Development of a nomogram for severe influenza in previously healthy children: a retrospective cohort study

Wenyun Huang[1,2], Wensi Niu[2], Hongmei Chen[1], Wujun Jiang[2], Yanbing Fu[1,2], Xiuxiu Li[1,2], Minglei Li[1,2], Jun Hua[1,2],* and **Chunxia Hu[2],***

Abstract

Objective: We aimed to develop a nomogram to predict the risk of severe influenza in previously healthy children.

Methods: In this retrospective cohort study, we reviewed the clinical data of 1135 previously healthy children infected with influenza who were hospitalized in the Children's Hospital of Soochow University between 1 January 2017 and 30 June 2021. Children were randomly assigned in a 7:3 ratio to a training or validation cohort. In the training cohort, univariate and multivariate logistic regression analyses were used to identify risk factors, and a nomogram was established. The validation cohort was used to evaluate the predictive ability of the model.

Result: Wheezing rales, neutrophils, procalcitonin > 0.25 ng/mL, *Mycoplasma pneumoniae* infection, fever, and albumin were selected as predictors. The areas under the curve were 0.725 (95% CI: 0.686–0.765) and 0.721 (95% CI: 0.659–0.784) for the training and validation cohorts, respectively. The calibration curve showed that the nomogram was well calibrated.

Conclusion: The nomogram may predict the risk of severe influenza in previously healthy children.

[1]Department of Emergency Medicine, Children's Hospital of Soochow University, Suzhou, China
[2]Department of Pediatrics, Suzhou Wujiang District Children's Hospital (Children's Hospital of Soochow University Wujiang Branch), Suzhou, China

*These authors contributed equally to this work.

Corresponding author:
Jun Hua, Children's Hospital of Soochow University, No. 92 Zhongnan Street, Suzhou Industrial Park, Suzhou, Jiangsu 215025, China.
Email: huajun@suda.edu.cn

Introduction

All populations are susceptible to infection with influenza viruses. Seasonal influenza epidemics occur worldwide every year and can lead to millions of cases of severe illness. Children are a high-risk group for influenza. A 2018 meta-analysis showed that approximately 109.5 million children aged <5 years were infected with influenza and approximately 870,000 children were hospitalized with influenza-related acute lower respiratory tract infection. Moreover, up to 34,800 children aged <5 years died from influenza-related acute lower respiratory tract infection, accounting for 4% of all deaths from acute lower respiratory tract infection in this age group.[1] The high incidence of influenza among children aged <5 years in China is a considerable burden.[2] Children <5 years of age or those with underlying medical conditions are also at increased risk for influenza-related extrapulmonary complications such as acute renal failure, myocarditis/myositis, rhabdomyolysis, and encephalitis/encephalopathy.[3–5] The American Academy of Pediatrics states that individuals with underlying medical conditions including asthma, cardiovascular disease (except hypertension), and renal, hematologic, metabolic, and neurologic diseases (including moderate to severe developmental delay) may have a higher risk of contracting critical influenza illness.[6] However, most children with influenza infection were previously healthy. Few studies have been conducted on the risk factors for severe influenza in previously healthy children.

In this study, to determine risk factors for severe influenza in previously healthy children, we collected and summarized the clinical data of influenza-infected children who were previously healthy and were hospitalized at the Children's Hospital of Soochow University. We developed a predictive model of severe influenza in previously healthy children to help clinicians identify severe influenza early, reduce the incidence of severe influenza, and improve outcomes for pediatric patients.

Methods

This was a retrospective cohort study. This study was approved by the Ethics Committee of the Children's Hospital of Soochow University (Suzhou, China; approval no. 2021CS128) in accordance with the Declaration of Helsinki. The requirement for informed consent was waived by the Ethics Committee of the Children's Hospital of Soochow University because of the retrospective nature of the study. Details of all patients were removed. The reporting of this study conforms to STROBE guidelines.[7]

Patients and definitions

Patients. We collected data on children who were influenza-infected, previously healthy, and hospitalized at the Children's Hospital of Soochow University between 1 January 2017 and 30 June 2021. All personal patient details were deleted.

Definitions. Influenza diagnosis was based on the positive result of an influenza virus nucleic acid test or an influenza virus rapid antigen test.

Previously healthy children were defined as children without one or more (but not limited to) the following underlying medical conditions: autoimmune disease; use of immunosuppressants, connective tissue disease; tumors; hematological, renal, or cardiovascular diseases (except hypertension); endocrine and metabolic disease; neurologic disease (including moderate to severe developmental delay); bronchial foreign body; congenital bronchopulmonary dysplasia; bronchomalacia; asthma; and tuberculosis.

Influenza infections within our study population were divided into three types

of illness: mild, severe, and critical. Children with mild influenza illness were defined as the non-severe group, and those with severe and critical illness were defined as the severe group.

Severe influenza illness was confirmed with one or more of the following presentations:

(1) dyspnea and/or increased respiratory rate: children aged >5 years (>30 breaths/minute), children aged 1 to 5 years (>40 breaths/minute), infants aged 2 to 12 months (>50 breaths/minute), and neonates to 2-month-old infants (>60 breaths/minute);
(2) changes in consciousness: unresponsiveness, lethargy, restlessness, and convulsions;
(3) severe vomiting, severe diarrhea, or severe dehydration;
(4) oliguria: urine volume in children <0.8 mL/kg/hour (i.e., for children aged over 14 years <17 mL/hour, infants <200 mL/24 hours, preschool children <300 mL/24 hours, and school-aged children 400 mL/24 hours) or acute renal failure;
(5) pneumonia confirmed by X-ray or computed tomography;
(6) the original underlying medical condition is seriously aggravated.[8]

Critical influenza illness was confirmed with one or more of the following presentations: (1) respiratory failure, (2) acute necrotizing encephalopathy, (3) septic shock, (4) multiple organ insufficiency, and (5) other serious clinical conditions requiring admission to the intensive care unit.[8]

Clinical data collection

Clinical data included demographic data (age, sex, and underlying medical condition), clinical presentation and complications (fever, highest temperature, fever duration, hyperpyrexia ≥39.1°C, cough, wheezing, gastrointestinal symptoms, convulsions/disturbances of consciousness, headaches, sore throat, rash, myalgia/fatigue, hoarseness, tachypnea, three-concave sign, dyspnea, wet rales, wheezing rales, and pneumonia), laboratory findings (white blood cell count, neutrophils, hemoglobin, platelets, C-reactive protein, erythrocyte sedimentation rate, procalcitonin, serum sodium, serum potassium, lactic acid, albumin, prealbumin, alanine aminotransferase, aspartate aminotransferase, creatine kinase isoenzyme, creatine kinase, lactate dehydrogenase, serum creatinine, blood urea nitrogen, lymphocyte subsets, and immunoglobulins A, G, and M), and radiologic findings.

Etiological detection

We collected nasopharyngeal secretions within 24 hours of admission from all children. Because of their medical conditions, some children were treated with electronic bronchoscopy after obtaining their parents' consent; samples of bronchoalveolar lavage fluid were collected at the same time. All samples were subjected to the following: antigen detection for adenovirus, respiratory syncytial virus, and influenza A and B viruses; nucleic acid amplification testing for influenza A and B viruses, human bocavirus, *Mycoplasma pneumoniae*, cytomegalovirus, human metapneumovirus, human rhinovirus; and bacterial culture identification (including *Streptococcus pneumoniae* and *Haemophilus influenzae*).

Statistical analysis

In this study, model establishment and all statistical analyses were performed using IBM SPSS 26.0 (IBM Corp., Armonk, NY, USA) and R version 4.1.2 (http://www.R-project.org). Categorical data were expressed as frequency (percentage) and normally distributed continuous measurement

data were expressed as mean ± standard deviation. Non-normally distributed continuous measurement data were expressed as median (interquartile range). The chi-square test or Fisher's exact test was used for categorical data. For comparisons of normally distributed continuous variables, t-tests, one-way analysis of variance, and Welsh's analysis of variance were used. Additionally, for non-normally distributed continuous variables, the Mann–Whitney U test, the Kruskal–Wallis H test, and the Bonferroni post hoc test were applied. Only a two-tailed P value of <0.05 was considered statistically significant.

The study population was randomly assigned in a 7:3 ratio to the training cohort or the validation cohort. The training and validation cohorts were used to derive and validate the model, respectively.

In the training cohort, univariate analysis was applied to investigate the risk factors for severe influenza among previously healthy children, whereas independent risk factors and a regression equation prediction model were obtained using multivariate logistic regression (with a 0.05 probability of inclusion and a 0.10 probability of exclusion). The logistic regression prediction model was visualized as a nomogram using the rms package in R.

In the validation cohort, the area under the receiver operating characteristic curve (AUC) was used to quantify the model's prediction ability, and the calibration curve was applied to evaluate accuracy and discriminativeness.

Results

Patient characteristics

The study included a total of 1135 children, among which 803 had mild influenza illness, 281 had severe illness, and 51 had critical illness. Three children with critical illness died from influenza-associated encephalopathy.

Pediatric patients were randomized into training (777 patients) and validation (358 patients) cohorts. The general characteristics and clinical data of the children with influenza in training and validation cohorts are shown in Table 1. The P values suggested that variables were not statistically different between training and validation cohorts, signifying that the partition of data across the training and validation cohorts was balanced.

Risk factors for severe influenza

Univariate analysis

General information and clinical features. No significant differences were observed by sex and average age across the three types of illness (P > 0.05; Table 2).

As shown in Table 2, significant differences were observed across the types of illness in fever, highest temperature, wheezing, gastrointestinal symptoms, convulsions/disturbances of consciousness, headache, rash, hoarseness, tachypnea, three-concave sign, dyspnea, wet rales, wheezing rales, and pneumonia (P < 0.05).

Laboratory and radiologic findings. Significant differences were observed across the three illness types in white blood cell counts, neutrophils, hemoglobin, platelets, C-reactive protein, procalcitonin >0.25 ng/mL, serum sodium, lactic acid, albumin, alanine aminotransferase, creatine kinase isoenzyme, creatine kinase, serum creatinine, $CD3^-/CD16^+\%$ and immunoglobulin M (P < 0.05). Table 3 illustrates that the more severe the disease, the higher the levels of neutrophils, C-reactive protein, alanine aminotransferase, and serum creatinine, and the lower the levels of hemoglobin, serum sodium, lactic acid, albumin, and immunoglobulin M.

As for radiological findings, all cases of hydrothorax and consolidation visible on X-ray or computed tomography scans

Table 1. Variables of the children with influenza.

Variables	Training cohort (n = 777)	Validation cohort (n = 358)	Statistics	P
General Information				
Sex (male/female)	454/323	215/143	$\chi^2 = 0.268$	0.605
Age (months)	38.69 ± 28.59	39.52 ± 32.14	$T = -0.415$	0.678
Clinical Features				
Fever	723 (93.1%)	335 (93.6%)	$\chi^2 = 0.107$	0.744
Highest temperature	39.3 (39.0; 39.9)	39.4 (39.0; 40.0)	$Z = -1.015$	0.310
Fever duration	5.18 ± 3.43	5.32 ± 3.65	$T = -0.638$	0.524
Hyperpyrexia $\geq 39.1^\circ$C	464 (59.7%)	217 (60.6%)	$\chi^2 = 0.082$	0.774
Cough	771 (99.2%)	356 (99.4%)	–*	1.000
Wheezing	233 (30.0%)	104 (29.1%)	$\chi^2 = 0.103$	0.748
Gastrointestinal symptoms	141 (18.1%)	76 (21.2%)	$\chi^2 = 1.506$	0.220
Convulsions/disturbances of consciousness	41 (5.3%)	17 (4.7%)	$\chi^2 = 0.141$	0.707
Headache	13 (1.7%)	2 (0.6%)	–*	0.166
Sore throat	10 (1.3%)	2 (0.6%)	–*	0.359
Rash	19 (2.4%)	7 (2.0%)	$\chi^2 = 0.263$	0.608
Myalgia/fatigue	12 (1.5%)	2 (0.6%)	–*	0.247
Hoarseness	10 (1.3%)	4 (1.1%)	–*	1.000
Tachypnea	17 (2.2%)	5 (1.4%)	$\chi^2 = 0.807$	0.369
Three-concave signs	12 (1.5%)	8 (2.2%)	$\chi^2 = 0.675$	0.411
Dyspnea	26 (3.3%)	13 (3.6%)	$\chi^2 = 0.060$	0.806
Wet rales	291 (37.5%)	121 (33.8%)	$\chi^2 = 1.414$	0.234
Wheezing rales	166 (21.4%)	76 (21.2%)	$\chi^2 = 0.003$	0.959
Pneumonia	195 (25.1%)	93 (26.0%)	$\chi^2 = 0.100$	0.751
Degree of Illness				
Mild	548 (70.5%)	255 (71.2%)	$\chi^2 = 0.207$	0.902
Severe	195 (25.1%)	86 (24.0%)		
Critical	34 (4.4%)	17 (4.7%)		
Radiologic Findings				
Hydrothorax	16 (2.1%)	5 (1.4%)	$\chi^2 = 0.592$	0.441
Consolidation	86 (11.1%)	38 (10.6%)	$\chi^2 = 0.052$	0.820
Laboratory Findings				
WBC ($\times 10^9$)	8.30 ± 4.49	8.40 ± 4.87	$t = -0.323$	0.747
N (%)	49.24 ± 20.63	47.89 ± 20.17	$t = 1.029$	0.304
Hb (g/L)	123.0 (116.0; 130.0)	122.0 (115.0; 129.2)	$Z = -0.671$	0.503
Platelets ($\times 10^9$)	299.80 ± 130.90	298.43 ± 119.54	$t = 0.169$	0.866
CRP (mg/L)	3.0 (0.7; 10.4)	3.2 (0.7; 10.4)	$Z = -0.005$	0.996
ESR >15 mm/hour	103 (13.3%)	40 (11.2%)	$\chi^2 = 0.966$	0.326
PCT >0.25 ng/mL	103 (13.3%)	43 (12.0%)	$\chi^2 = 0.339$	0.560
Na$^+$ (mmol/L)	137.11 ± 2.80	137.07 ± 2.88	$t = 0.230$	0.818
K$^+$ (mmol/L)	4.01 ± 0.55	4.04 ± 0.51	$t = -0.823$	0.411
Lac (mmol/L)	2.3 (1.8; 3.0)	2.4 (1.9; 3.1)	$Z = -1.240$	0.215
Albumin (g/L)	42.61 ± 2.93	42.75 ± 3.53	$t = -0.688$	0.492
Prealbumin (mg/L)	150.90 ± 40.30	149.86 ± 37.74	$t = 0.412$	0.680
ALT (U/L)	16.1 (12.1; 22.6)	16.7 (12.5; 25.6)	$Z = -1.337$	0.181

Table I. Continued.

Variables	Training cohort (n = 777)	Validation cohort (n = 358)	Statistics	P
AST (U/L)	39.9 (32.8; 50.4)	42.2 (33.4; 52.3)	$Z = -1.799$	0.072
CKMB (ng/mL)	1.6 (1.0,3.1)	1.8 (1.0,3.3)	$Z = -1.166$	0.243
CK (U/L)	91.5 (66.2; 132.3)	98.3 (67.7; 141.1)	$Z = -1.732$	0.083
LDH (U/L)	361.1 (312.2; 445.4)	545 (311.7; 463.3)	$Z = -0.814$	0.416
Cr (µmmol/L)	26.29 ± 9.46	27.37 ± 10.48	$t = -1.719$	0.086
BUN (mmol/L)	3.41 ± 1.35	3.46 ± 1.24	$t = -0.596$	0.551
$CD3^+$ (%)	61.40 ± 11.36	61.72 ± 10.46	$t = -0.457$	0.648
$CD3^+/CD4^+$ (%)	33.5 (27.6; 40.0)	33.8 (27.9; 39.3)	$Z = -0.199$	0.843
$CD3^+/CD8^+$ (%)	24.30 ± 9.15	24.33 ± 7.95	$t = -0.039$	0.969
$CD4^+/CD8^+$ (%)	1.4 (1.0; 2.0)	1.4 (1.0; 2.0)	$Z = -0.341$	0.733
$CD3^-/CD19^+$ (%)	22.1 (16.3; 30.6)	22.5 (16.1; 30.3)	$Z = -0.224$	0.823
$CD3^-/CD16^+$ (%)	13.19 ± 9.60	13.23 ± 9.77	$t = -0.065$	0.949
$CD19^+/CD23^+$ (%)	9.64 ± 5.63	10.01 ± 5.63	$t = -1.039$	0.299
IgA (g/L)	0.80 ± 0.58	0.79 ± 0.58	$t = 0.419$	0.675
IgG (g/L)	7.88 ± 2.49	7.74 ± 2.71	$t = 0.822$	0.411
IgM (g/L)	1.17 ± 0.54	1.13 ± 0.56	$t = 1.090$	0.276
Etiological Findings				
IV-A	393 (50.6%)	180 (50.3%)	$\chi^2 = 0.009$	0.925
IV-B	386 (49.7%)	178 (49.7%)	$\chi^2 = 0.000$	0.989
MP	93 (12%)	32 (8.9%)	$\chi^2 = 2.297$	0.130
RSV	26 (3.3%)	15 (4.2%)	$\chi^2 = 0.501$	0.479
HBoV	30 (3.9%)	8 (2.2%)	$\chi^2 = 2.003$	0.157
HMPV	11 (1.4%)	6 (1.7%)	$\chi^2 = 0.113$	0.737
CMV	3 (0.4%)	0 (0.0%)	–*	0.556
HRV	7 (0.9%)	4 (1.1%)	–*	0.749
ADV	2 (0.3%)	1 (0.3%)	–*	1.000
SP	174 (22.4%)	94 (26.3%)	$\chi^2 = 2.028$	0.154
HI	175 (22.5%)	67 (18.7%)	$\chi^2 = 2.118$	0.146

t, test; Z, Mann–Whitney U test; χ^2 ,Chi-square test; *, Fisher's exact test, SPSS26.0 did not display statistics. WBC, white blood cells; N, neutrophils; Hb, hemoglobin; Plt, platelets; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PCT, procalcitonin; Na^+, serum sodium; K^+, serum potassium; Lac, lactic acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKMB, creatine kinase isoenzyme; CK, creatine kinase; LDH, lactate dehydrogenase; Cr, serum creatinine; BUN, blood urea nitrogen; IgA, immunoglobulin A; IgM, immunoglobulin M; IgG, immunoglobulin G; ADV, adenovirus; RSV, respiratory syncytial virus; IV-A, influenza A virus; IV-B, influenza B virus; HBoV, human bocavirus; MP, *Mycoplasma pneumoniae*; CMV, cytomegalovirus; HMPV, human metapneumovirus; HRV, human rhinovirus; SP, *Streptococcus pneumoniae*; HI, *Haemophilus influenzae*.

occurred in the severe and critical illness groups. The consolidation rate was higher in the severe (40.0%) than in critical illness group, whereas the proportion of hydrothorax was higher in the critical (8.8%; P < 0.05) than in the severe illness group (Table 3).

Etiological findings. No significant differences (P > 0.05) were observed in the rates of infection with the respiratory syncytial virus, influenza A and B viruses, human bocavirus, cytomegalovirus, human metapneumovirus, human rhinovirus, *Streptococcus pneumoniae*, and *Haemophilus influenzae* across the three illness types groups, whereas infection rates for *Mycoplasma pneumoniae* and adenovirus were significantly different across the groups (P < 0.05; Table 4).

Table 2. Comparison of general patient information and clinical features.

Variables	Mild (n = 548)[a]	Severe (n = 195)[b]	Critical (n = 34)[c]	Statistics	P
General Information					
Sex (male/female)	328/220	106/89	20/14	$\chi^2 = 1.790$	0.409
Age (months)	37.61 ± 28.01	41.78 ± 29.85	38.38 ± 30.08	F* = 1.531	0.217
Clinical Features					
Fever	515 (94%)[b]	174 (89.2%)[ac]	34 (100%)[b]	F = 7.116	**0.023**
Highest temperature	39.3 (39,39.9)	39.2 (38.7; 39.7)[c]	39.9 (39.0; 40.0)[b]	H = 8.372	**0.015**
Fever duration	5.11 ± 3.16	5.29 ± 3.85	5.74 ± 4.52	W = 0.447	0.641
Hyperpyrexia ≥39.1°C	332 (60.6%)	107 (54.9%)	25 (73.5%)	$\chi^2 = 4.771$	0.092
Cough	546 (99.6%)	192 (98.5%)	33 (97.1%)	F = 5.374	0.054
Wheezing	143 (26.1%)[b]	83 (42.6%)[ac]	7 (20.6%)[b]	$\chi^2 = 20.077$	**<0.001**
Gastrointestinal symptoms	104 (19.0%)	26 (13.3%)[c]	11 (32.4%)[b]	$\chi^2 = 7.916$	**0.019**
Convulsions/ disturbances of consciousness	0 (0.0%)[bc]	23 (11.8%)[ac]	18 (52.9%)[ab]	F = 129.725	**<0.001**
Headache	6 (1.1%)[c]	4 (2.1%)	3 (8.8%)[a]	F = 8.203	**0.009**
Sore throat	9 (1.6%)	1 (0.5%)	0 (0.0%)	F = 1.066	0.661
Rash	17 (3.1%)[b]	0 (0.0%)[ac]	2 (5.9%)[b]	F = 9.568	**0.005**
Myalgia/fatigue	10 (1.8%)	2 (1%)	0 (0.0%)	F = 0.385	0.849
Hoarseness	4 (0.7%)[c]	2 (1%)[c]	4 (11.8%)[ab]	F = 14.133	**0.001**
Tachypnea	0 (0.0%)[bc]	10 (5.1%)[ac]	7 (20.6%)[ab]	F = 48.707	**<0.001**
Three-concave signs	0(0.0%)[bc]	8 (4.1%)[a]	4 (11.8%)[a]	F = 31.475	**<0.001**
Dyspnea	0 (0.0%)[bc]	15 (7.7%)[ac]	11 (32.4%)[ab]	F = 77.220	**<0.001**
Wet rales	191 (34.9%)[bc]	97 (49.7%)[ac]	3 (8.8%)[ab]	F = 27.487	**<0.001**
Wheezing rales	95 (17.3%)[b]	65 (33.3%)[a]	6 (17.6%)	$\chi^2 = 22.202$	**<0.001**
Pneumonia	0 (0.0%)[bc]	177 (90.8%)[ac]	18 (52.9%)[ab]	F = 701.461	**<0.001**

F*, one-way analysis of variance; W, Welsh's analysis of variance; F, Fisher's exact test; H, nonparametric Kruskal–Wallis H test; χ^2, Chi-square test. Different combinations of a, b, and c represent statistically significant differences across illness types.

Multivariate analysis. We divided the training cohort into non-severe (mild illness) and severe (severe illness and critical illness) groups. Taking whether the patient was included in the severe group as the response variable, we performed a logistic regression analysis on variables with statistical significance in the above univariate analysis. The results showed that wheezing rales (odds ratio [OR]: 2.959, 95% confidence interval [CI]: 1.977–4.428), neutrophils (OR: 1.024, 95% CI: 1.015–1.033), procalcitonin >0.25 ng/mL (OR: 2.338, 95% CI:1.462–3.739), and *Mycoplasma pneumoniae* (OR: 4.351, 95% CI: 2.695–7.026) were independent risk factors for severe influenza in previously healthy children; fever (OR: 0.440, 95% CI: 0.229–0.843) and albumin (OR: 0.929, 95% CI: 0.876–0.985) were protective factors (Table 5).

Development of a predictive model for severe influenza

Logistic regression model. We established a logistic regression model as follows: logistic $(P) = e^X/(1 + e^X)$, $X = 1.216 - 0.882$ fever (no = 0, yes = 1) + 1.085 wheezing rales (no = 0, yes = 1) + 0.024 neutrophils (%) + 0.849 PCT > 0.25 ng/mL (no = 0, yes = 1)

Table 3. Comparison of laboratory and radiologic findings.

Variables	Mild (n = 548)[a]	Severe (n = 195)[b]	Critical (n = 34)[c]	Statistics	P
Laboratory Findings					
WBC ($\times 10^9$)	8.06 ± 4.43[b]	9.05 ± 4.68[a]	7.96 ± 3.89	F*=3.576	**0.028**
N (%)	46.51 ± 20.02[bc]	53.42 ± 20.12[ac]	69.11 ± 18.78[ab]	F*=26.155	**<0.001**
Hb (g/L)	123.0 (116.0; 130.0)[c]	123.0 (116.0; 129.0)[c]	115.0 (102.8; 123.0)[ab]	H=12.525	**0.002**
Platelets ($\times 10^9$)	296.18 ± 131.13[bc]	323.00 ± 128.63[ac]	225.18 ± 107.34[ab]	F*=8.977	**<0.001**
CRP (mg/L)	2.4 (0.7; 8.5)[bc]	4.2 (0.8; 21.4)[a]	6.2 (1.4; 21.3)[a]	H=14.622	**0.001**
ESR >15 mm/hour	70 (12.8%)	32 (16.4%)	1 (2.9%)	F=5.022	0.076
PCT >0.25 ng/mL	56 (10.2%)[c]	29 (14.9%)[c]	18 (52.9%)[ab]	F=36.093	**<0.001**
Na^+ (mmol/L)	137.29 ± 2.50[c]	136.89 ± 3.37[c]	135.56 ± 3.27[ab]	F*=7.065	**0.001**
K^+ (mmol/L)	4.03 ± 0.56	3.97 ± 0.49	3.94 ± 0.71	W=1.102	0.337
Lac (mmol/L)	2.4 (1.9; 3.0)[c]	2.4 (1.8; 3.0)[c]	1.9 (1.4; 2.8)[ab]	H=7.102	**0.029**
Albumin (g/L)	42.78 ± 2.73[a]	42.36 ± 3.20	41.27 ± 3.96[c]	W=3.468	**0.036**
Prealbumin (mg/L)	151.17 ± 37.64	151.80 ± 46.84	141.04 ± 41.25	W=1.016	0.367
ALT (U/L)	15.9 (12.0; 22.1)[c]	15.8 (12.5; 22.7)[c]	19.3 (15.9; 32.0)[ab]	H=8.876	**0.012**
AST (U/L)	40.5 (33.3; 50.6)	38.6 (30.7; 49.0)	44.6 (35.4; 52.4)	H=5.995	0.050
CKMB (ng/mL)	1.6 (1.0; 2.9)[c]	1.5 (0.8; 3.2)[c]	2.8 (1.5; 4.9)[ab]	H=9.135	**0.010**
CK (U/L)	91.6 (66.5; 129.8)[c]	81.9 (63.5; 125.3)[c]	181.9 (130.1; 265.0)[ab]	H=33.363	**<0.001**
LDH (U/L)	359.4 (313.9; 432.4)	376.3 (308.8; 469.0)	354.6 (292.0; 482.9)	H=1.070	0.586
Cr (µmmol/L)	25.3 (19.5; 30.8)[c]	25.4 (21.0; 32.0)	29.4 (21.3; 34.9)[a]	H=6.692	**0.035**
BUN (mmol/L)	3.37 ± 1.37	3.44 ± 1.11	3.99 ± 2.06	W=1.664	0.196
CD3+ (%)	61.85 ± 10.86[c]	61.05 ± 11.82	56.16 ± 15.01[a]	W=2.552	0.084
CD3+/CD4+ (%)	34.07 ± 9.43	33.89 ± 9.93	30.64 ± 12.53	F*=2.007	0.135
CD3+/CD8+ (%)	24.23 ± 7.90	24.84 ± 12.23	22.37 ± 6.95	F*=1.126	0.325
CD4+/CD8+ (%)	1.5 (1.0; 2.0)	1.4 (1.0; 1.9)	1.4 (1.0; 1.8)	H=1.252	0.535
CD3−/CD19+ (%)	23.74 ± 10.32	25.71 ± 12.13	23.23 ± 11.58	W=2.136	0.124
CD3−/CD16+ (%)	11.4 (7.1; 17.4)[b]	10.2 (5.7; 15.7)[ac]	13.2 (6.2; 28.6)[b]	H=7.202	**0.027**
CD19+/CD23+ (%)	9.63 ± 5.56	10.04 ± 5.91	7.48 ± 4.67	F*=3.009	0.050
IgA (g/L)	0.78 ± 0.54	0.88 ± 0.64	0.86 ± 0.80	W=2.139	0.124
IgG (g/L)	7.85 ± 2.38	7.96 ± 2.75	7.80 ± 2.74	W=0.124	0.884
IgM (g/L)	1.15 ± 0.49	1.26 ± 0.65[c]	1.00 ± 0.46[b]	W=4.843	**0.010**
Radiologic Findings					
Hydrothorax	0 (0.0%)[bc]	13 (6.7%)[a]	3 (8.8%)[a]	F=38.431	**<0.001**
Consolidation	0 (0.0%)[bc]	78 (40.0%)[a]	8 (23.5%)[a]	F=236.814	**<0.001**

F*, one-way analysis of variance; W, Welsh's analysis of variance; F, Fisher's exact test; H, nonparametric Kruskal–Wallis H test. Different combinations of a, b, and c represent statistically significant differences across illness types. WBC, white blood cells; N, neutrophils; Hb, hemoglobin; Plt, platelets; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PCT, procalcitonin; Na^+, serum sodium; K^+, serum potassium; Lac, lactic acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKMB, creatine kinase isoenzyme; CK, creatine kinase; LDH, lactate dehydrogenase; Cr, serum creatinine; BUN, blood urea nitrogen; IgA, immunoglobulin A; IgM, immunoglobulin M; IgG, immunoglobulin G.

− 0.074 albumin (g/L) + 1.470 *Mycoplasma pneumoniae* infection (no = 0, yes = 1).

Nomogram for severe influenza. We visualized the logistic regression prediction model as a nomogram (Figure 1). Each factor in the nomogram had a scoring scale, with a high total score indicating a great risk of severe influenza. The application method of the nomogram was as follows. First, based on the level of severe influenza risk items, we found the point on the corresponding axis in the nomogram and drew a straight line perpendicular to the score axis, with the point as the origin. The point of intersection was the corresponding score for this item. Then, we added the scores for each predictor to obtain the

Table 4. Etiological findings.

Variables	Mild (n = 548)[a]	Severe (n = 195)[b]	Critical (n = 34)[c]	Statistics	P
IV-A	281 (51.3%)	95 (48.7%)	17 (50%)	$\chi^2 = 0.382$	0.826
IV-B	269 (49.1%)	100 (51.3%)	17 (50%)	$\chi^2 = 0.279$	0.870
MP	40 (7.3%)[b]	50 (25.6%)[a]	3 (8.8%)	$F = 40.480$	**<0.001**
RSV	14 (2.6%)	10 (5.1%)	2 (5.9%)	$F = 4.151$	0.096
HBoV	17 (3.1%)	10 (5.1%)	3 (8.8%)	$F = 4.260$	0.100
HMPV	10 (1.8%)	1 (0.5%)	0 (0.0%)	$F = 1.393$	0.492
CMV	2 (0.4%)	1 (0.5%)	0 (0.0%)	$F = 0.956$	1.000
HRV	5 (0.9%)	2 (1.0%)	0 (0.0%)	$F = 0.236$	1.000
ADV	0 (0.0%)[c]	1 (0.5%)	1 (2.9%)[a]	$F = 7.438$	**0.024**
SP	127 (23.2%)	44 (22.6%)	3 (8.8%)	$F = 3.917$	0.137
HI	121 (22.1%)	50 (25.6%)	4 (11.8%)	$F = 3.323$	0.194

F, Fisher's exact test; χ^2, Chi-square test. Different combinations of a, b, and c represent statistically significant differences across illness types. ADV, adenovirus; RSV, respiratory syncytial virus; IV-A, influenza A virus; IV-B, influenza B virus; HBoV, human bocavirus; MP, *Mycoplasma pneumoniae*; CMV, cytomegalovirus; HMPV, human metapneumovirus; HRV, human rhinovirus; SP, *Streptococcus pneumoniae*; HI, *Haemophilus influenzae*.

Table 5. Risk factors for severe influenza in previously healthy children.

Variables	B	SE	Wald	OR	OR (95% CI)	P
Fever	−0.822	0.332	6.122	0.440	**0.229–0.843**	**0.013**
Wheezing rales	1.085	0.206	27.799	2.959	**1.977–4.428**	**<0.001**
N (%)	0.024	0.004	28.554	1.024	**1.015–1.033**	**<0.001**
PCT >0.25 ng/mL	0.849	0.240	12.567	2.338	**1.462–3.739**	**<0.001**
Albumin (g/L)	−0.074	0.030	6.030	0.929	**0.876–0.985**	**0.014**
MP	1.470	0.244	36.191	4.351	**2.695–7.026**	**<0.001**
Constant	1.216	1.372	0.786	–	–	0.375

Because hydrothorax and consolidation were the diagnostic criteria for pneumonia, and tachypnea, three-concave signs, and dyspnea were the diagnostic criteria for severe influenza, these were not included in logistic regression. B, regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval; N, neutrophils; PCT, procalcitonin; MP, *Mycoplasma pneumoniae*.

total score. Finally, based on the total score on the risk probability axis, we obtained the current risk probability of severe influenza in a previously healthy child.

Validation of a prediction model for severe influenza

Performance of nomogram in the training cohort. The receiver operating characteristic curve showed that the AUC of the predicted probability was 0.725 (95% CI: 0.686–0.765), the sensitivity of the prediction model was 0.651, and the specificity was 0.690 (Table 6, Figure 2a).

After 1000 bootstrap resamples, the calibration curve showed that the predicted probability was consistent with the actual probability and was close to the ideal state (Figure 2b).

Performance of nomogram in the validation cohort. In the validation cohort, the AUC was 0.721 (95% CI: 0.659–0.784) and the sensitivity and specificity were 0.631 and 0.737, respectively (Figure 3a). The calibration
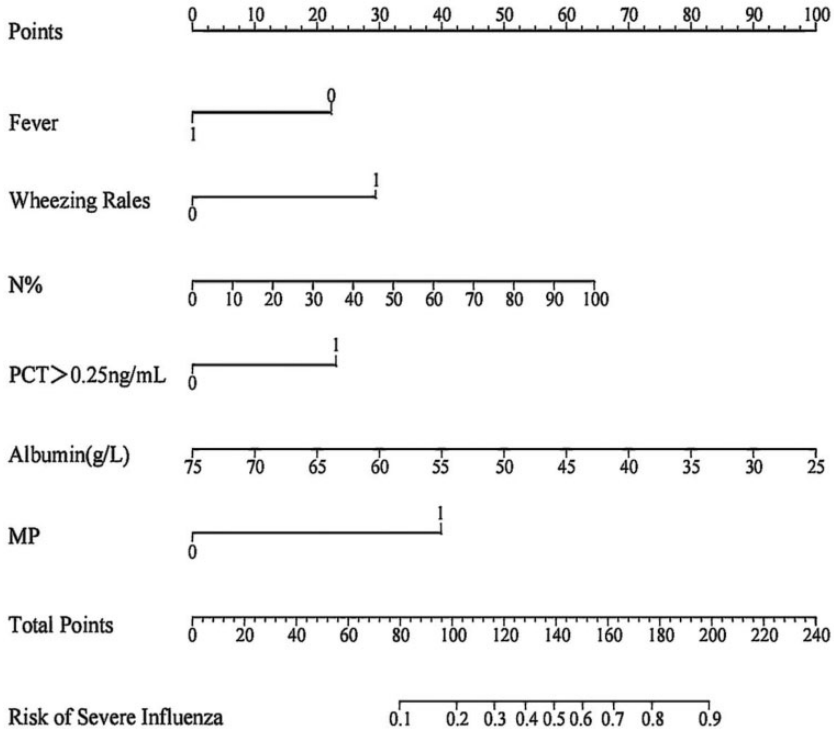
Figure 1. Nomogram for predicting severe influenza risk in previously healthy children. On the axes of wheezing rales, PCT > 0.25 ng/mL, MP 0 means the predicted one does not have the factor, and 1 means the one has the factor.

N, neutrophils; PCT, procalcitonin; MP, *Mycoplasma pneumoniae*.

Table 6. Comparison of ROC curve performance.

Variables	Cutoff value	AUC (95% CI)	Sensitivity	Specificity	Youden Index
N (%)	55.85	0.628 (0.584–0.671)	0.537	0.668	0.205
Albumin (g/L)	39.55	0.552 (0.507–0.597)	0.197	0.894	0.091
Predicted probability	0.284	0.725 (0.686–0.765)	0.651	0.690	0.341

The cut-off value was calculated according to the Youden index maximization.

Youden index = sensitivity + specificity − 1. ROC, receiver operating characteristic; AUC, area under the receiver operating characteristic curve; N, neutrophils, CI, confidence interval.

curves showed good agreement between predicted and actual probabilities in the validation cohort (Figure 3b).

Discussion

In our study, we found that independent risk factors for severe influenza were wheezing rales, neutrophils, procalcitonin > 0.25 ng/mL, and *Mycoplasma pneumoniae* infection, whereas fever and albumin were protective factors. We established and verified a nomogram for severe influenza based on the factors. The sensitivity of the prediction model was 0.651 and the specificity was 0.690. The calibration
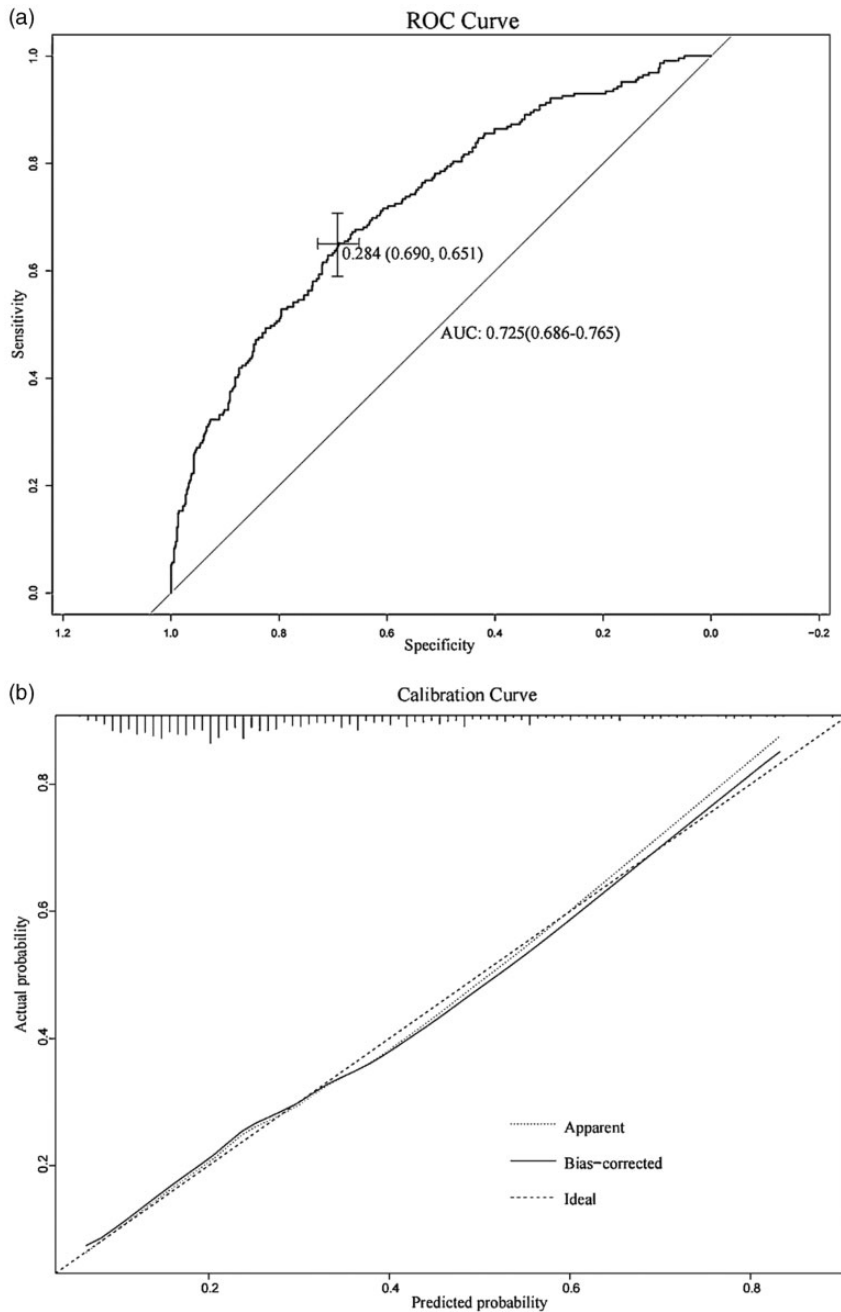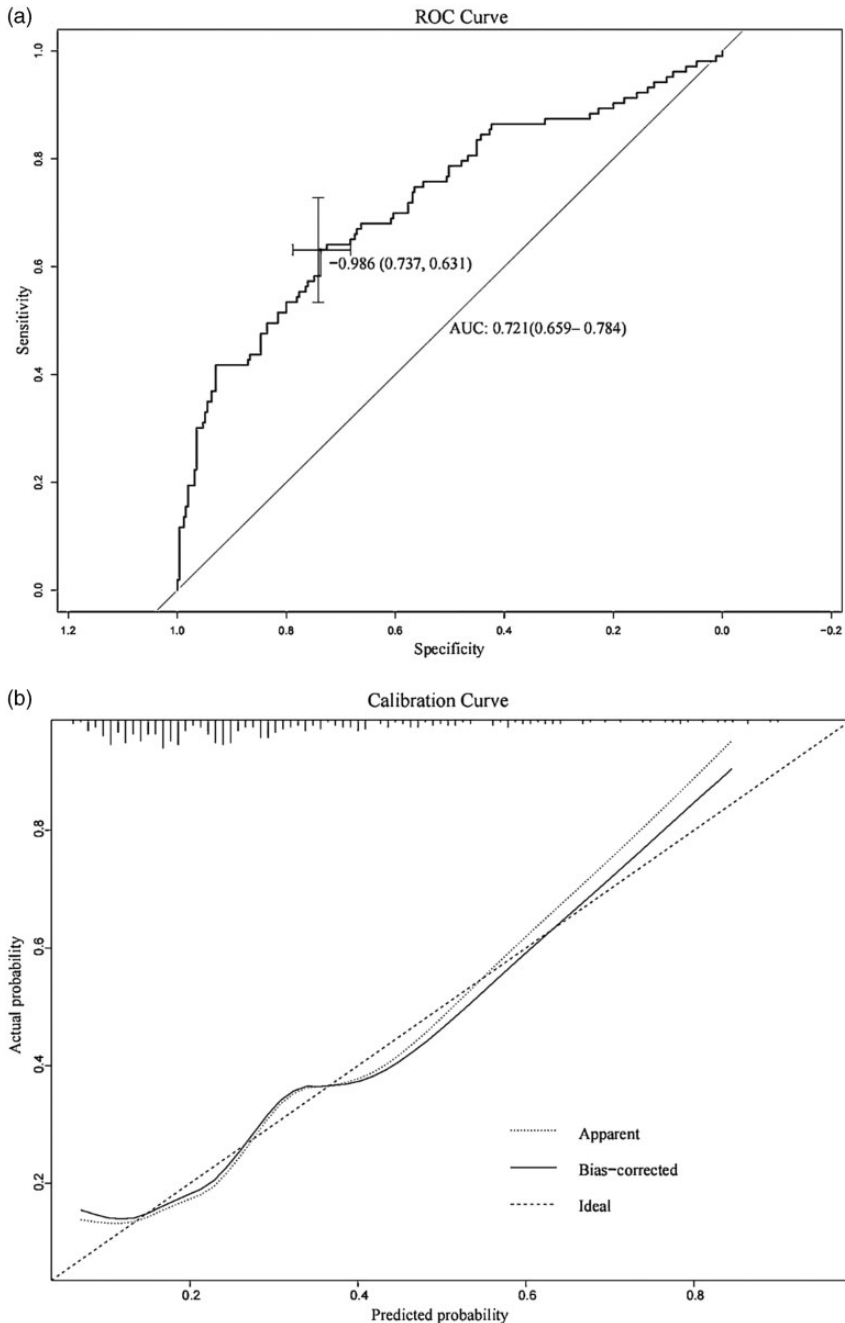
(a)

(b)

Figure 2. Receiver operator characteristic curve and a calibration curve of the nomogram prediction in the training cohort. (a) The area under the receiver operating characteristic curve was 0.725 (95% confidence interval: 0.686–0.765), with a sensitivity of 0.651 and a specificity of 0.690 and (b) The x-axis represents the estimated probability and the y-axis represents the actual probability of severe influenza. The solid line represents the performance of the nomogram, with a closer fit to the diagonal dotted line representing a better prediction.

(a)

(b)

Figure 3. Receiver operator characteristic curve and calibration curve of the nomogram prediction in the training set. (a) The area under the receiver operating characteristic curve was 0.721 (95% confidence interval: 0.659–0.784), with a sensitivity of 0.631 and a specificity of 0.737 and (b) The x-axis represents the estimated probability and the y-axis represents the actual probability of severe influenza. The predicted probability was consistent with the actual probability and was close to the ideal state.

curves in the training and validation cohorts showed that the predicted probability was consistent with the actual predicted probability and was close to the reference line. Results showed that the nomogram had strong predictive ability.

The main symptoms of influenza in our study population were fever and cough, followed by wheezing and gastrointestinal symptoms; a small number of children had headaches, sore throat, myalgia/fatigue, rash, and other symptoms. The 2020 Chinese version of the childhood influenza expert consensus[8] indicated that children with influenza are more likely to have a fever—especially hyperpyrexia—and often have symptoms of cough, sore throat, runny/stuffy nose, vomiting, and diarrhea, occasionally with additional symptoms including headache, myalgia, and fatigue. The difference in symptomology may be attributable to the fact that most children in this study were young and had a poor ability to express pain or discomfort. Additionally, severe influenza is more common in children under 5 years of age.[8] Moreover, Rao observed that children with severe influenza were younger and had a higher rate of influenza A virus infection compared with those who had mild influenza.[9]

We observed that in the critical illness group, the rates of tachypnea (17.6%), three-concave sign (15.7%), dyspnea (35.3%), and hydrothorax (7.8%) were significantly higher than in the severe illness group, whereas the proportions of wet rales (17.6%), wheezing rales (21.6%), pneumonia (60.8%), and consolidation (25.5%) were lower than in the severe illness group. Shi et al. reported that 46.2% of patients with influenza who died had acute respiratory distress syndrome and 38.5% had pneumonia,[10] indicating that flu-related pulmonary complications may cause serious consequences. Song et al. concluded that influenza may cause severe neurological complications in previously healthy children.[11] Moreover, the influenza A virus can cause isolated or predominantly neurologic manifestations that can lead to poor outcomes.[12] In a study of 100 pediatric patients with febrile seizures (FS), Esen et al. observed that the influenza A virus was detectable in 16% of patients with simple FS and 30% of patients with complex FS.[13] Nevertheless, because convulsions/disturbances of consciousness were the diagnostic criteria for severe influenza in our study, these symptoms were not included in the logistic regression. Notably, 52.9% (13/34) of the critically ill patients in our training cohort had convulsions/disorders of consciousness—significantly higher than that of children with severe influenza illness (11.8%)—and the three children in this study who died had influenza-related encephalopathy. Furthermore, the rate of convulsions/disturbances of consciousness was significantly different between the severe illness (11.4%) and critical illness (51.0%) groups, signifying that this symptom might be a risk factor for critical illness ($P < 0.01$). In radiologic findings, compared with that in the severe illness group, the proportion of patients in the training cohort with hydrothorax (8.8%) was significantly higher in the critical illness group whereas the proportion with consolidation (23.5%) was significantly lower. Ma et al. observed that shadows or pleural effusions on radiographs were associated with severe influenza infection ($P = 0.02$).[14] Similarly, we found that fever was a protective factor for severe influenza. This may be because children are more likely to receive early treatment when fever attracts the attention of parents.

A study of 36,047 patients in Shanghai reported[15] that levels of hemoglobin, C-reactive protein, alanine aminotransferase, aspartate aminotransferase, serum creatinine, blood urea nitrogen, and lactic acid in those with severe influenza were significantly higher than levels in patients without

severe influenza. Moreover, decreased hemoglobin and increased alanine aminotransferase, blood urea nitrogen, and lactic acid levels were risk factors for death in children with influenza. In our study, the mean/median values of neutrophils, C-reactive protein, alanine aminotransferase, and serum creatinine increased with disease severity, whereas the mean/median of hemoglobin, serum sodium, lactic acid, albumin, and immunoglobulin M showed the opposite trend. Among these laboratory findings, risk factors for severe influenza were neutrophils and procalcitonin >0.25 ng/mL, whereas albumin was a protective factor.

One study concluded that the aggravation of influenza may be related to an inflammatory factor storm.[16] However, in our study, all of the immunological indicators were not independent risk factors for severe influenza. Among them, immunoglobulin M decreased with disease severity. This might be related to the complicated immune response in severe influenza. Furthermore, this study only collected known clinical data on cellular and humoral immunity and many immune factors were not included; therefore, our findings are only partially informative.

In terms of etiology, only *Mycoplasma pneumoniae* was an independent risk factor for severe influenza in our study. In children under 5 years of age, *Mycoplasma pneumoniae* coinfection with influenza B virus was common and was an important cause of community-acquired pneumonia, which itself could lead to severe disease.[17,18] Hypoxemia, acute lung injury, and multiorgan involvement are also reported to occur in *Mycoplasma pneumoniae*-associated pneumonia.[19] Although respiratory syncytial virus, human bocavirus, cytomegalovirus, human metapneumovirus, and human rhinovirus were not independent risk factors, the infection rates of respiratory syncytial virus (5.9%), human bocavirus

(8.8%), and adenovirus (2.9%) were significantly higher in the critical illness group than in those in the other two groups. In addition, children with critical illness were more likely to be coinfected with respiratory syncytial virus and human bocavirus. Edyta et al.[20] further observed a high incidence of respiratory syncytial virus infection combined with influenza virus infection; patients with coinfection had more severe disease and were at high risk for complications, especially when the influenza A virus was involved. Furthermore, severe influenza-associated pneumonia is often complicated with bacterial infection and is associated with a high mortality rate.[21] The most common bacterial coinfection with influenza A virus is *Streptococcus pneumoniae* infection;[22] this may be because the influenza A virus promotes the attachment of *Streptococcus pneumoniae* to alveolar epithelial cells, resulting in severe post-influenza bacterial pneumonia.[23] However, no statistically significant differences were observed between *Streptococcus pneumoniae* and *Haemophilus influenzae* and the proportions of *Streptococcus pneumoniae* and *Haemophilus influenzae* in the critical illness group were lower than those in the other two types. This may be because *Streptococcus pneumoniae* and *Haemophilus influenzae* were the only bacterial pathogens studied.

The study had some limitations. Because of the single-center design of the current study, we could not avoid potential selection bias. Second, the fact that data for both the training and validation cohorts were from retrospective studies may have led to additional bias. Therefore, further studies based on another independent prospective cohort are required. When applying the model in practice, the issue of overfitting requires vigilance. We will seek multi-center collaboration in follow-up studies to further examine the performance of the model.

Conclusion

In this study, we investigated risk factors for severe influenza among previously healthy children using univariate analysis and obtained predictive factors using multivariate logistic regression. On this basis, we established a novel nomogram prediction model. The model has good discrimination and validity and can help clinicians identify the risk of severe influenza in previously healthy children and provide early treatment.

Author contributions

WYH: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Software, Visualization, Writing-original draft.
WSN: Conceptualization, Methodology, Validation, Supervision
HMC: Resources, Methodology, Supervision
WJJ: Methodology, Supervision, Funding acquisition
YBF, XXL, and MLL: Investigation, Data curation
JH: Conceptualization, Project administration, Supervision, Writing-review& editing
CXH: Funding acquisition, Supervision

Declaration of conflicting interests

The authors declare that they have no competing interests.

ORCID iD

Jun Hua https://orcid.org/0000-0002-9610-807X

References

1. Wang X, Li Y, O'Brien KL, et al. Global burden of respiratory infections associated with seasonal influenza in children under 5 years in 2018: a systematic review and modelling study. *Lancet Glob Health* 2020; 8: e497–e510.
2. Li J, Chen Y, Wang X, et al. Influenza-associated disease burden in mainland China: a systematic review and meta-analysis. *Sci Rep* 2021; 11: 2886.
3. National Immunization Advisory Committee Technical Working Group. Influenza Vaccination Technical Working Group. Technical guidelines for seasonal influenza vaccination in China (2021–2022). *Zhonghua Liu Xing Bing Xue Za Zhi.* 2021; 42: 1722–1749. (In Chinese with English abstract).
4. Teutsch SM, Zurynski YA, Nunez C, et al. Ten Years of National Seasonal Surveillance for Severe Complications of Influenza in Australian Children. *Pediatr Infect Dis J* 2021; 40: 191–198.
5. Sellers SA, Hagan RS, Hayden FG, et al. The hidden burden of influenza: A review of the extra-pulmonary complications of influenza infection. *Influenza Other Respir Viruses* 2017; 11: 372–393.
6. Committee on infectious diseases. Recommendations for Prevention and Control of Influenza in Children, 2021–2022. *Pediatrics* 2021; 148: e2021053745.
7. Von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Ann Intern Med* 2007; 147: 573–577.
8. China National Clinical Research Center for Respiratory Diseases; Group of Respirology, Chinese Pediatric Society, Chinese Medical Association. Expert consensus on diagnosis and treatment of influenza in children (2020 edition). *Chin J of Appl Clin Pediatr* 2020; 35: 1281–1288. (In Chinese with English abstract).
9. Rao S, Yanni E, Moss A, et al. Evaluation of a New Clinical Endpoint for Moderate to Severe Influenza Disease in Children: A

Prospective Cohort Study. *J Pediatric Infect Dis Soc* 2020; 9: 460–467.

10. Shi T, Nie Z, Huang L, et al. Mortality risk factors in children with severe influenza virus infection admitted to the pediatric intensive care unit. *Medicine (Baltimore)* 2019; 98: e16861.

11. Song Y, Li S, Xiao W, et al. Influenza-Associated Encephalopathy and Acute Necrotizing Encephalopathy in Children: A Retrospective Single-Center Study. *Med Sci Monit* 2021; 27: e928374.

12. Takia L, Saini L, Keshavan S, et al. Neurological Manifestations of Influenza A (H1N1): Clinical Features, Intensive Care Needs, and Outcome. *Indian J Pediatr* 2020; 87: 803–809.

13. Esen FH, Secilmis Y, Dogan M, et al. Influenza A as a Common Viral Cause of Complex Febrile Seizures. *J Pediatr Infect Dis* 2021; 16: 200–204.

14. Ma HY, Wu JL, Lu CY, et al. Risk factors associated with severe influenza virus infections in hospitalized children during the 2013 to 2014 season. *J Microbiol Immunol Infect* 2016; 49: 387–393.

15. Shi Y, Chen W, Zeng M, et al. Clinical features and risk factors for severe influenza in children: A study from multiple hospitals in Shanghai. *Pediatr Neonatol* 2021; 62: 428–436.

16. Gu Y, Zuo X, Zhang S, et al. The Mechanism behind Influenza Virus Cytokine Storm. *Viruses* 2021; 13: 1362.

17. Ma YJ, Wang SM, Cho YH, et al. Clinical and epidemiological characteristics in children with community-acquired mycoplasma pneumonia in Taiwan: A nationwide surveillance. *J Microbiol Immunol Infect* 2015; 48: 632–638.

18. Guan WD, Gong XY, Mok CK, et al. Surveillance for seasonal influenza virus prevalence in hospitalized children with lower respiratory tract infection in Guangzhou, China during the post-pandemic era. *PLoS One* 2015; 10: e120983.

19. Zhang T, Han C, Guo W, et al. Case Report: Clinical Analysis of Fulminant Mycoplasma pneumoniae Pneumonia in Children. *Front Pediatr* 2021; 9: 741663.

20. Edyta Z, Małgorzata C, August W, et al. P388 RSV and influenza co-infections in children in three consecutive influenza epidemic seasons. *Archives of Disease in Childhood* 2019; 104: A311.

21. Chen YF, Wu PF, Yang CL, et al. Risk factors and outcomes of severe influenza pneumonia with bacterial co-infection in critically ill patients. *Eur Respir J* 2017; 50: PA2113. ERS International Congress 2017 abstracts, 2017[C].

22. MacIntyre CR, Chughtai AA, Barnes M, et al. The role of pneumonia and secondary bacterial infection in fatal and serious outcomes of pandemic influenza a (H1N1) pdm09. *BMC Infect Dis* 2018; 18: 637.

23. Sumitomo T, Nakata M, Nagase S, et al. GP96 Drives Exacerbation of Secondary Bacterial Pneumonia following Influenza a Virus Infection. *mBio* 2021; 12: e3269.