Original Article

The blood routine test holds screening values for influenza A in 2023: a retrospective study

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Background: Influenza A is the most common viral pathogen isolated from pediatric clinics during influenza seasons. Some young patients with influenza manifest rapid progression with high fever and severe sequelae, such as pneumonia and meningitis. Therefore, early diagnosis and prompt treatment are highly important. Specific diagnostic tests currently include antigen detection, antibody detection, nucleic acid test and virus isolation. Rapid antigen testing is the most commonly adopted method in the outpatient setting, but false negative results are frequently observed, which causes delayed treatment and severe outcome. Routine blood test is the most commonly used detection for the outpatients. Incorporating specific blood cell counts into rapid antigen test may overcome some technical issues and enable accurate early diagnosis.

Methods: We enrolled 537 children with influenza-like symptoms like fever or respiratory symptoms from pediatric outpatients and 110 children without infectious diseases for control. Routine blood tests detected by a routine analyzer and influenza A virus antigen detection were performed in the patients. Significant blood routine parameters between groups were examined by statistical tests. Parameters in routine blood test were assessed by the receiver operating characteristic curve to find the screening indicators of influenza A. Multivariate logistic regression were used to establish the optimal combinations of blood routine parameters in our screening model.

Results: Two subgroups were set according to age: ≤6 years old group and >6 years old group. In each group, patients were further divided into three subgroups: the influenza A-positive-result group (A+ group) (n=259), influenza A-negative-result group (A– group) (n=277) and healthy control group (H group) (n=110). Most routine blood parameters showed significant differences among the three subgroups in each age group. Notably, lymphocyte (LYM) number, platelet (PLT) number, lymphocyte-to-monocyte ratio (LMR) and LYM multiplied by PLT (LYM*PLT) exhibited extremely significant differences. Using A– group as a reference based on the area under the curve (AUC), both age groups had a similar trend. For A– group, the optimal cutoff value of LYM*PLT was 221.6, the AUC, the sensitivity and specificity were 0.6830, 55.71% and 76.92% in the ≤6 years old group. Meanwhile, the cutoff value of LYM*PLT was 196.7, and the AUC, the sensitivity and specificity were 0.6448, 53.97% and 70.81%, respectively in the >6 years old group. Screening model based on multivariate logistic regression model revealed that LYM*PLT was the optimal parameter combinations in ≤6 years old group (AUC =0.7202), while LYM and PLT were the optimal parameter combinations in >6 years old group (AUC =0.6760).

Conclusions: Several blood routine parameters in children with influenza A demonstrate differential levels in both age subgroups. The LYM*PLT exhibits the potential screening value of influenza infection.

Keywords: Influenza A; rapid antigen testing; routine blood tests; children

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Introduction

Influenza is an acute and sometimes very severe respiratory infectious disease worldwide, accounting for up to one billion infections annually, with 3–5 million severe patients and approximately 400,000 to 500,000 deaths. The major symptoms are fever, cough, sore throat, runny nose, muscle pain, headache, and fatigue (1). Influenza A is the most common type virus isolated from clinic visitors during the flu seasons. Despite its essence of self-healing, the disease in some patients rapidly develops into severe pneumonia and meningitis. Therefore, early diagnosis is essential for decreasing morbidity, hospitalization time, and mortality.

Diagnosis of influenza largely relies on symptoms, clinical signs, epidemiological knowledge and laboratory tests. Laboratory methods mainly are antigen detection, antibody detection, nucleic acid tests and virus isolation. Although nucleic acid tests and virus isolation are the gold standard (2), they are time-consuming, relatively expensive and technically difficult, which limit their wide employment in outpatients and emergency department. Rapid antigen testing is specific, but negative results are common in early phase of mild influenza A infection. On the other hand, blood test is commonly performed in most outpatients, and is an important guide for diagnosing the presence, type, and severity of infection. It is commonly applied for differing viral infections from bacterial infections in practice (3). In addition to complete blood panel (4,5), related hematological parameters, such as neutrophil-to-lymphocyte ratio (NLR) (6,7), platelet-to-lymphocyte ratio (PLR) (8), and lymphocyte (LYM) multiplied by platelet (LYM*PLT) (9), are reported to be important screening indicators in infectious diseases. A number of previous studies have confirmed that routine blood parameters are helpful for early identification of influenza (8,10,11). However, their predictive value in influenza A has not been well studied in pediatric patients. Moreover, these studies were grouping according to the results of nucleic acid test, which is inconsistent with the practical clinical situation (6,8,12). It is necessary to combine blood routine tests with rapid antigen testing for influenza A screening. We present this article in accordance with the STARD reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-23-435/rc).

Highlight box

Key findings

- Children with influenza A always demonstrate decreased lymphocyte multiplied by platelet. It exhibits the best predictive value of influenza infection.

What is known and what is new?

- Previous studies have found that routine blood parameters are helpful for early identification of influenza. However, these studies are grouped according to the results of nucleic acid test and focus on adults.
- In the present study, grouped patient subjects by the results of rapid antigen tests (positive and negative), we have found that the blood routine test still holds screening values for influenza A.

What is the implication, and what should change now?

- Blood routine tests combined with rapid antigen tests would lead to early diagnosis of influenza A in the children. It could decrease the morbidity, hospitalization time, and mortality of influenza A.

Methods

Study design

The detailed study design was demonstrated in *Figure 1*. The minimal sample size in this study were estimated by PASS (version: 21.0.3) in analysis module of one-way ANOVA.

Patients

In this study, we included patients less than sixteen years old with influenza-like symptoms who presented at the outpatient pediatric clinic of Ruijin Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China, from February to April 2023. All of the patients underwent routine blood tests and rapid antigen testing. Influenza-like symptoms included: fever, sore throat, headache, cough or muscle pain. Inclusion criteria

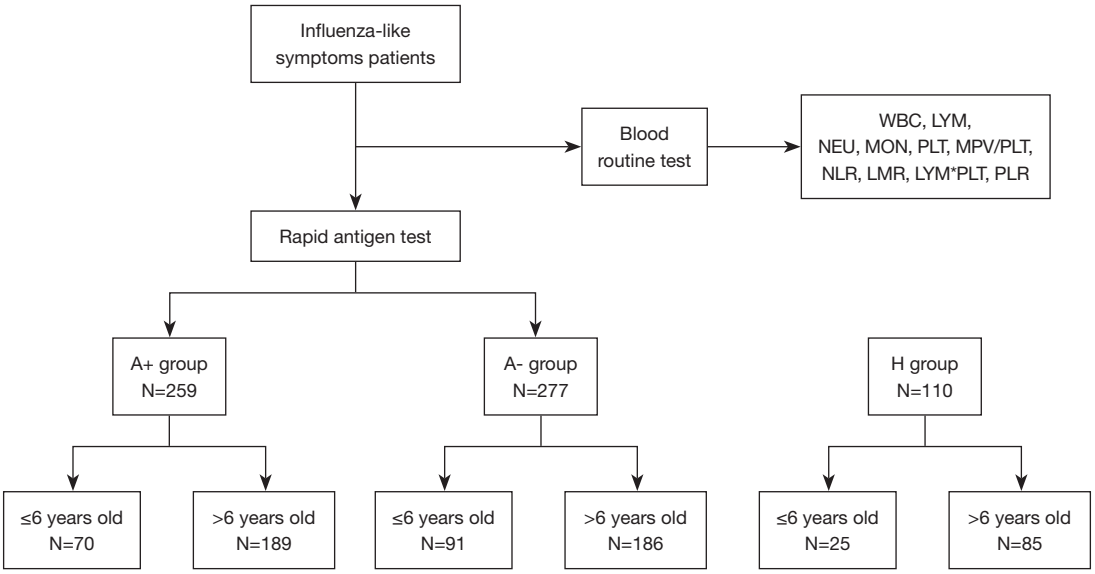

Figure 1 Study flow chart. A+ group, influenza A-positive-result group; A– group, influenza A-negative-result group; H group, healthy control group. WBC, white blood cell count; LYM, lymphocyte; NEU, neutrophil; MON, monocyte; PLT, platelet; MPV, mean platelet volume; MPV/PLT, MPV divided by PLT; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; LYM*PLT, LYM multiplied by PLT; PLR, platelet-to-lymphocyte ratio.

included fever, sore throat, headache, cough, muscle pain or respiratory tract infection (1,13). The exclusion criteria were: (I) systemic chronic diseases, for example, cardiovascular diseases, liver disease, nephropathy, anemia, hematological disease, and cancer; (II) immunodeficiency or immunosuppression; (III) patients who declined blood routine examination or rapid antigen testing; (IV) long-term use of any medications that may affect the result of the blood routine; (V) patients who had undergone antiviral therapy before the initial clinic visit.

Routine blood parameters

Capillary blood samples of blood routine tests were obtained by finger prick (12,14) and detected by a routine analyzer (BC-6800, Mindray, Shenzhen, China). The complete blood count results included white blood cell (WBC), neutrophil (NEU), LYM, monocyte (MON), platelet (PLT), and mean PLT volume (MPV). In addition, other hematological parameters were calculated, including NLR, lymphocyte-to-monocyte ratio (LMR), LYM*PLT, PLR and MPV divided by PLT (MPV/PLT).

Influenza A detection by rapid antigen testing

The type of samples submitted for testing was nasal swab of both nasal mucosae obtained by trained physicians. Colloidal gold immunochromatography was applied in the influenza A virus antigen detection kit (Guangzhou Wondfo Biotech Co., Ltd, Guangzhou, China). Double antibody sandwich method was used to detect influenza A antigen. 80 mL of the treated sample extract were added dropwise to the sample well of the test card and the displayed results were recorded within 15–20 minutes.

Statistical analyses

GraphPad Prism version 8.0 was applied to statistical analysis and P value <0.05 was considered as statistically significant. Continuous variables were expressed as mean ± standard deviation when conforming to a normal distribution. Categorical variables were expressed as frequencies. *t*-test was used for between two-groups comparisons. Chi-squared test or Fisher's exact test were applied to the comparison of frequencies. Kruskal-Wallis H test or one-way ANOVA were applied to continuous variables between multiple groups. Using influenza A negative-result group (A– group) or healthy control group (H group) as a reference, the sensitivity, specificity and the area under the curve (AUC) of LYM, MON, PLT, NLR, LMR, LYM*PLT, PLR and MPV/PLT were calculated with the receiver operating characteristic (ROC) curve model. Logistic regression model was used to explore the optimal combinations of different blood routine parameters for

Table 1 The baseline characteristics of patients

Variables	≤6 years old				>6 years old			
	A+ group (n=259)	A– group (n=277)	H group (n=110)	P	A+ group (n=259)	A– group (n=277)	H group (n=110)	P
Male/female	32/38	46/45	15/10	0.466	109/80	101/85	37/48	0.092
Age, years (mean ± SD)	4.70±1.52	4.37±1.66	5.04±0.92	0.060	10.10±2.04	9.78±2.00	10.02±1.87	0.293
Fever	70	89		0.505	187	164		9.679e–06
Cough	58	73		0.690	162	116		2.823e–07
Sore throat	12	16		1	81	72		0.462
Abdominal pain	5	3		0.296	5	6		0.770
Diarrhea	4	1		0.168	6	3		0.503
Vomit	12	11		0.374	20	19		1
Dizzy	2	5		0.451	27	32		0.480
Headache	6	6		0.765	30	32		0.781
Fatigue	11	9		0.337	21	27		0.356
Muscle pain	4	5		1	21	15		0.381

A+ group, influenza A-positive-result group; A– group, influenza A-negative-result group; H group, healthy control group.

predicting influenza A. this logistic model was developed by *glm* function in R software (version: 4.1.1). Then, ROC analysis was performed to examine the accuracy of this model in GraphPad Prism version 8.0.

Ethical statement

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Wuxi Branch of Shanghai Ruijin Hospital Ethics Committee (No. 001). Informed consent was taken from all the patients' legal guardians.

Results

Patient characteristics

In brief, the minimal sample size calculated in PASS using one-way ANOVA module is 27. To improve the accuracy and reliability of our study, we collected more samples than this estimated sample size. Totally, 537 patients were enrolled in the study and were divided into influenza A-positive-result patients (A+ group) (n=259) and influenza A-negative-result patients (A– group) (n=277) according to rapid antigen tests. Simultaneously, 110 children without infectious diseases were selected as the healthy control group (H group). Meanwhile, children were divided into two age groups: the ≤6 years old and the >6 years old group. No statistically significant difference was found in gender nor age among the three groups (A+ group/A– group/H group) in each age group (P>0.05) (*Table 1*).

Results of routine blood parameters

Tables 2,*3* list the parameters in the routine blood tests of the influenza A children. In both the ≤6 years old group and >6 years old group, there were significant differences in WBC, NEU, LYM, MON, PLT, NLR, LMR, LYM*PLT, PLR and MPV/PLT between A+ group, A– group and H group. The WBC, NEU, LYM, MON, PLT, NLR, LMR, LYM*PLT, PLR and MPV/PLT level in the A+ group, A– group and H group were significantly different in the subgroups of patients ≤6 years old (*Table 2*) and >6 years old (*Table 3*). Compared to the H group, patients in the A+ group had significantly decreased number in WBC, LYM, PLT, LMR and LYM*PLT values, while NEU, MON, NLR, PLR and MPV/PLT values were significantly increased (*Tables 2*,*3*) in both age subgroups. In the ≤6 years old group, WBC, LYM, PLT, NLR, LMR, and LYM*PLT were significantly different between the A+ group and the A– group (*Figure 2*, Figure S1 and *Table 2*). In the >6 years

Table 2 Hematological parameters of the three groups in the ≤6 years old group

Parameters	A+ group	A– group	H group	P	P[a]	P[b]
WBC (10^9/L)	6.28±2.30	7.49±3.78	6.92±1.72	0.0430	0.0136	0.2110
NEU (%)	65.4±17.1	61.4±17.4	41.0±14.5	<0.0001	0.0600	<0.0001
LYM (%)	23.3±15.3	27.4±15.6	49.0±13.7	<0.0001	0.0147	<0.0001
MON (%)	9.8±3.5	9.2±3.2	6.2±1.7	<0.0001	0.2651	<0.0001
NEU (10^9/L)	4.27±2.17	4.85±3.25	2.78±0.98	0.0001	0.1824	<0.0001
LYM (10^9/L)	1.34±0.99	1.86±1.09	3.46±1.40	<0.0001	0.0022	<0.0001
MON (10^9/L)	0.54±0.24	0.63±0.28	0.42±0.13	0.0001	0.2903	<0.0001
PLT (10^9/L)	206.90±50.31	230.10±67.06	312.80±70.48	<0.0001	0.0132	<0.0001
MPV (fL)	9.53±1.08	9.58±0.93	9.38±1.39	0.6690	0.6970	0.5853
LMR	9.53±1.96	3.26±2.16	8.40±2.91	<0.0001	0.0308	<0.0001
NLR	4.64±3.70	3.69±3.91	1.20±1.53	<0.0001	0.0158	<0.0001
MPV/PLT	0.049±0.015	0.046+0.016	0.032±0.009	<0.0001	0.2197	<0.0001
LYM*PLT	271.7±189.3	436.2±295.2	1,118.0±534.7	<0.0001	<0.0001	<0.0001
PLR	212.7±130.0	177.8±156.2	111.5±66.4	<0.0001	0.1236	<0.0001

Data are presented as mean ± SD. [a], compared to the A– group, significance P<0.05; [b], compared to the H group, significance P<0.05. A+ group, influenza A-positive-result group; A– group, influenza A-negative-result group; H group, healthy control group. WBC, white blood cell count; LYM, lymphocyte; NEU, neutrophil; MON, monocyte; PLT, platelet; MPV, mean platelet volume; MPV/PLT, MPV divided by PLT; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; LYM*PLT, LYM multiplied by PLT; PLR, platelet-to-lymphocyte ratio.

Table 3 Hematological parameters of the three groups in the >6 years old group

Parameters	A+ group	A– group	H group	P	P[a]	P[b]
WBC (10^9/L)	5.93±2.40	7.12±3.88	6.53±1.44	<0.0001	0.0004	0.0111
NEU (%)	68.3±14.3	66.2±16.2	43.5±9.3	<0.0001	0.3271	<0.0001
LYM (%)	20.5±13.0	23.0±15.1	45.0±9.8	<0.0001	0.0839	<0.0001
MON (%)	10.0±3.7	9.2±3.5	6.8±1.3	<0.0001	0.0369	<0.0001
NEU (10^9/L)	4.22±2.23	5.02±3.54	2.88±1.06	<0.0001	0.0097	<0.0001
LYM (10^9/L)	1.08±0.58	1.41±0.80	2.93±0.75	<0.0001	<0.0001	<0.0001
MON (10^9/L)	0.56±0.24	0.60±0.27	0.44±0.11	<0.0001	0.1739	<0.0001
PLT (10^9 /L)	213.20±59.19	235.60±60.07	288.00±65.17	<0.0001	0.0003	<0.0001
MPV (fL)	9.52±1.15	9.58±1.16	9.81±1.15	0.1527	0.6012	0.0895
LMR	2.27±1.19	2.83±2.34	6.89±2.14	<0.0001	0.0352	<0.0001
NLR	5.23±4.24	4.96±4.56	1.07±0.62	<0.0001	0.2004	<0.0001
MPV/PLT	0.049±0.019	0.045±0.018	0.036±0.012	<0.0001	0.0191	<0.0001
LYM*PLT	233.1±153.2	340.6±249.9	857.2±336.4	<0.0001	<0.0001	<0.0001
PLR	252.1±147.4	230.4±159.0	103.9±34.51	<0.0001	0.1712	<0.0001

Data are presented as mean ± SD. [a], compared to the A– group, significance P<0.05; [b], compared to the H group, significance P<0.05. A+ group, influenza A-positive-result group; A– group, influenza A-negative-result group; H group, healthy control group. WBC, white blood cell count; LYM, lymphocyte; NEU, neutrophil; MON, monocyte; PLT, platelet; MPV, mean platelet volume; MPV/PLT, MPV divided by PLT; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; LYM*PLT, LYM multiplied by PLT; PLR, platelet-to-lymphocyte ratio.

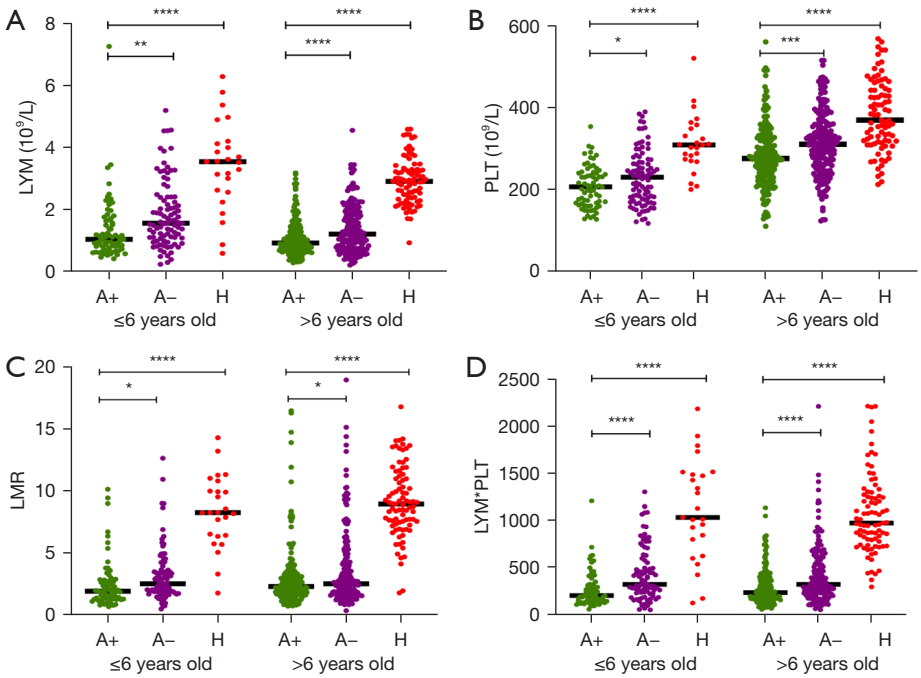

Figure 2 Differences in LYM, PLT, LMR, LYM*PLT values in the A+, A– and H group. *, P <0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001. A+ group, influenza A-positive-result group; A– group, influenza A-negative-result group; H group, healthy control group. LYM, lymphocyte; PLT, platelet; LMR, lymphocyte-to-monocyte ratio; LYM*PLT, LYM multiplied by PLT.

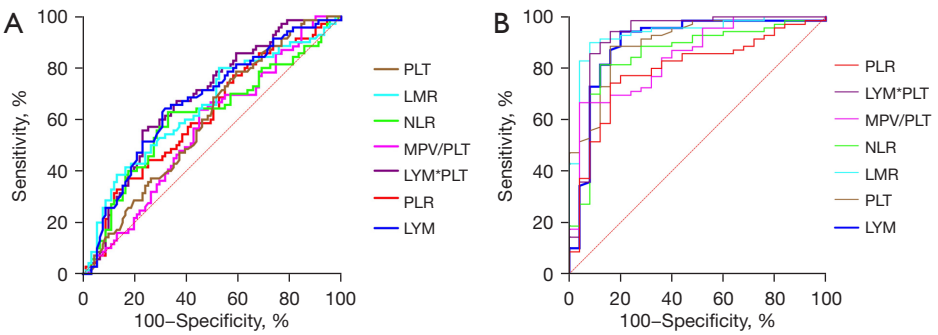

Figure 3 ROC curves of LYM, PLT, LMR, NLR, MPV/PLT, LYM*PLT and PLR values in the ≤6 years old A+ group. (A) The A– group as a reference; (B) the H group as the reference. LYM, lymphocyte; PLT, platelet; MPV, mean platelet volume; MPV/PLT, MPV divided by PLT; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; LYM*PLT, LYM multiplied by PLT; PLR, platelet-to-lymphocyte ratio; ROC, receiver operating characteristic.

old group, WBC, NEU, LYM, PLT, LMR, LYM*PLT and MPV/PLT were significantly different between the A+ group and the A– group (*Figure 2*, Figure S1 and *Table 3*).

Screening values of blood parameters in influenza A

In patients ≤6 years old A+ group, when referred to the A– group, based on the AUC, the best parameter that predicted influenza A was the LYM*PLT. The optimal cutoff value of LYM*PLT was 221.6, the AUC, the sensitivity and specificity were 0.6830, 55.71% and 76.92%, respectively. The cutoff value of LMR was 1.917, the AUC, the sensitivity and specificity were 0.6489, 47.14% and 75.82%, respectively. When referred to the H group, the maximum AUC was obtained for the LMR; the cutoff value of the LMR was 4.712, the AUC, the sensitivity

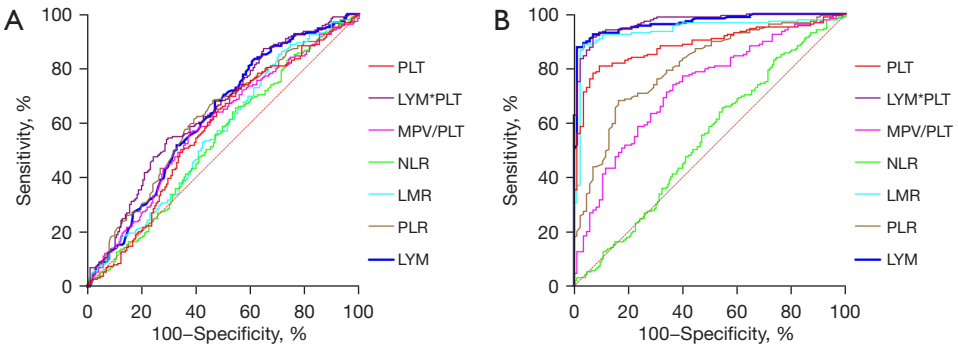

Figure 4 ROC curves of LYM, PLT, LMR, NLR, MPV/PLT, LYM*PLT and PLR values in the >6 years old A+ group. (A) The A– group as a reference; (B) the H group as the reference. LYM, lymphocyte; PLT, platelet; MPV, mean platelet volume; MPV/PLT, MPV divided by PLT; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; LYM*PLT, LYM multiplied by PLT; PLR, platelet-to-lymphocyte ratio; ROC, receiver operating characteristic.

and specificity were 0.9377, 90% and 92%, respectively (*Figure 3*). The cutoff value of LYM*PLT was 598.4, the AUC, the sensitivity and specificity were 0.9211, 94.29% and 84%, respectively.

In patients >6 years old group, the best performance was identified by LYM*PLT regardless of A– group or H group as a reference. When referred to the A– group, the cutoff value was 196.7, the AUC, the sensitivity and specificity were 0.6448, 53.97% and 70.81%, respectively. When referred to the H group was used as a reference, the cutoff value was 461.9, the AUC, the sensitivity and specificity were 0.9713, 91.53% and 92.94%, respectively (*Figure 4*).

Predictive model for children with influenza A based on multivariate logistic regression

To further improve the screening accuracy of influenza A, we used multivariate logistic regression to find the optimal combinations of different blood routine parameters and developed a screening model based on our results. For children in ≤6 years old, the method that included all independent variables using the ENTER method achieved the highest Akaike information criterion (AIC) value and AUC. The results of logistic regression were displayed in *Table 4*. This predictively model was described as: Influenza A = 6.40 + WBC×–0.95 + NEU×0.81 + LYM×2.69 + MON×1.55 + PLT×–0.005 + MPV×0.037 + LMR×–0.071 + NLR×0.167 + (MPV/PLT) ×–79.58 + (LYM*PLT) ×–0.012 + PLR×–0.005. Interestingly, LYM*PLT was still the most significant of all parameters, which was consistent with our above analysis. Notably, using 0.399 as the optimal cutoff value, the sensitivity, specificity and AUC have reached 0.857, 0.5111, 0.7202 in this screening model, respectively and obviously achieved better performance than single blood routine parameter (*Figure 5A*). For children in >6 years old, the method that included all independent variables using the ENTER method also achieved the highest AIC value and AUC. The results of logistic regression were displayed in *Table 5*. This predictively model was described as: Influenza A = 4.32 + WBC×–0.57 + NEU×0.47 + LYM×–0.35 + MON×1.28 + PLT×–0.003 + MPV×–0.24 + LMR×–0.06 + NLR×–0.057 + (MPV/PLT)×3.40 + (LYM*PLT)×–0.00004 + PLR×–0.003. Interestingly, although LYM*PLT was not significant in this model, using 0.386 as the optimal cutoff value, the sensitivity, specificity and AUC have reached 0.56, 0.70, 0.6760 in this screening model, respectively and obtained better performance than single blood routine parameter (*Figure 5B*).

Discussion

Clinically, the common manifestations of influenza A infection are fever, cough and sore throat. Based on these typical symptoms, physicians further employ routine blood tests and rapid antigen tests to confirm the infection (*Table 1*). Although rapid influenza test is routinely performed, delayed diagnosis is common. Influenza A mostly causes mild symptoms and is self-limited, while critical cases cause severe economic and social burden. Routine blood tests in combination with rapid antigen test are commonly used and have proved to be effective for early diagnosis of influenza A (15). In this study, we analyzed and compared the parameters of the routine blood tests in influenza patients in order to find a better way for early

Table 4 Results of logistic regression in the ≤6 years old group

Parameters	Estimate	Std. error	z value	Crude OR (95% CI)	Crude P value	Adj. OR (95% CI)	P (Wald's test)
(Intercept)	6.40	3.22	1.99				0.0469*
WBC	−0.95	1.12	−0.85	0.9 (0.8, 1)	0.03	0.4 (0, 3.4)	0.39
NEU	0.81	1.13	0.72	0.9 (0.8, 1)	0.21	2.3 (0.2, 20.5)	0.47
LYM	2.69	1.57	1.71	0.6 (0.4, 0.8)	0.00	14.7 (0.7, 319.9)	0.09
MON	1.55	1.88	0.83	0.5 (0.1, 1.6)	0.22	4.7 (0.1, 187.7)	0.41
PLT	−0.01	0.01	−0.40	0.99 (0.99, 1.0)	0.03	0.994 (0.969, 1.020)	0.69
MPV	0.04	0.30	0.12	0.9 (0.7, 1.3)	0.60	1 (0.6, 1.9)	0.90
LMR	−0.07	0.23	−0.31	0.8 (0.7, 1)	0.05	0.9 (0.6, 1.5)	0.76
NLR	0.17	0.18	0.91	1.1 (1, 1.2)	0.14	1.2 (0.8, 1.7)	0.36
MPV/PLT	−79.58	43.68	−1.82	2.30E+05 (0, 1.58E+14)	0.23	0 (0, 412.5)	0.07
LYM*PLT	−0.01	0.01	−2.14	0.997 (0.99, 0.999)	<0.001	1 (1, 1)	0.03*
PLR	0.00	0.00	−1.02	1.00 (0.999, 1.004)	0.15	0.995 (0.986, 1.004)	0.31

Significance: *, P<0.05. AIC: 213.79. OR, odds risk; CI, confidence interval; WBC, white blood cell count; LYM, lymphocyte; NEU, neutrophil; MON, monocyte; PLT, platelet; MPV, mean platelet volume; MPV/PLT, MPV divided by PLT; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; LYM*PLT, LYM multiplied by PLT; PLR, platelet-to-lymphocyte ratio; AIC, Akaike information criterion.

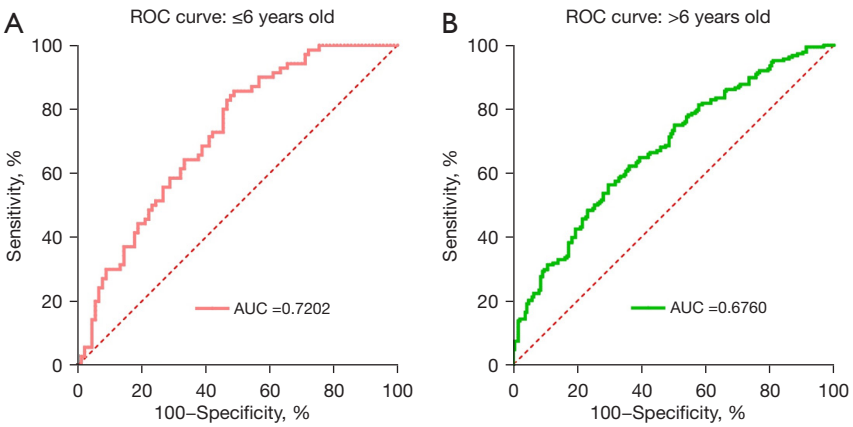

Figure 5 ROC curves of the logistic regression model for influenza A in both age subgroups. (A) ≤6 years old group; (B) >6 years old group. AUC, area under the curve; ROC, receiver operating characteristic.

diagnosis. We found that LYM, PLT, LMR and LYM*PLT had significant differences between A+ group and A− group or healthy group regardless of age. Furthermore, the LYM*PLT and LMR exhibited the best predictive value of influenza infection according to the AUC.

Although children less than 16 years were chosen in our study, subgroups of ≤6 years old and >6 years old were still set. Physiologically, the count of LYM and NEU have their second crossover between 4 and 6 years old, then LYM decreases and NEU increases to the levels commonly observed in adults (16). Moreover, children generally start primary school at the age of six and the odd of cross-infections substantially elevates at the same time. This is why our study applied 6 years as the age cut-off. Either in ≤6 years old and >6 years old group, patients were divided into A+ group and A− group based on the results of rapid antigen testing.

A significant decrease in LYM was found in A+ group in children of both age subgroups. This is consistent with previous studies showing decreased LYM counts in

Table 5 Results of logistic regression in the >6 years old group

Parameters	Estimate	Std. error	z value	Crude OR (95% CI)	Crude P value	Adj. OR (95% CI)	P (Wald's test)
(Intercept)	4.32	1.80	2.40				0.0164*
WBC	−0.57	1.00	−0.57	0.9 (0.8, 1)	<0.001	0.6 (0.1, 4)	0.57
NEU	0.47	1.01	0.46	0.9 (0.8, 1)	0.01	1.6 (0.2, 11.4)	0.64
LYM	−0.35	1.30	−0.27	0.5 (0.4, 0.7)	<0.001	0.7 (0.1, 9)	0.79
MON	1.28	1.24	1.03	0.6 (0.3, 1.4)	0.22	3.6 (0.3, 40.5)	0.30
PLT	0.00	0.01	−0.41	0.993 (0.99, 0.997)	<0.001	0.9974 (0.985, 1.01)	0.68
MPV	−0.24	0.15	−1.62	1 (0.8, 1.1)	0.66	0.8 (0.6, 1.1)	0.11
LMR	0.06	0.13	0.46	0.9 (0.8, 1)	0.01	1.1 (0.8, 1.4)	0.65
NLR	0.06	0.09	0.66	1 (1, 1.1)	0.54	1.1 (0.9, 1.3)	0.51
MPV/PLT	3.40	15.82	0.22	1,061,983.3 (10.5, 1.08E+11)	0.02	29.9 (0, 8.69E+14)	0.83
LYM*PLT	0.00	0.00	−0.15	0.997 (0.996, 0.999)	<0.001	0.9996 (0.99, 1.01)	0.88
PLR	0.00	0.00	−1.09	1.001 (0.9996, 1.002)	0.17	0.997(0.99, 1.002)	0.28

Significance: *, P<0.05. AIC: 503.09. OR, odds risk; CI, confidence interval; WBC, white blood cell count; LYM, lymphocyte; NEU, neutrophil; MON, monocyte; PLT, platelet; MPV, mean platelet volume; MPV/PLT, MPV divided by PLT; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; LYM*PLT, LYM multiplied by PLT; PLR, platelet-to-lymphocyte ratio; AIC, Akaike information criterion.

children with influenza A by inducing the apoptosis of LYMs (12,17,18). Meanwhile, LYMs redistribution and migration in respiratory system further aggravate the decrease in circulating LYMs (19). Lymphopenia is helpful for distinguishing influenza A infection from other virus infection that causes increased LYM count including respiratory syncytial virus, rhinovirus, adenovirus, Epstein-Barr virus, cytomegalovirus (11,20-24). Our study achieved the sensitivity, specificity and AUC of 64.29%, 68.13%, 0.669 respectively compared to A– group in the ≤6 years old group. Similarly, in the >6 years old group, sensitivity 68.25%, specificity 52.97%, AUC 0.622 were observed versus A– group. Severe lymphopenia is useful to recognize patients at risk of severe complications and poor outcomes (25).

Another finding was decreased PLT and increased MPV/PLT in the A+ group compared to those in the A– and H group in both age subgroups, which is consistent with previous study (12). Our study achieved the sensitivity, specificity and AUC of 68.57%, 49.45%, 0.5962, respectively compared to A– group in the ≤6 years old group. Similarly, in the >6 years old group, sensitivity, specificity, and AUC were 61.38%, 60.75%, and 0.6162, respectively versus A– group. PLT consumption is caused by inflammation-induced coagulation and phagocytosing influenza virus particles that would be cleared from circulation (26). Other possible reasons include activation of PLT by virus antibodies (27) and influenza-induced immune thrombocytopenia (28). Dysregulated PLTs or thrombocytopenia may result in systemic inflammation and organ damage (29). Decreased PLT and increased WBC counts are often found in non-survivor influenza-infected patients (30). These findings suggest that PLT count can be recognized as a key characteristics of influenza and helps identify high-risk cases and predict prognosis.

A significant increase in MON was found in the A+ group compared to H group in both age subgroups, consistent with previous studies (31,32). Increased MONs coincide with the role of MONs in host defense function against influenza infection (33). MON populations peak on at second and fourth day of symptomatic illness (32), which explains decreased LYMs but increased MONs in our study. In fact, most outpatients receive routine blood tests in the first 3 days since the onset of symptoms, when MONs are yet to reach its peak. Because we found decreased LYMs in A+ group, we assumed that a combination of LYM and MON may be a practical biomarker for the prediction of influenza A infection. According to our results, the application of LMR to predictive diagnosis is more meaningful for children at six years old and younger with healthy children as reference.

Besides LMR, we also selected LYM*PLT as a predictive parameter of influenza A. AUC was 0.683 in ≤6 years old

group compared to A– group, which is in line with an AUC in the previous studies (9,12). According to our results, LYM*PLT is more efficient for influenza A diagnosis in children, and has higher predictive value and screening value. A retrospective study found that LYM*PLT had the largest AUC and the best screening value, with the sensitivity and specificity of 57.59% and 72.60%, respectively (9). Moreover, a logistic regression model based on all blood routine parameters achieved better predictive performance than single parameters both in ≤6 years old group and >6 years old group, which demonstrates that combinations of different blood routine parameters could improve the improve the screening accuracy. Collectively, our study suggests that it is feasible to use routine blood tests in combination with rapid antigen test to assist in the early diagnosis of influenza A infection.

The innovative point of the present study is that we grouped patient subjects by the results of rapid antigen tests (positive and negative), which is different from previous studies grouping according to the results of nucleic acid test. The benefit of this is its being close to the real clinical setting because blood routine test combined with antigen is commonly used for influenza A screening. The limitation of this study is that there was a difference in the time from symptom onset to initial physician visit, thus some subjects only showed little changes in their routine blood testing and with the negative result of the rapid antigen. Finally, the sample size of the current study is relatively small, which can be improved by further investigation.

Conclusions

Blood routine tests are convenient, inexpensive, rapid and easy to follow up. Blood routine tests coupled with rapid antigen testing are useful for screening of influenza A patients at early stage. A significantly lower LMR*PLT was seen in influenza A children, regardless of age groups. The LYM*PLT demonstrated the best performance either using A– group or H group as a reference. Logistic regression model based on all blood routine parameters significantly improves the screening accuracy.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://tp.amegroups.com/article/view/10.21037/tp-23-435/rc

Data Sharing Statement: Available at https://tp.amegroups.com/article/view/10.21037/tp-23-435/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tp.amegroups.com/article/view/10.21037/tp-23-435/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Wuxi Branch of Shanghai Ruijin Hospital Ethics Committee (No. 001). Informed consent was taken from all the patients' legal guardians.

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