Research Square

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Association of hematological and inflammatory markers with influenza A virus infection in children: a retrospective study of 1,379 antigen-confirmed cases

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Research Article

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Abstract

Background

Influenza A virus (IAV) infection remains a significant cause of pediatric morbidity worldwide. Rapid antigen detection tests are widely used but have limited sensitivity. This study evaluated complete blood count (CBC)-derived parameters and composite inflammatory indices associated with IAV infection in children and examined age-dependent variations.

Methods

A retrospective study of 1,379 pediatric patients (685 IAV-positive, 694 IAV-negative) with influenza-like illness admitted to People's Hospital of Deyang City who underwent both rapid antigen detection tests and CBC testing. Patients were stratified into five age groups. Logistic regression, ROC analysis, and formal age-interaction testing were performed.

Results

IAV-positive children exhibited lower lymphocyte counts (1.17 vs. 1.78, $P < 0.001$) and higher MLR (0.64 vs. 0.49, $P < 0.001$). MLR showed the strongest independent association (aOR = 4.823, $P < 0.001$). Significant age interactions were observed for WBC, LYM, MLR, and CRP.

Conclusions

Hematological markers are significantly associated with IAV infection in children, but their associations vary by age. Age-stratified thresholds should be considered.

1. Introduction

Influenza A virus (IAV) infection imposes a substantial health burden on children worldwide, accounting for an estimated 90 million new infections and up to 1 million hospital admissions annually among children under 5 years [1, 2]. Children, particularly those under 5 years of age, are at increased risk for severe influenza-related complications, including pneumonia, encephalopathy, and secondary bacterial infections [3]. The clinical presentation of influenza in children is often nonspecific, overlapping with other respiratory viral infections such as respiratory syncytial virus (RSV), human metapneumovirus, and SARS-CoV-2, thereby making an accurate etiological diagnosis challenging in the absence of laboratory confirmation [4].

Rapid antigen detection tests (RADTs) have been widely adopted as point-of-care diagnostic tools for influenza owing to their operational simplicity, rapid turnaround time (typically 10–15 min), and low cost.

However, the sensitivity of RADTs in pediatric populations is suboptimal, ranging from 50% to 80% compared with reverse transcription-polymerase chain reaction (RT-PCR), the gold standard [5, 6]. Factors such as younger age, lower viral load, and variable specimen quality further reduce RADT sensitivity in children [7]. Although RT-PCR offers superior diagnostic accuracy, its widespread implementation is constrained by higher cost, longer turnaround time, and the need for specialized laboratory infrastructure. There remains a clear need for accessible, low-cost adjunctive tools that can augment the diagnostic yield of RADTs in pediatric settings.

The complete blood count (CBC) is among the most commonly ordered laboratory tests in pediatric emergency departments and outpatient clinics. In recent years, a growing body of evidence has suggested that routine CBC parameters and derived composite indices may have diagnostic value in differentiating influenza from other respiratory infections in children [8–10]. Lymphopenia has been consistently reported as a hallmark of influenza infection, reflecting virus-induced depletion of circulating lymphocytes through direct infection and apoptosis of lymphoid cells [11]. Thrombocytopenia has also been observed in influenza patients, potentially mediated by viral interactions with megakaryocytes and altered platelet production [12].

Beyond individual CBC parameters, composite inflammatory indices that integrate multiple cell lineages have garnered increasing attention. The neutrophil-to-lymphocyte ratio (NLR) and monocyte-to-lymphocyte ratio (MLR) have been investigated as biomarkers for various infectious and inflammatory conditions, including pediatric tuberculosis [13], appendicitis [14], and viral respiratory infections [8, 15]. More recently, the systemic immune-inflammation index (SII) and the systemic inflammation response index (SIRI) have emerged as novel composite markers reflecting the interplay between innate immunity, adaptive immunity, and thrombopoiesis [16, 17]. These indices have demonstrated prognostic value in adult oncology and cardiovascular disease, but their utility in pediatric infectious diseases — particularly influenza — remains largely unexplored.

A critical consideration insufficiently addressed in prior studies is the influence of age on hematological parameters in children. Pediatric CBC reference ranges undergo profound changes from infancy through adolescence, driven by the maturation of the hematopoietic and immune systems [18, 19]. Lymphocyte counts are physiologically elevated in infants and young children compared with adolescents, whereas neutrophil counts show the opposite trend. These age-dependent variations may confound the association between CBC-derived markers and IAV infection and may also modify the diagnostic performance of these markers across different pediatric age groups. Failure to account for age effects could lead to misleading conclusions and inappropriate application of uniform diagnostic thresholds. To address these knowledge gaps, the present study was designed with four specific objectives: (1) to compare CBC parameters and composite inflammatory indices (NLR, MLR, SII, SIRI) between IAV-positive and IAV-negative children presenting with influenza-like illness; (2) to evaluate the independent associations of these markers with IAV infection using logistic regression with age adjustment; (3) to assess the discriminatory performance of individual markers and multivariable models using ROC

analysis; and (4) to investigate age-dependent modifications of marker–IAV associations through formal interaction testing and age-stratified analyses.

2. Materials and methods

2.1 Study design and population

This retrospective study included pediatric patients (0–18 years) who presented with influenza-like illness (ILI) at the pediatric outpatient clinic and emergency department of the People's Hospital of Deyang City between January 2022 and December 2023. ILI was defined as the presence of fever (axillary temperature ≥ 38°C) and cough or sore throat, with onset within the preceding 72 h. Patients were included if they had undergone both a nasopharyngeal swab-based RADT for influenza A virus and a CBC within 24 h of presentation [20]. Exclusion criteria were: (1) incomplete clinical or laboratory data; (2) a history of immunosuppressive therapy or known immunodeficiency; (3) underlying hematological disorders; (4) co-infection confirmed by additional microbiological testing; and (5) use of antibiotics or antiviral medications within 48 h prior to presentation. A total of 1,379 patients (685 IAV-positive and 694 IAV-negative) met the inclusion criteria. To account for the well-established age-dependent changes in pediatric hematological parameters, patients were classified into five age groups based on developmental stages of the immune system [18, 19]: Group 1 (Infants): 0 to < 1 year (n = 80); Group 2 (Toddlers): 1 to < 3 years (n = 286); Group 3 (Preschool): 3 to < 6 years (n = 393); Group 4 (School-age): 6 to < 12 years (n = 476); Group 5 (Adolescents): 12 to 18 years (n = 144).

The research related to human use has complied with all the relevant national regulations, institutional policies, and in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' Institutional Review Board (Medical Ethics Committee of People's Hospital of Deyang City, 2023-04-102-K01). Given the retrospective nature of the study and the absence of any intervention, the ethics committee waived the requirement for separate informed consent.

2.2 Laboratory testing

Nasopharyngeal swab specimens were collected from each patient and tested using a commercially available RADT for influenza A and B (Wondfo Biotech Co., Ltd, Guangzhou, China). The test was performed according to the manufacturer's instructions, and results were read within 15–20 min Only patients with positive RADT results for influenza A were classified as the IAV-positive group. The IAV-negative group comprised patients who tested negative for both influenza A and influenza B.

Venous blood samples were collected at the time of clinical evaluation and analyzed using an BC7500 blood cell analyzer (Mindray Bio-Mdical Electronics Co., Ltd, Shenzhen, China). The following CBC parameters were recorded: white blood cell count (WBC), neutrophil count (NEUT), lymphocyte count (LYM), monocyte count (MONO), hemoglobin (Hb), hematocrit (HCT), platelet count (PLT), mean platelet volume (MPV), and platelet distribution width (PDW). C-reactive protein (CRP) levels were measured

using an Latex-enhanced immunonephelometry assay (Mindray Bio-Mdical Electronics Co., Ltd, Shenzhen, China). The NLR, MLR, SII and SIRI values were calculated using the above parameters.

2.3 Statistical analysis

All statistical analyses were performed using R 4.3.2 software (R Foundation for Statistical Computing, Vienna, Austria). Continuous variables were presented as median with interquartile range (IQR) owing to non-normal distributions, as assessed by the Shapiro–Wilk test. Comparisons between IAV-positive and IAV-negative groups were performed using the Mann–Whitney U test. Categorical variables were compared using the chi-square test or Fisher's exact test. Univariate logistic regression was performed to estimate crude odds ratios (OR) and 95% confidence intervals (CI) for the association between each hematological or inflammatory marker and IAV infection.

Three multivariable logistic regression models were constructed to assess the independent associations of markers with IAV infection after mutual adjustment. Model 1 (Traditional CBC model): Included WBC, NEUT, LYM, MONO, HCT, PLT, and CRP, along with age group as a forced covariate. Model 2 (Composite index model 1): Included WBC, HCT, PLT, CRP, NLR, and MLR, with age group as a forced covariate. This model replaced individual neutrophil, lymphocyte, and monocyte counts with their ratios to reduce collinearity while capturing essential biological information. Model 3 (Composite index model 2): Included WBC, HCT, PLT, CRP, SII, and SIRI, with age group as a forced covariate. This model evaluated the novel systemic inflammation indices. For all multivariable models, variables were selected based on clinical relevance and prior evidence; automated stepwise selection was not employed. Model discrimination was assessed using the area under the receiver operating characteristic curve (AUC). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) at the optimal cut-off were reported. Model fit was evaluated using Nagelkerke's pseudo-R^2. To formally test whether the associations between hematological markers and IAV infection varied by age, interaction terms (marker × age group) were introduced into separate logistic regression models for each marker. The significance of the interaction was assessed using likelihood ratio tests comparing models with and without the interaction term. Markers with interaction $P < 0.10$ were considered to have suggestive evidence of age-dependent effects, and those with $P < 0.05$ were considered statistically significant.

3. Results

3.1 Baseline characteristics

A total of 1,379 pediatric patients were included in the final analysis, comprising 685 IAV-positive (49.7%) and 694 IAV-negative (50.3%) cases. Baseline demographic and clinical characteristics are presented in Table 1. The age was 5.8 (3.1–9.4) in the IAV-positive group and 5.5 (2.8–9.6) in the IAV-negative group ($P = 0.482$). The sex distribution was similar between the two groups ($P = 0.493$). Fever duration at presentation was comparable (median 1.8 vs. 1.7 days, $P = 0.215$).

Table 1
Baseline characteristics and clinical demographic data.

Parameter	IAV-positive	IAV-negative	*P*
Sample N	685	694	--
Sex	361 (52.7%)	353 (50.9%)	0.493
Age	5.8 (3.1–9.4)	5.5 (2.8–9.6)	0.482
WBC ($\times 10^9$/L)	6.86 (5.52–8.51)	8.32 (6.30–11.10)	< 0.001
NEUT ($\times 10^9$/L)	4.78 (3.47–6.19)	5.31 (3.56–7.95)	< 0.001
LYM ($\times 10^9$/L)	1.17 (0.84–1.59)	1.78 (1.18–2.56)	< 0.001
MONO ($\times 10^9$/L)	0.78 (0.59–0.98)	0.82 (0.62–1.14)	< 0.001
HCT (%)	42.90 (40.80–45.20)	42.10 (40.00-44.60)	< 0.001
PLT ($\times 10^9$/L)	223 (188–259)	233 (195–275)	< 0.001
NLR	4.12 (2.49–6.36)	3.12 (1.64–5.29)	< 0.001
MLR	0.64 (0.48–0.87)	0.49 (0.34–0.70)	< 0.001
CRP (mg/L)	6.29 (5.00-12.59)	8.06 (5.00-20.70)	< 0.001
SII	875.39 (563–1403)	698.44 (393–1298)	< 0.001
SIRI	3.05 (1.80–4.90)	2.59 (1.33–4.99)	0.002

IAV-positive children had significantly lower total WBC counts ($P < 0.001$), lower neutrophil counts ($P < 0.001$), and markedly lower lymphocyte counts ($P < 0.001$) compared with IAV-negative children. Monocyte counts were also lower in the IAV-positive group ($P < 0.001$). Platelet counts were significantly lower in IAV-positive children ($P < 0.001$), whereas hematocrit was slightly higher ($P < 0.001$). The NLR was significantly elevated in IAV-positive children ($P < 0.001$). The MLR showed a more pronounced elevation ($P < 0.001$), with the largest relative difference (approximately 31%) among all markers examined. The SII was also higher in the IAV-positive group ($P < 0.001$), as was the SIRI ($P = 0.002$). CRP levels were significantly lower in IAV-positive children ($P < 0.001$), a finding consistent with the typical pattern observed in viral infections (Fig. 1).

3.2 Age-stratified analysis

Infants (0–1 year): IAV-positive infants showed substantially lower lymphocyte counts (1.82 vs. 3.63 × 10^9/L) and elevated MLR (0.54 vs. 0.30), with an MLR difference of 0.24. Neutrophil counts were similar between groups (2.97 vs. 2.89 $\times 10^9$/L).

Toddlers (1–3 years): Similar patterns persisted, with significantly lower lymphocyte counts (1.38 vs. 2.18 × 10^9/L) and elevated MLR (0.62 vs. 0.45) and NLR (2.74 vs. 1.73) in IAV-positive children.

Preschool (3–6 years): The NLR difference between IAV-positive and IAV-negative children was minimal (4.06 vs. 3.96), suggesting poor discriminatory ability of NLR in this age group. Conversely, MLR maintained a clear separation (0.62 vs. 0.54).

School-age (6–12 years): More pronounced differences emerged, with IAV-positive children showing higher NLR (4.91 vs. 3.99) and MLR (0.70 vs. 0.53).

Adolescents (12–18 years): The widest separation between groups was observed in this age subgroup. IAV-positive adolescents exhibited the highest NLR (5.85 vs. 3.78), highest MLR (0.80 vs. 0.44), and highest SII (1166.09 vs. 736.51) among all age groups. The MLR in IAV-positive adolescents was approximately 82% higher than that in IAV-negative adolescents (Table 2).

Table 2
Age-stratified comparison of hematological and inflammatory markers in children with and without influenza A virus infection.

Group	Parameter	IAV-positive	IAV-negative
Infants	WBC ($\times 10^9$/L)	6.15 (4.73–7.25)	7.99 (5.45–10.50)
	NEUT ($\times 10^9$/L)	2.97 (2.09–3.62)	2.89 (1.86–4.89)
	LYM ($\times 10^9$/L)	1.82 (1.21–2.79)	3.63 (2.34–4.83)
	MONO ($\times 10^9$/L)	0.91 (0.73–1.15)	1.03 (0.76–1.40)
	NLR	1.69 (1.18–2.04)	1.06 (0.58–1.68)
	MLR	0.54 (0.40–0.66)	0.30 (0.23–0.39)
	SII	412.39 (260.32-568.79)	261.43 (125.55-424.36)
	SIRI	1.31 (1.02–2.49)	0.96 (0.56–1.63)
	CRP (mg/L)	5.00 (5.00-8.24)	7.87 (5.00-23.07)
Toddlers	WBC ($\times 10^9$/L)	6.28 (5.18–7.99)	7.44 (5.59–10.24)
	NEUT ($\times 10^9$/L)	4.00 (2.89–5.38)	4.00 (2.74–6.26)
	LYM ($\times 10^9$/L)	1.38 (0.98–2.02)	2.18 (1.58–2.94)
	MONO ($\times 10^9$/L)	0.85 (0.71–1.08)	0.92 (0.70–1.37)
	NLR	2.74 (1.69–4.46)	1.73 (1.26–3.16)
	MLR	0.62 (0.44–0.83)	0.45 (0.32–0.62)
	SII	658.27 (354.52-1085.72)	443.80 (246.66-788.06)
	SIRI	2.30 (1.53–3.92)	1.81 (0.99–3.46)
	CRP (mg/L)	5.28 (5.00-11.69)	7.41 (5.00-17.96)
Preschool	WBC ($\times 10^9$/L)	6.97 (5.50–8.54)	9.02 (6.75–11.98)
	NEUT ($\times 10^9$/L)	4.92 (3.54–6.18)	6.15 (4.24-9.00)
	LYM ($\times 10^9$/L)	1.23 (0.86–1.70)	1.66 (1.11–2.38)
	MONO ($\times 10^9$/L)	0.78 (0.57–0.96)	0.87 (0.64–1.15)
	NLR	4.06 (2.56–5.82)	3.96 (2.23–6.36)
	MLR	0.62 (0.48–0.84)	0.54 (0.40–0.74)

Group	Parameter	IAV-positive	IAV-negative
	SII	871.43 (563.27-1406.60)	947.36 (521.63-1531.83)
	SIRI	2.97 (1.81–4.69)	3.30 (1.90–5.52)
	CRP (mg/L)	6.83 (5.00-12.24)	11.29 (5.00-24.70)
School-age	WBC ($\times 10^9$/L)	7.11 (5.65–8.66)	8.40 (6.51–10.96)
	NEUT ($\times 10^9$/L)	5.14 (3.80–6.54)	5.69 (4.13–8.45)
	LYM ($\times 10^9$/L)	1.1 (0.78–1.35)	1.55 (1.07–2.03)
	MONO ($\times 10^9$/L)	0.71 (0.56–0.91)	0.72 (0.58–1.04)
	NLR	4.91 (3.04–7.08)	3.99 (2.56–6.16)
	MLR	0.7 (0.51–0.90)	0.53 (0.38–0.72)
	SII	1048.24 (648.91-1578.67)	891.43 (518.12-1569.18)
	SIRI	3.64 (2.12–5.45)	3.06 (1.59–5.29)
	CRP (mg/L)	6.06 (5.00-12.66)	8.15 (5.00-23.64)
Adolescents	WBC ($\times 10^9$/L)	7.44 (5.92–8.57)	8.38 (6.89–10.37)
	NEUT ($\times 10^9$/L)	5.61 (4.22–6.75)	5.89 (4.54–7.96)
	LYM ($\times 10^9$/L)	0.96 (0.66–1.27)	1.71 (1.09–2.24)
	MONO ($\times 10^9$/L)	0.78 (0.61–0.92)	0.68 (0.56–0.94)
	NLR	5.85 (4.00-8.39)	3.78 (2.05–6.53)
	MLR	0.8 (0.57–1.08)	0.44 (0.27–0.79)
	SII	1166.09 (731.70-1646.58)	736.51 (456.05-1427.64)
	SIRI	4.01 (2.81–5.84)	2.88 (1.23–6.29)
	CRP (mg/L)	9.5 (5.00-16.91)	5.00 (5.00-10.37)

3.3 Logistic regression analysis

Crude univariate logistic regression identified multiple markers significantly associated with IAV infection (Table 3, Fig. 2). The strongest positive associations were observed for MLR (OR = 4.129, 95% CI: 2.913–5.853, $P < 0.001$), followed by NLR (OR = 1.057, 95% CI: 1.026–1.089, $P < 0.001$), and HCT (OR = 1.047, 95% CI: 1.016–1.079, $P = 0.003$). The strongest protective factors were LYM (OR = 0.411, 95% CI: 0.355–0.477, $P < 0.001$), MONO (OR = 0.458, 95% CI: 0.343–0.611, $P < 0.001$), WBC (OR = 0.816, 95% CI: 0.784–0.849, $P < 0.001$), and CRP (OR = 0.970, 95% CI: 0.961–0.979, $P < 0.001$). Notably, SII showed a

weak but statistically significant positive association (OR = 1.0001, 95% CI: 1.0000–1.0002, *P* = 0.024), whereas SIRI was not significantly associated with IAV infection in univariate analysis (OR = 0.998, 95% CI: 0.969–1.026, *P* = 0.862).

Table 3
Univariate logistic regression analysis.

Variable	**OR**	**95% CI**	***P***
WBC	0.8158	0.7844–0.8485	< 0.001
NENE	1.0208	1.0132–1.0285	< 0.001
NEUT	0.8712	0.8378–0.9060	< 0.001
LY	0.9659	0.9570–0.9749	< 0.001
LYM	0.4113	0.3548–0.4769	< 0.001
MO	1.0546	1.0276–1.0822	< 0.001
MONO	0.4578	0.3430–0.6111	< 0.001
HCT	1.0471	1.0159–1.0793	0.0029
PLT	0.9969	0.9951–0.9987	< 0.001
PDW	0.9977	0.9498–1.0481	0.9274
MPV	1.0626	0.9682–1.1662	0.2007
NLR	1.0571	1.0259–1.0893	< 0.001
MLR	4.1291	2.9130–5.8528	< 0.001
CRP	0.9701	0.9611–0.9793	< 0.001
SII	1.0001	1.0000-1.0002	0.0239
SIRI	0.9975	0.9694–1.0264	0.862

After adjustment for age group, the OR estimates for most markers were modestly attenuated but remained statistically significant. The age-adjusted OR for MLR was 3.642 (95% CI: 2.476–5.358, *P* < 0.001), and that for LYM was 0.427 (95% CI: 0.360–0.506, *P* < 0.001), confirming that the associations of these markers with IAV infection were independent of age effects.

As shown in Table 4, three multivariable logistic regression models were constructed, all with age group included as a forced covariate.

Table 4
Multivariable logistic regression analysis.

Model	Variable	OR	95% CI	*P*
Model 1	WBC	0.5334	0.3108–0.9155	0.0226
	NEUT	1.5327	0.8919–2.6340	0.1222
	LYM	0.6259	0.3559–1.1010	0.104
	MONO	4.6996	2.4559–8.9928	< 0.001
	HCT	1.0501	1.0145–1.0869	0.0054
	PLT	1.0018	0.9996–1.0040	0.1111
	CRP	0.9812	0.9706–0.9918	< 0.001
Model 2	WBC	0.7815	0.7425–0.8226	< 0.001
	HCT	1.0537	1.0189–1.0897	0.0023
	PLT	1.0012	0.9991–1.0034	0.2489
	CRP	0.9848	0.9743–0.9953	0.0047
	NLR	1.0383	0.9843–1.0952	0.1675
	MLR	4.8226	2.8313–8.2143	< 0.001
Model 3	WBC	0.7253	0.6832–0.7699	< 0.001
	HCT	1.0569	1.0224–1.0926	0.0011
	PLT	1.0006	0.9983–1.0028	0.6244
	CRP	0.9859	0.9757–0.9962	0.0075
	SII	1.0002	1.0000-1.0005	0.0781
	SIRI	1.1446	1.0614–1.2345	< 0.001

Model 1 (Traditional CBC parameters): This model included WBC, NEUT, LYM, MONO, HCT, PLT, CRP, and age group. WBC demonstrated a negative association (OR = 0.533, $P = 0.023$), whereas MONO showed a strong positive association (OR = 4.700, $P < 0.001$).

Model 2 (Composite index model 1): This model included WBC, HCT, PLT, CRP, NLR, MLR, and age group. MLR emerged as the strongest independent predictor (OR = 4.823, 95% CI: 2.831–8.214, $P < 0.001$), whereas NLR was not independently significant (OR = 1.038, $P = 0.167$), indicating that MLR captured most of the composite index information relevant to IAV discrimination. WBC remained negatively associated (OR = 0.782, $P < 0.001$).

Model 3 (Composite index model 2): This model included WBC, HCT, PLT, CRP, SII, SIRI, and age group. Notably, in this model, SIRI demonstrated a significant positive association with IAV infection (OR = 1.145, 95% CI: 1.061–1.235, $P < 0.001$), contrasting sharply with its non-significant association in univariate analysis (OR = 0.998, $P = 0.862$). This pattern — in which a variable becomes significant only after adjusting for other covariates — is a classic suppression effect: the crude association of SIRI with IAV infection was masked by confounding influences of other CBC parameters, and adjustment for these covariates unmasked its independent contribution. SII was not independently significant in this model (OR = 1.0002, $P = 0.078$).

3.4 ROC analysis

As shown in Table 5 and Fig. 3, LYM (AUC = 0.705, 95% CI: 0.681–0.729) demonstrated the highest discriminatory performance. At the optimal cut-off of 1.60 $\times 10^9$/L, sensitivity was 75.6% and specificity was 59.1%, with PPV of 64.6% and NPV of 71.1%. MLR (AUC = 0.659, 95% CI: 0.634–0.684) was the second-best individual marker. At the optimal cut-off of 0.53, sensitivity was 70.2% and specificity was 55.9%, with PPV of 61.1% and NPV of 65.5%. WBC (AUC = 0.648, 95% CI: 0.623–0.673) showed moderate discriminatory capacity. At the cutoff of 9.30 $\times 10^9$/L, sensitivity was 85.1% but specificity was only 39.5%, reflecting its role as a screening rather than a diagnostic marker. NLR (AUC = 0.594, 95% CI: 0.568–0.620) and SII (AUC = 0.577, 95% CI: 0.551–0.603) showed weak discriminatory performance. SIRI (AUC = 0.549, 95% CI: 0.523–0.575) showed the poorest discrimination among the composite indices, consistent with its marginal univariate association. Among the multivariable models, Model 1 achieved the highest AUC (0.746, 95% CI: 0.723–0.769), followed by Model 2 (AUC = 0.735, 95% CI: 0.712–0.759) and Model 3 (AUC = 0.713, 95% CI: 0.689–0.737). The difference between Model 1 and Model 2 was not statistically significant (DeLong test $P = 0.183$), suggesting that the composite index model achieved comparable discriminatory performance with substantially fewer collinearity concerns.

Table 5
Diagnostic efficacy of hematological indicators and multivariable models for IAV infection in children

Variable	AUC	95% CI	Cut-off	Se	Sp	PPV	NPV
WBC	0.6478	0.6226–0.6730	9.3	0.8511	0.3948	0.5813	0.7287
NENE	0.5727	0.5466–0.5988	61.6	0.7504	0.4006	0.5527	0.6192
NEUT	0.5731	0.5470–0.5992	7.89	0.9226	0.2536	0.5496	0.7686
LY	0.6059	0.5801–0.6316	25.4	0.7796	0.4049	0.5639	0.6505
LYM	0.7051	0.6810–0.7291	1.6	0.7562	0.5908	0.6459	0.7106
MO	0.579	0.5529–0.6050	10.3	0.6102	0.5259	0.5596	0.5775
MONO	0.565	0.5388–0.5912	0.99	0.7635	0.3732	0.5459	0.6152
HCT	0.558	0.5318–0.5842	41.7	0.6701	0.4438	0.5432	0.5768
PLT	0.5542	0.5280–0.5804	272	0.8248	0.2666	0.5261	0.6066
PDW	0.5077	0.4813–0.5340	15.2	0.8307	0.2118	0.5099	0.5589
MPV	0.5198	0.4935–0.5462	10.7	0.3255	0.7233	0.5373	0.5207
NLR	0.5944	0.5684–0.6203	2.31	0.781	0.3948	0.5602	0.6462
MLR	0.6594	0.6344–0.6844	0.53	0.7022	0.5591	0.6112	0.6554
CRP	0.5641	0.5379–0.5903	16.2	0.8438	0.3184	0.55	0.6738
SII	0.5769	0.5509–0.6030	539.2626	0.7737	0.4006	0.5603	0.642
SIRI	0.5488	0.5226–0.5751	1.9713	0.7255	0.3991	0.5438	0.5957
Model 1	0.7463	0.7233–0.7692	0.4454	0.8307	0.5648	0.6533	0.7717
Model 2	0.7352	0.7119–0.7585	0.5029	0.7066	0.6599	0.6722	0.695
Model 3	0.7129	0.6890–0.7368	0.4635	0.7869	0.5533	0.6349	0.7245

3.5 Age interaction analysis

LYM × age group: The interaction was highly significant ($\chi^2 = 50.53$, df = 4, $P < 0.001$), indicating that the protective association of higher LYM with IAV infection differs substantially across age groups. The LYM difference between IAV-positive and IAV-negative children was most pronounced in adolescents (0.96 vs. 1.71 $\times 10^9$/L) and least pronounced in preschoolers, although the protective pattern was consistent across all age strata (Table 2).

WBC × age group: A significant interaction was observed (χ^2 = 19.61, P < 0.001). The WBC difference between IAV groups was largest in the preschool group (6.97 vs. 9.02 ×10^9/L) and smallest in the adolescent group (7.44 vs. 8.38 ×10^9/L).

CRP × age group: A significant interaction was found (χ^2 = 14.46, P = 0.006). Notably, in adolescents, CRP levels were higher in IAV-positive compared with IAV-negative children (9.50 vs. 5.00 mg/L), which was the opposite pattern from other age groups in which CRP was generally lower in IAV-positive children.

MLR × age group: The interaction was significant (χ^2 = 12.64, P = 0.013). The MLR difference between IAV-positive and IAV-negative children increased progressively with age, from a 0.24 difference in infants (0.54 vs. 0.30) to a 0.36 difference in adolescents (0.80 vs. 0.44).

HCT × age group (P = 0.034) and PLT × age group (P = 0.038) also showed significant interactions. NLR × age group (P = 0.067) and SIRI × age group (P = 0.052) showed suggestive but non-significant interactions at the conventional α = 0.05 threshold. The NLR interaction was driven by the near absence of an NLR difference in preschool children (IAV-positive: 4.06 vs. IAV-negative: 3.96) contrasted with the substantial difference in adolescents (5.85 vs. 3.78).

3.6 Correlation analysis

A Spearman correlation heatmap (Fig. 4) revealed expected relationships among hematological markers. Strong correlations were observed between WBC and NEUT (r = 0.89), between NLR and SII (r = 0.94), and between NLR and SIRI (r = 0.86). MLR showed moderate correlations with NLR (r = 0.72) and SII (r = 0.67), and a strong correlation with SIRI (r = 0.80), suggesting that MLR captures a dimension of the inflammatory response partially distinct from NEUT indices. CRP showed weak to modest correlations with all markers, indicating its independent contribution beyond the CBC-derived measures.

4. Discussion

This retrospective study of 1,379 pediatric patients provides a comprehensive evaluation of hematological and inflammatory markers associated with IAV infection in children, with particular emphasis on age-dependent effects. The principal findings are as follows: (1) IAV-positive children exhibit a distinct hematological profile characterized by leukopenia, lymphopenia, and elevated MLR; (2) MLR is the strongest independent predictor of IAV infection among the markers examined, with an adjusted OR of 4.823 in multivariable models; (3) lymphocyte count demonstrates the best single-marker discriminatory performance (AUC = 0.705); and (4) the associations of WBC, lymphocyte count, MLR, and CRP with IAV infection exhibit significant age-dependent variations, underscoring the importance of age stratification in applying these markers clinically.

4.1 MLR as a robust marker of IAV infection

The strong and consistent association of MLR with IAV infection merits particular attention. In this study, MLR achieved the highest odds ratio among all markers in both univariate (OR = 4.129) and multivariable (OR = 4.823) analyses, and its discriminatory performance (AUC = 0.659) was second only to that of lymphocyte count. The biological plausibility of this association is supported by well-characterized roles of monocytes and lymphocytes in the immune response to influenza. Lymphopenia in influenza results from a combination of direct viral cytopathic effects on lymphocytes, virus-induced apoptosis, and sequestration of activated lymphocytes in the respiratory tract [11]. Concurrently, monocytes are actively recruited to infected lung tissue via CCL2–CCR2 signaling, where they differentiate into macrophages and dendritic cells that contribute to viral clearance through phagocytosis, antigen presentation, and cytokine production [21]. The simultaneous decrease in lymphocyte count and the relative preservation or increase in monocyte count amplifies the MLR, rendering it a sensitive composite indicator of the host's immune response to IAV infection.

Importantly, MLR outperformed NLR in our analyses. In Model 2, where both ratios were included, MLR remained highly significant (OR = 4.823, $P < 0.001$), whereas NLR was not independently associated ($P = 0.167$). This superiority of MLR over NLR likely reflects the greater specificity of monocyte dynamics in viral infection. Although neutrophil elevation can occur in both viral and bacterial infections (making NLR less specific), the interplay between monocyte recruitment and lymphocyte depletion is more characteristic of the antiviral response [8]. Our findings are consistent with those of Temel et al. [8], who reported that MLR was superior to NLR in differentiating influenza from other respiratory infections in children. The present study extends these observations by demonstrating the robustness of the MLR association after rigorous adjustment for age and other confounding factors.

4.2 Age-dependent modifications of hematological markers

The age-dependent variations in marker–IAV associations represent a critical finding with direct clinical implications. Our formal interaction testing revealed that the associations of WBC, lymphocyte count, MLR, and CRP with IAV infection differ significantly across pediatric age groups, indicating that uniform diagnostic thresholds applied across all ages would be inappropriate.

The interaction for MLR showed a particularly instructive pattern: the difference between IAV-positive and IAV-negative children increased progressively from infancy through adolescence. This monotonic increase likely reflects the physiological maturation of the immune system. In infants and young children, the immune system is dominated by adaptive immunity with physiologically elevated lymphocyte counts [18]. As children mature, the innate immune system becomes relatively more prominent, and the balance between lymphocyte and monocyte compartments shifts. Consequently, the same viral infection generates a more exaggerated MLR response in older children. This progressive pattern suggests that MLR may serve as a more reliable marker across a broader age range than NLR — but only if age-specific thresholds are applied.

NLR, in contrast, showed a non-monotonic age-dependent pattern. The NLR difference between IAV-positive and IAV-negative children was negligible in the preschool group (4.06 vs. 3.96), rendering NLR of minimal diagnostic value in children aged 3–6 years. However, the NLR difference was substantial in adolescents (5.85 vs. 3.78). The age interaction for NLR did not reach formal statistical significance (P = 0.067), suggesting that this pattern should be interpreted cautiously and validated in independent cohorts. The near absence of an NLR difference in preschoolers likely reflects the counterbalancing effects of two age-related phenomena: (1) young children have higher baseline lymphocyte counts, which attenuate the relative lymphopenia-driven NLR increase; and (2) their neutrophil response to viral infection is less robust than that in older children.

The CRP–age interaction (P = 0.006) revealed an intriguing pattern worthy of further investigation. In younger children, CRP levels were consistently lower in IAV-positive compared with IAV-negative children — a pattern consistent with the typical acute-phase response to viral infection, in which interferons suppress IL-6-driven CRP production [22]. However, in adolescents, CRP was paradoxically higher in IAV-positive children (9.50 vs. 5.00 mg/L). Several mechanisms might explain this reversal. First, hormonal changes during puberty — particularly the increase in sex steroids — may modulate the acute-phase response, potentially amplifying IL-6 signaling in response to viral infection. Second, the maturing immune system in adolescents may mount a more vigorous inflammatory response to IAV, including a stronger IL-6-driven acute-phase reaction [23]. Third, although we excluded confirmed co-infections, the possibility of undetected concurrent bacterial colonization or secondary inflammatory processes in older children cannot be completely ruled out. Further studies with comprehensive cytokine profiling are needed to clarify the biological basis of this age-dependent CRP response.

4.3 Clinical implications and diagnostic utility

Our findings have several important clinical implications. Given the limited sensitivity of RADTs — particularly in children with lower viral loads [7] — hematological markers could serve as adjunctive tools to support clinical decision-making in settings where RADT results are equivocal or where molecular testing is unavailable. Based on our results, we propose the following considerations: First, lymphocyte count < 1.60 $\times 10^9$/L provides moderate supportive evidence for IAV infection, with an AUC of 0.705 and a sensitivity of 75.6%. This threshold is clinically practical, as lymphocyte count is a standard component of any CBC report and requires no additional calculation. Second, MLR > 0.53 offers incremental supportive evidence, particularly when lymphocyte count alone is borderline. Although MLR requires a simple calculation, it can be derived from any CBC report and carries the advantage of capturing the synergistic information from two cell lineages. Third, diagnostic performance varies substantially by age. In infants and toddlers, MLR cut-offs should be lower (reflecting their lower baseline MLR), whereas in adolescents, higher MLR thresholds are warranted. For NLR, our data suggest it should be used with caution in preschool children (3–6 years), where it showed negligible discriminatory ability. Fourth, multivariable models combining CBC parameters can achieve AUC values approaching 0.75, which may be clinically useful when RADT results are negative but clinical suspicion remains high. However, the incremental gain from multivariable models over lymphocyte count alone (AUC difference approximately

0.04) is modest, suggesting that in most clinical scenarios, lymphocyte count and MLR may offer sufficient adjunctive information without complex calculations.

It is essential to emphasize that CBC-derived markers are not a substitute for virological confirmation. The moderate AUC values (0.55–0.71 for individual markers) indicate that these markers have limited standalone diagnostic accuracy and should be interpreted in conjunction with clinical assessment and RADT results. Their greatest value likely lies in their negative predictive role: in settings where RADT is unavailable or impractical, a normal lymphocyte count and MLR may help reduce the probability of IAV infection, whereas markedly abnormal values may strengthen the case for empiric antiviral therapy pending confirmatory testing.

4.4 Strengths and limitations

This study has several strengths. First, the large sample size (n = 1,379) provides adequate statistical power for stratified analyses and interaction testing, enabling robust detection of age-dependent effects that smaller studies could not have identified. Second, the comprehensive assessment of multiple hematological markers — including both traditional CBC parameters and novel composite indices (SII, SIRI) — allows for direct comparison of their relative diagnostic value within a single, well-characterized cohort. Third, the formal age-interaction analysis, rarely performed in prior studies, addresses a critical gap in the literature by demonstrating that marker–IAV associations are not uniform across pediatric development.

Several limitations must be acknowledged. First, the retrospective single-center design may limit the generalizability of our findings. The patient population and clinical practices at a single tertiary care center may not be representative of other healthcare settings. Second, IAV infection was diagnosed by RADT rather than RT-PCR, the gold standard. RADT has imperfect sensitivity (estimated at 50–80% [5, 6]), and some patients classified as IAV-negative may have been false negatives, which would bias our results toward the null. However, this limitation also reflects the real-world clinical context in which these markers would be applied — RADT remains the most widely used initial diagnostic tool — making our findings directly relevant to actual clinical practice. Third, co-infections with other respiratory viruses were not systematically excluded through multiplex PCR testing; some IAV-negative patients may have had other viral infections (e.g., RSV, rhinovirus, SARS-CoV-2), which could have influenced their hematological profiles and reduced between-group differences. Fourth, the cross-sectional design of marker assessment at a single time point precludes analysis of temporal changes in these markers and limits causal inference.

5. Conclusions

In conclusion, hematological and inflammatory markers — particularly lymphocyte count and MLR — are significantly associated with IAV infection in children presenting with influenza-like illness. Among the markers examined, MLR showed the strongest independent association with IAV infection, outperforming NLR, SII, and SIRI in both univariate and multivariable analyses. The diagnostic

performance of these markers is moderate but age-dependent. The significant age interactions for WBC, lymphocyte count, MLR, CRP, HCT, and PLT indicate that uniform diagnostic thresholds applied across all pediatric ages are unlikely to be optimal. While routine CBC-derived indices cannot replace virological confirmation, they may serve as cost-effective adjunctive tools, particularly when rapid antigen test results are equivocal or molecular testing is unavailable.

Abbreviations

IAV, influenza A virus; RADT, rapid antigen detection test; CBC, complete blood count; RT-PCR, reverse transcription-polymerase chain reaction; WBC, white blood cell; NEUT, neutrophil; LYM, lymphocyte; MONO, monocyte; HCT, hematocrit; PLT, platelet; CRP, C-reactive protein; NLR, neutrophil-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; SII, systemic immune-inflammation index; SIRI, systemic inflammation response index; OR, odds ratio; CI, confidence interval; AUC, area under the ROC curve; ROC, receiver operating characteristic; FDR, false discovery rate; IQR, interquartile range; ILI, influenza-like illness

Declarations

Ethics approval and consent to participate: The research related to human use has complied with all the relevant national regulations, institutional policies, and in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' Institutional Review Board (Medical Ethics Committee of People's Hospital of Deyang City, 2023-04-102-K01).

Consent for publication: Given the retrospective nature of the study and the absence of any intervention, the ethics committee waived the requirement for separate informed consent.

Availability of data and materials: All data generated or analysed during this study are included in this published article.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions:

E jianfei: Conceptualization, Methodology, Formal analysis, Writing — Original Draft

Shu yao: Data curation, Investigation, Validation, Writing — Original Draft

Yang hao: Software, Visualization, Writing — Review & Editing

Zheng mao: Supervision, Project administration, Funding acquisition, Writing — Original Draft

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References

1. Iuliano AD, Roguski KM, Chang HH, Muscatello DJ, Palekar R, Tempia S, et al. Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. Lancet. 2018;391(10127):1262. 10.1016/S0140-6736(18)30105-3.
2. Nair H, Brooks WA, Katz M, Roca A, Berkley JA, Madhi SA, et al. Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis. Lancet. 2011;378(9807):1917–30. 10.1016/S0140-6736(11)61051-9.
3. Uyeki TM, Bernstein HH, Bradley JS, Englund JA, File TM, Fry AM, et al. Clinical Practice Guidelines by the Infectious Diseases Society of America: 2018 Update on Diagnosis, Treatment, Chemoprophylaxis, and Institutional Outbreak Management of Seasonal Influenzaa. Clin Infect Dis. 2019;68(10):1790. 10.1093/cid/ciz044.
4. Merckx J, Wali R, Schiller I, Caya C, Gore GC, Chartrand C, et al. Diagnostic Accuracy of Novel and Traditional Rapid Tests for Influenza Infection Compared With Reverse Transcriptase Polymerase Chain Reaction: A Systematic Review and Meta-analysis. Ann Intern Med. 2017;167(6):394–409. 10.7326/M17-0848.
5. Chartrand C, Leeflang MM, Minion J, Brewer T, Pai M. Accuracy of rapid influenza diagnostic tests: a meta-analysis. Ann Intern Med. 2012;156(7):500–11. 10.7326/0003-4819-156-7-201204030-00403.
6. Ferrani S, Prazuck T, Béchet S, Lesne F, Cohen R, Levy C. Diagnostic accuracy of a rapid antigen triple test (SARS-CoV-2, respiratory syncytial virus, and influenza) using anterior nasal swabs versus multiplex RT-PCR in children in an emergency department. Infect Dis Now. 2023;53(7):104769. 10.1016/j.idnow.2023.104769.
7. Bell C, Birstler J, Goss MD, Temte E, Barlow S, Chen G, et al. Factors influencing sensitivity of a rapid influenza diagnostic test in a community-based population of kindergarten through 12th-grade students: Wisconsin 2015–2020. Influenza Other Respir Viruses. 2023;17(1):e13064. 10.1111/irv.13064.
8. Temel H, Gündüz M, Tosun AI, Celebi M, Okur M. The Importance of Neutrophil/Lymphocyte and Lymphocyte/Monocyte Ratios in The Diagnosis of Influenza in Children. Clin Lab. 2021;67(4). 10.7754/Clin.Lab.2020.200907.
9. Zhu R, Chen C, Wang Q, Zhang X, Lu C, Sun Y. Routine blood parameters are helpful for early identification of influenza infection in children. BMC Infect Dis. 2020;20(1):864. 10.1186/s12879-020-05584-5.

10. Hong J, Lv J, Wu M, Shao J, Wu Q. The blood routine test holds screening values for influenza A in 2023: a retrospective study. Transl Pediatr. 2024;13(2):236–47. 10.21037/tp-23-435. . Epub 2024 Feb 23. PMID: 38455751; PMCID: PMC10915438.
11. Nichols JE, Niles JA, Roberts NJ Jr. Human lymphocyte apoptosis after exposure to influenza A virus. J Virol. 2001;75(13):5921–9. 10.1128/JVI.73.13.5921-5929.2001.
12. Bunce PE, High SM, Nadjafi M, Stanley K, Liles WC, Christian MD. Pandemic H1N1 influenza infection and vascular thrombosis. Clin Infect Dis. 2011;52(2):e14–7. 10.1093/cid/ciq125.
13. Kissling M, Fritschi N, Baumann P, Buettcher M, Bonhoeffer J, Naranbhai V, et al. Monocyte, Lymphocyte and Neutrophil Ratios - Easy-to-Use Biomarkers for the Diagnosis of Pediatric Tuberculosis. Pediatr Infect Dis J. 2023;42(6):520–7. 10.1097/INF.0000000000003901.
14. Duman L, Karaibrahimoğlu A, Büyükyavuz Bİ, Savaş MÇ. Diagnostic Value of Monocyte-to-Lymphocyte Ratio Against Other Biomarkers in Children With Appendicitis. Pediatr Emerg Care. 2022;38(2):e739–42. 10.1097/PEC.0000000000002347.
15. Dulkadir R, Oztelcan Gunduz B, Differentiating. COVID-19 and influenza in children: hemogram parameters as diagnostic tools. Front Public Health. 2024;12:1377785. 10.3389/fpubh.2024.1377785.
16. Hu B, Yang XR, Xu Y, Sun YF, Sun C, Guo W, et al. Systemic immune-inflammation index predicts prognosis of patients after curative resection for hepatocellular carcinoma. Clin Cancer Res. 2014;20(23):6212–22. 10.1158/1078-0432.CCR-14-0442.
17. Qi Q, Zhuang L, Shen Y, Geng Y, Yu S, Chen H, et al. A novel systemic inflammation response index (SIRI) for predicting the survival of patients with pancreatic cancer after chemotherapy. Cancer. 2016;122(14):2158–67. 10.1002/cncr.30057.
18. Higgins V, Tahmasebi H, Bohn MK, Hall A, Adeli K. CALIPER Hematology Reference Standards (II). Am J Clin Pathol. 2020;154(3):342–52. 10.1093/ajcp/aqaa057.
19. Zierk J, Hirschmann J, Toddenroth D, Arzideh F, Haeckel R, Bertram A, et al. Next-generation reference intervals for pediatric hematology. Clin Chem Lab Med. 2019;57(10):1595–607. 10.1515/cclm-2018-1236.
20. Guidelines for the Diagnosis and Treatment of Influenza. (2025 Edition)[J]. Clinical Education of General Practice, 2025, 23(7): 581–589. 10.13558/j.cnki.issn1672-3686.2025.007.002
21. Lin KL, Suzuki Y, Nakano H, Ramsburg E, Gunn MD. CCR2 + monocyte-derived dendritic cells and exudate macrophages produce influenza-induced pulmonary immune pathology and mortality. J Immunol. 2008;180(4):2562–72. 10.4049/jimmunol.180.4.2562.
22. Huang L, Ye C, Zhou R, Ji Z. Diagnostic value of routine blood tests in differentiating between SARS-CoV-2, influenza A, and RSV infections in hospitalized children: a retrospective study. BMC Pediatr. 2024;24(1):328. 10.1186/s12887-024-04822-y.
23. Coates BM, Staricha KL, Wiese KM, Ridge KM. Influenza A Virus Infection, Innate Immunity, and Childhood. JAMA Pediatr. 2015;169(10):956–63. 10.1001/jamapediatrics.2015.1387.

Figures

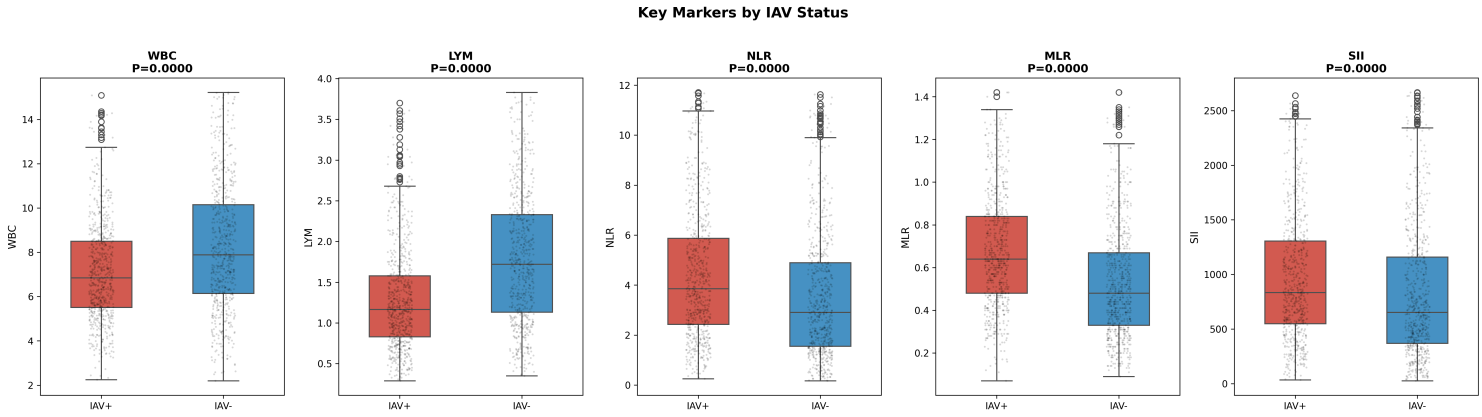


Figure 1

Boxplot comparison of key hematological markers between IAV-positive and IAV-negative groups.

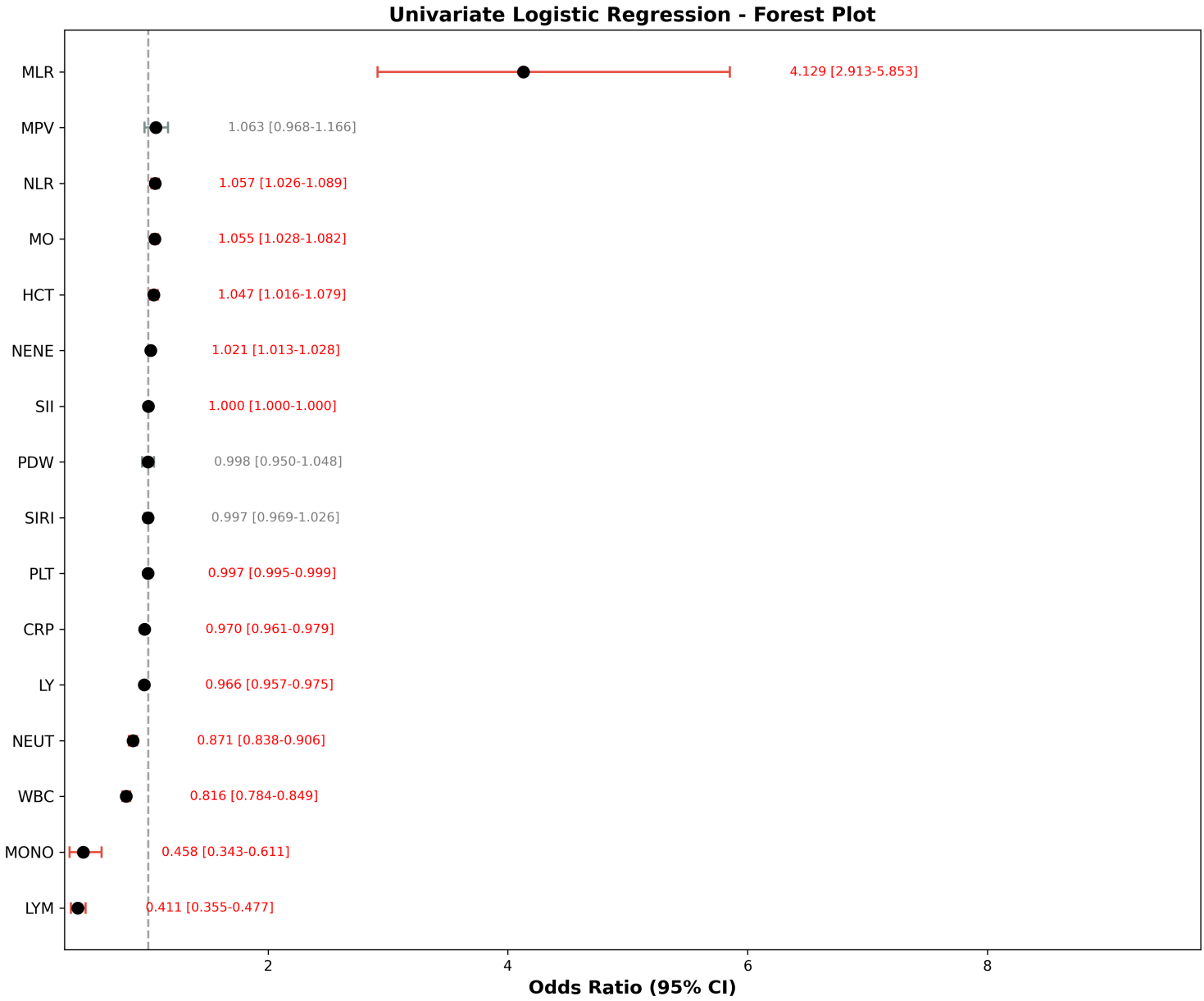


Figure 2

Forest plot of univariate logistic regression.

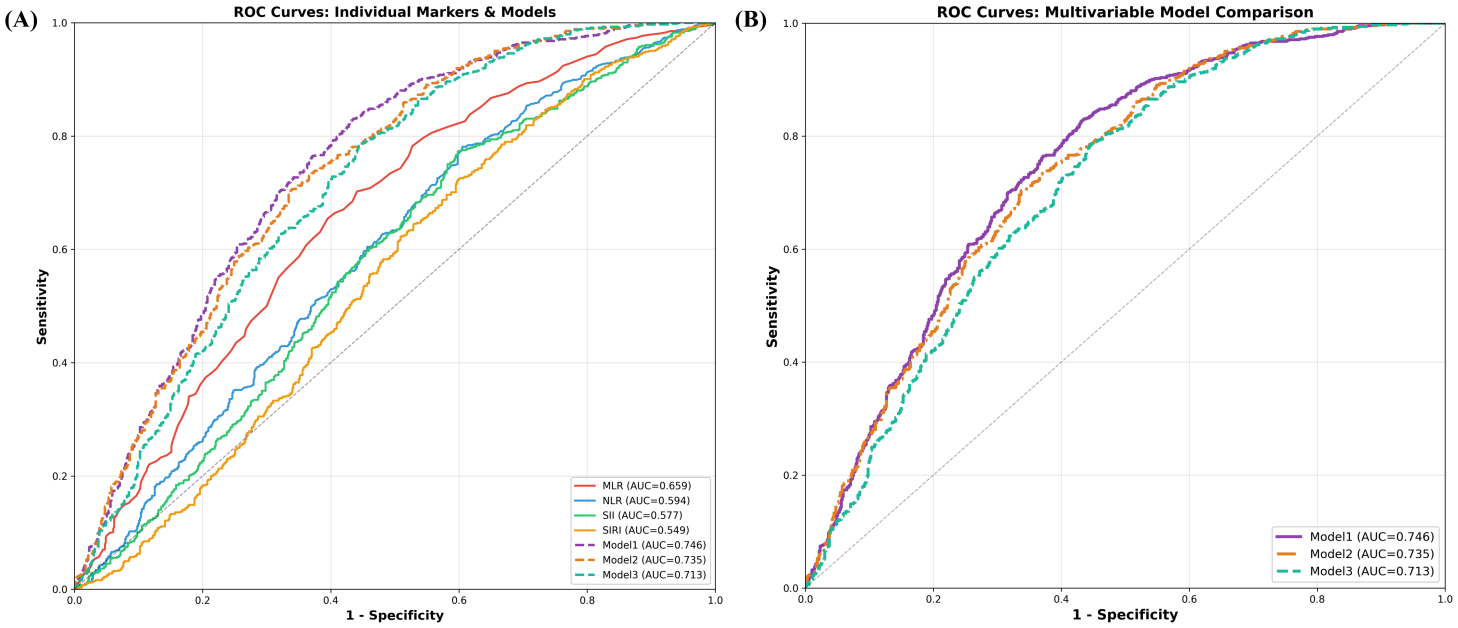


Figure 3

ROC curve analysis. (A) ROC curves of individual hematological markers; (B) ROC curves of three multivariable models.

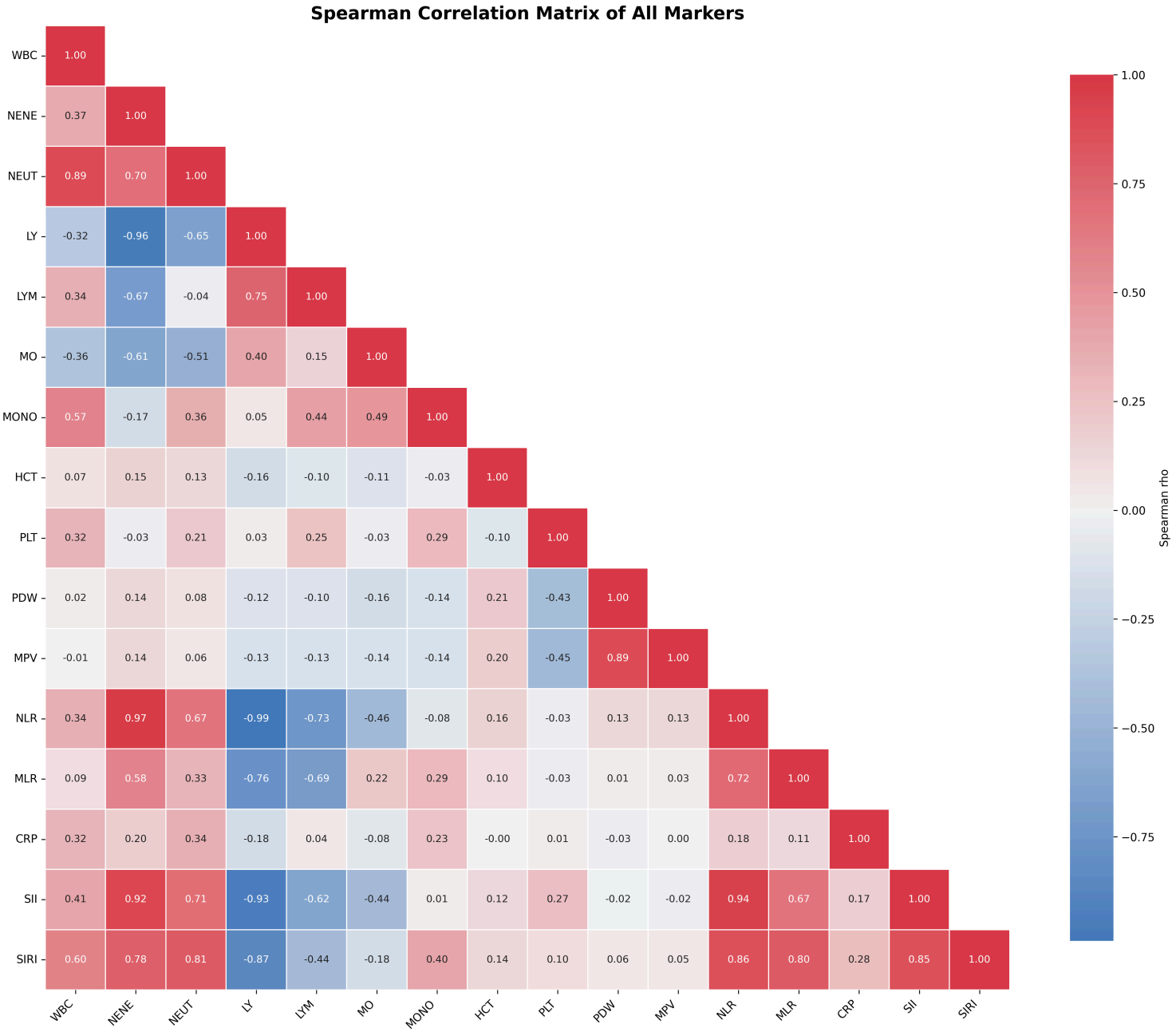


Figure 4

Spearman correlation heatmap of hematological and inflammatory markers.