.40±65.54 | 322.91±69.79 | 65.139 | .000    |
| LYM*PLT | 382.42±239.52 | 421.07±224.71 | 566.23±367.67 | 1301.14±509.80 | 113.119 | .000    |
| MPV/PLT | 0.050±0.017 | 0.046±0.015 | 0.043±0.013 | 0.031±0.008 | 71.223 | .000    |

Table 4. Hematological parameters of the four groups in the >6-year-old group.
| Parameters | A+ group | B+ group | A-/B- group | H group | F   | P-value |
|------------|----------|----------|-------------|---------|-----|---------|
| WBC $10^9$/L | 6.86±2.11 | 6.70±2.20 | 7.44±2.68 | 6.90±1.75 | 1.347 | .260 |
| NEU % | 69.16±11.95 | 65.21±12.56 | 62.46±15.42 | 48.37±10.03 | 86.847 | .000 |
| LYM % | 18.80±10.02 | 22.34±10.82 | 25.28±12.95 | 40.83±9.18 | 125.259 | .000 |
| LYM $10^9$/L | 1.22±0.61 | 1.41±0.67 | 1.74±1.01 | 2.74±0.71 | 117.736 | .000 |
| MON % | 10.97±3.80 | 11.23±4.23 | 10.35±4.26 | 7.09±2.21 | 54.511 | .000 |
| MON $10^9$/L | 0.74±0.32 | 0.73±0.31 | 0.70±0.26 | 0.48±0.16 | 41.786 | .000 |
| NLR | 5.18±3.42 | 3.94±2.83 | 3.63±2.98 | 1.35±0.90 | 94.10 | .000 |
| LMR | 1.85±1.09 | 2.21±1.33 | 7.99±6.27 | 6.69±6.36 | 43.845 | .000 |
| RBC $10^{12}$/L | 4.72±0.49 | 4.76±0.55 | 4.69±0.31 | 4.78±0.36 | .351 | .788 |
| Hb g/dl | 13.29±0.88 | 13.37±0.88 | 13.26±0.88 | 13.44±0.95 | .819 | .484 |
| PLT $10^9$/L | 227.70±52.55 | 218.50±53.60 | 235.36±56.33 | 291.42±61.12 | 38.839 | .000 |
| LYM*PLT | 281.40±171.42 | 312.74±178.98 | 423.72±291.34 | 812.95±317.72 | 86.43 | .000 |
| MPV/PLT | 0.048±0.015 | 0.049±0.018 | 0.046±0.015 | 0.036±0.011 | 19.940 | .000 |

**Figures**
Figure 1

Differences in LYM count, PLT count, LMR, NLR, LYM*PLT and MPV/PLT values in the A+ group, B+ group, A-B- group and H group.
**Figure 2**

ROC curves of LYM count, PLT count, LMR, NLR, LYM*PLT and MPV/PLT values in the 1- to 6-year-old A+ group (A. the A-B- group as a reference; B. the H group as the reference).

**Figure 3**

ROC curves of LYM count, PLT count, LMR, NLR, LYM*PLT and MPV/PLT values in the 1- to 6-year-old B+ group (A. the A-B- group as a reference; B. the H group as the reference).
Figure 4

ROC curves of LYM count, PLT count, LMR, NLR, LYM*PLT and MPV/PLT values in the >6-year-old A+ group (A. the A-B- group as a reference; B. the H group as the reference).
Figure 5

ROC curves of LYM count, PLT count, LMR, NLR, LYM*PLT and MPV/PLT values in the >6-year-old B+ group (A. the A-B- group as a reference; B. the H group as the reference).

Supplementary Files

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