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ORIGINAL RESEARCH

# Feasibility of Absent in Melanoma 2 as a Serological Marker in Relation to Complicated Community-Acquired Pneumonia in Children: A Prospective Cohort Study

Linjie Hu[1], Peng Zhou[1], Shanlin Wang[2], Yaping Shen[1], Suli Xing[1], Yuehan Zhang[1], Yanyan Zhu[2]

[1]Department of Pediatric, Shengzhou People's Hospital (Shengzhou Branch of The First Affiliated Hospital of Zhejiang University School of Medicine, The Shengzhou Hospital of Shaoxing University), Shengzhou, Zhejiang, 312400, People's Republic of China; [2]Department of Pediatric, Hangzhou Children's Hospital, Hangzhou, Zhejiang, 310014, People's Republic of China

Correspondence: Yanyan Zhu, Department of Pediatric, Hangzhou Children's Hospital, 195 Wenhui Road, Hangzhou, Zhejiang, 310014, People's Republic of China, Email zhyy0505@163.com



**Objective:** Absent in melanoma 2 (AIM2) is associated with inflammation. We intended to determine whether serum AIM2 levels are related to severity and complications of community-acquired pneumonia (CAP) in children.

**Methods:** In this prospective cohort study, serum AIM2 levels were measured in 305 children with CAP and in 100 healthy controls at the Hangzhou Children's Hospital between January 2022 and June 2023. CAP severity was assessed using the pediatric critical illness score (PCIS) and clinical pulmonary infection score (CPIS). In-hospital complicated CAP was identified as the outcome variable. Univariate and multivariate analyses were sequentially performed to determine the correlation between severity, outcome and serum AIM2 levels.

**Results:** Children with CAP had higher serum AIM2 levels than controls (median, 1.45 ng/mL versus 0.36 ng/mL; P<0.001). Serum AIM2 levels in diseased children were independently correlated with PCIS (beta=−0.020; P=0.001) and CPIS (beta=0.092; P=0.002), were linearly related to likelihood of complicated CAP (P for nonlinear =0.057), and were independently associated with complicated CAP (odds ratio= 6.162; P=0.005). The outcome association was robust by calculating the E-value at 11.8 and was not moderated by age, sex, weight and more (all P interaction >0.05). Serum AIM2 levels and two independent predictors, PCIS and CPIS, were integrated to construct the model. The model was pictorially represented by the nomogram and exhibited satisfactory discrimination capability, validity, and stability under the receiver operating characteristic (ROC) curve, decision curve, and calibration curve. By computing the net reclassification improvement and integrated discrimination improvement indices and comparing the area under the ROC curve, the model significantly outperformed the combination of the PCIS and CPIS.

**Conclusion:** Markedly enhanced serum AIM2 levels following CAP in children are highly linked to severity and complicated CAP, substantializing serum AIM2 as a biochemical metric for assessing the severity and identifying adverse outcomes of childhood CAP.

**Keywords:** community-acquired pneumonia, children, absent in melanoma 2, outcomes, severity, biomarkers

## Introduction

Community-acquired pneumonia (CAP), a significant health concern around the globe, represents a leading cause of hospitalization in children.[1,2] The overall incidence rate of CAP was 15.97 per 1000 person-years in children below 5 years old in southeastern China from January 1, 2015 to December 31, 2020.[3] Pediatric CAP is characterized by heterogeneous clinical presentations, ranging from mild respiratory or systemic symptoms (eg, fever, cough, and wheezing) to severe complications, such as acute renal injury, sepsis, and multiorgan failure.[4] Intricate molecular mechanisms including inflammatory responses, oxidative reactions, and cellular apoptosis play pivotal roles in the progression of childhood CAP.[5] The pediatric critical illness score (PCIS) is summed based on 10 indicators from laboratory tests and physical examination.[6] The clinical pulmonary infection score (CPIS) is calculated at a basis of

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clinical, analytical, imaging and microbiological data.[7] Both PICS and CPIS are conventionally used to evaluate CAP severity in children.[8,9] Complicated CAP, a severe form manifested by local or systemic complications, signifies disease progression and necessitates aggressive treatments.[10–12] Accordingly, early identification of complicated CAP may be of utmost significance in the clinical practice of CAP treatment in children. However, complexities of PICS and CPIS calculations may limit their clinical feasibility in clinical work, necessitating continued search for blood biomarkers owning to easy obtainability of blood samples in terms of discrimination of complicated CAP in children.

Inflammasomes have been implicated in a spectrum of pathophysiological processes, including the occurrence and development of pulmonary infections.[13,14] Absent in melanoma 2 (AIM2), a key component of the inflammasome complex, is a critical mediator of the inflammatory responses in various inflammation-related diseases.[15,16] AIM2 expression in the lung tissues was substantially elevated.[17] In addition, lung injury was attenuated, and survival was significantly improved in AIM2-deficient mice with influenza-induced lung injury.[18] Similarly, AIM2-driven alveolar macrophage pyroptosis markedly aggravated experimental lung injury, whereas genetic silencing of AIM2 notably diminished inflammation.[19] Moreover, higher AIM2 levels in the bronchoalveolar lavage fluid were associated with pulmonary fibrosis.[20] Intriguingly, increased serum AIM2 levels were independently associated with stroke-associated pneumonia in adults with acute intracerebral hemorrhage.[21] These data suggest that AIM2 could be specifically derived from lung injury, therefore leading to the conception that serum AIM2 may be a potential biomarker of lung injury. Here, serum AIM2 levels were measured in a group of children with CAP to investigate serum AIM2 as a biomarker for assessing severity and identifying complicated CAP in children.

# Materials and Methods

## Study Design and Subject Selection

This prospective cohort study was done at the Hangzhou Children's Hospital between January 2022 and June 2023. All children with CAP were enrolled consecutively. The inclusion criteria were as follows: (1) newly diagnosed CAP, (2) 3 months < age <14 years in consideration of blood-sampling obtainability and physiological traits of children, and (3) admission of children with CAP to the hospital. The exclusion criteria were (1) other respiratory diseases, such as allergic pneumonia, asthma, or tuberculosis; (2) use of immunosuppressive drugs, underlying immune system disorders, congenital illnesses, and severe illness in other organs; and (3) other specific conditions, such as reluctance to participate, loss to follow-up, incomplete information, and unqualified blood samples. Children who underwent routine examinations at Hangzhou Children's Hospital were recruited as controls. This study was conducted in accordance with the principles of the Declaration of Helsinki, and the research protocol was approved by the Ethics Committee of the Hangzhou Children's Hospital (Ethics Approval Number: 2021–47) and written informed consent was obtained from the children's guardians.

## Data Collection

Some basic information, including age, sex, weight, height, preterm birth, family smoking status, vaccination, preadmission antibiotic use, preadmission fever and cough durations, were registered. Disease severity was assessed using the PCIS[6] and the CPIS.[7] Pathogens were classified into bacteria, virus, mycoplasma pneumoniae and mixed type. Complicated CAP was considered when any local or systemic complication was identified.[10–12] Local complications included parapneumonic effusion, empyema, necrotizing pneumonia, and lung abscess, and systemic complications included bacteremia, metastatic infection, multiorgan failure, acute respiratory distress syndrome, and disseminated intravascular coagulation and so forth.[10–12]

## Immune Analysis

Peripheral venous blood samples were collected at admission from children with CAP and at the entrance of the study from the control children. The blood samples were centrifuged to separate the serum for storage at −80 °C until subsequent testing. Serum AIM2 levels were measured using enzyme-linked immunosorbent assay (Catalog No. ZY-E6125H; Shanghai Zeye Biotechnology Co. Ltd., Shanghai, China). The detection range of this kit was 0.156–10 ng/mL with a sensitivity of 0.094 ng/mL, and both intra- and inter-assay coefficients of variation were less than 10%. All

samples were tested in duplicate by identical proficient technicians, who were inaccessible to clinical details. The two measurements were averaged for subsequent analyses.

## Statistical Analysis

Statistical analyses were completed applying SPSS 25.0 (IBM Corporation, Armonk, NY, USA), GraphPad Prism 9.0 (GraphPad Software, La Jolla, CA, USA), R 4.2.2 (https://www.r-project.org), and MedCalc 20.305 (MedCalc Software, Mariakerke, Belgium). The Kolmogorov–Smirnov test was used to determine the distribution normality of the quantitative variables. Normally distributed variables are presented as mean±standard deviation, whereas non-normally distributed variables are presented as median (25th-75th percentiles). Qualitative data are reported as counts (proportions). Based on the different data types, the $\chi 2$ test, Fisher's exact test, Mann–Whitney *U*-test, or *t*-test was employed for intergroup comparisons, as applicable. Bivariate correlation analysis was performed using the Spearman correlation test. A multivariate linear regression model was used to identify variables that were independently associated with serum AIM2 levels. Serum AIM2 levels were dichotomized according to their median values as high and low levels. The relevant variables were compared between the two groups to determine substantially different variables. These markedly different factors were included in the binary logistic regression model to reveal the independently associated parameters. In order to ascertain whether linear model was appropriate for statistical analysis, the restricted cubic spline was drawn to discern the possible linear correlation between serum AIM2 levels and risk of complicated CAP; and if P value was above 0.05 for nonlinear assumption, the linear model should be adopted for data analysis. To compare the differences of data between children with and without complicated CAP, a binary logistic regression model was used to investigate independently associated variables. Odds ratios (OR) and corresponding 95% confidence intervals (CI) were calculated to show associations. Subgroup analyses were performed to investigate whether the association was moderated by other variables, such as age, sex, weight, height and so forth. E-value, a component of sensitivity analysis, was computed based on OR value in regression analysis for reflecting the robustness of the association, with higher value signifying more strong result association.[22] A variance inflation factor (VIF) was generated to evaluate multicollinearity in the regression model; a VIF value < 10 indicates the absence of multicollinearity.[23] Receiver operating characteristic (ROC) curves were constructed to explore the discrimination efficiency. Z-test was used to compare the area under the curve. The independent predictors of complicated CAP were consolidated to develop the model. The model was pictorially represented by the nomogram, so as to predict CAP risk, in which each independent predictor corresponded to the respective point and all points were aggregated to mirror risk. A calibration curve was plotted to demonstrate the stability of the model and a decision curve was drawn to assess the clinical applicability of the model. Meanwhile, the Hosmer-Lemeshow test was done and brier score was computed in order to unveil whether the model was performed stably. Net reclassification improvement and integrated discrimination improvement indices were calculated to determine the improvement rate of the model. Here, the sample size was estimated at a type 1 error value (alpha) of 0.05, test power (1-beta) of 0.95, and Cohen's d of at least 0.8 for effect size in comparison of serum AIM2 levels across complicated CAP. A priori power analysis was performed to validate the adequate sample size by employing the G*Power 3.1.9.4 (Heinrich-Heine-Universität Düsseldorf, Universitätsstraße 1, Düsseldorf, Germany). Differences were considered statistically significant at a two-sided P-value of <0.05.

# Results

## Subject Selection and Features

An initial assessment was performed on 362 children with CAP who met pre-established inclusion criteria. In accordance with the prespecified exclusion criteria, fifty-seven children were excluded from this study because of other respiratory diseases (17 cases), use of immunosuppressive drugs (6 cases), underlying immune system disorders (7 cases), congenital illnesses (8 cases), severe sickness in other organs (8 cases), reluctance to participate in this study (3 cases), missed visits (2 cases), incomplete information (2 cases), and unqualified blood samples (4 cases). Ultimately, 305 children were included in the epidemiological survey. Baseline patient characteristics are outlined in Table 1. A group of 100 healthy children was used as a control. This group of controls consisted of 54 boys and 46 girls, encompassed 24 children

**Table 1** Baseline Characteristics of Diseased Children and Factors in Correlation with Serum Absent in Melanoma 2 Levels of Children with Community-Acquired Pneumonia

| Variables | All Patients | Spearman Test | |
|---|---|---|---|
| | | $\rho$ | P value |
| Age (months) | 42.8±32.4 | −0.016 | 0.774 |
| Gender (male/female) | 175/130 | 0.001 | 0.983 |
| Weight (kg) | 16.5±8.2 | 0.086 | 0.136 |
| Height (cm) | 100.3±23.9 | 0.046 | 0.426 |
| Preterm birth | 26 (8.5%) | 0.072 | 0.212 |
| Family smoking | 67 (22.0%) | 0.001 | 0.983 |
| Preadmission fever duration (days) | 3 (1–4) | 0.035 | 0.540 |
| Preadmission cough duration (days) | 5 (3–7) | 0.043 | 0.458 |
| Vaccination | 299 (98.0%) | 0.067 | 0.246 |
| Preadmission antibiotic use | 86 (28.2%) | 0.048 | 0.408 |
| Pathogens | | 0.021 | 0.718 |
| Bacterial infection | 42 (13.8%) | | |
| Viral infection | 157 (51.5%) | | |
| Mycoplasma pneumoniae infection | 91 (29.8%) | | |
| Mixed type | 15 (4.9%) | | |
| Heart rate (beats/min) | 115.1±17.9 | 0.011 | 0.844 |
| Respiration rate (times/min) | 29.0±6.4 | 0.044 | 0.442 |
| Body temperature (°C) | 38.6±1.3 | 0.148 | 0.010 |
| Blood white blood cell counts ($\times 10^9$/L) | 11.4 (8.4–14.4) | 0.236 | <0.001 |
| Blood procalcitonin levels (ng/mL) | 0.22 (0.08–0.34) | 0.259 | <0.001 |
| Blood C-reactive protein levels (mg/L) | 26.6 (18.8–37.3) | 0.297 | <0.001 |
| Pediatric critical illness scores | 96 (84–96) | −0.637 | <0.001 |
| Clinical pulmonary infection scores | 2 (2–5) | 0.685 | <0.001 |

**Notes**: Qualitative variables were presented as frequencies (proportions) and continuous variables were reported as medians (lower-upper quartiles) or means ± standard deviations as deemed suitable $\rho$ represents rho.

experiencing family smoking, included 10 suffering from preterm birth, were aged at mean value of 43.4 months (standard deviation, 33.4 months), had mean weight of 16.8 kg (standard deviation, 7.8 kg) and showed mean height of 101.8 cm (standard deviation, 25.3 cm). The above six variables did not differ significantly between the diseased children and the controls (all P>0.05).

## Serum AIM2 Levels and Disease Severity

Serum-based AIM2 levels were markedly higher in children with CAP than in the controls (P<0.001; Figure 1). Serum AIM2 levels were significantly negatively correlated with the PCIS (P<0.001; Figure 2) and were substantially positively related to CPIS (P< 0.001; Figure 3). In addition to the PCIS and CPIS, body temperature, blood procalcitonin levels, white blood cell counts, and blood C-reactive protein levels were closely related to serum AIM2 levels (all P<0.05; Table 1). By incorporating the six aforementioned factors, that is the PCIS, CPIS, body temperature, blood procalcitonin levels, white blood cell counts and blood C-reactive protein levels, into the multivariable linear regression model, the PCIS (beta, −0.020; 95% CI, −0.025–0.015; VIF, 1.408; P=0.001) and CPIS (beta, 0.092; 95% CI, 0.069–0.115; VIF, 1.553; P=0.002) were independently correlated with serum AIM2 levels. Next, diseased children were divided into two groups according to the median serum AIM2 level, that is the levels ≥ 1.45 ng/mL and < 1.45 ng/mL. As compared to children with serum AIM2 levels < 1.45 ng/mL, those with the levels ≥ 1.45 ng/mL displayed substantially elevated PCIS, CPIS, body temperature, blood procalcitonin levels, white blood cell counts and blood C-reactive protein levels (all P<0.05; Table 2). Subsequently, those significant variables, encompassing PCIS, CPIS, body temperature, blood procalcitonin levels, white blood cell counts and blood C-reactive protein levels, were included in the binary logistic

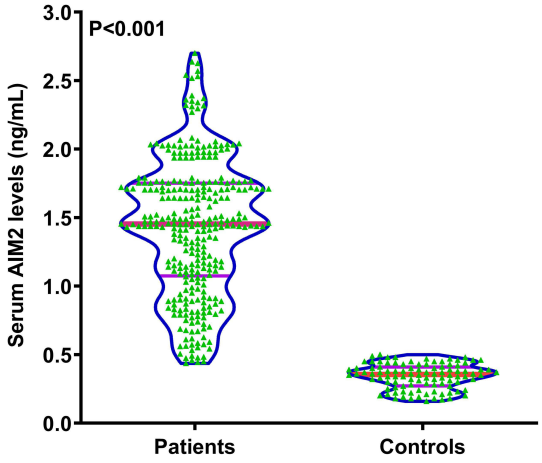


**Figure 1** Differences in serum levels of absent in melanoma 2 between healthy controls and children with community-acquired pneumonia. Serum absent in melanoma 2 levels are expressed as the median (upper quartile-lower quartile). Using the Mann–Whitney *U*-test, serum absent in melanoma 2 levels in children with community-acquired pneumonia were significantly higher than those in healthy controls (P<0.001). AIM2 indicates absent in melanoma 2.

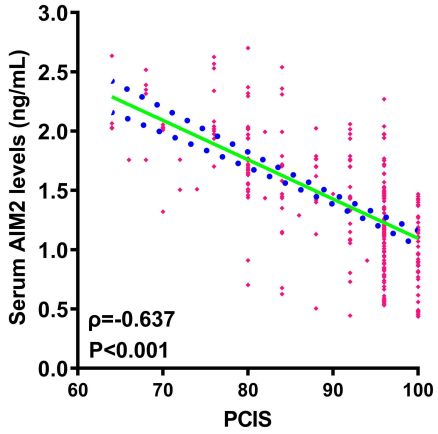


**Figure 2** Relationship between serum absent in melanoma 2 levels and pediatric critical illness score after community-acquired pneumonia in children. Using Spearman correlation coefficient, serum absent in melanoma 2 levels were strongly inversely correlated with the pediatric critical illness score after childhood community-acquired pneumonia (P<0.001). AIM2 means absent in melanoma 2.
**Abbreviation**: PCIS, pediatric critical illness score.

regression model, and then PCIS (OR, 0.864; 95% CI, 0.824–0.907; VIF, 1.982; P=0.002) and CPIS (OR, 1.924; 95% CI, 1.531–2.417; VIF, 2.103; P=0.003) were independently associated with serum AIM2 levels ≥ 1.45 ng/mL.

## Serum AIM2 Levels and Complicated CAP

In contrast to children without complicated CAP, those with the adverse event had notably increased serum AIM2 levels (P<0.001; Figure 4). Alternatively, serum-based AIM2 levels effectively anticipated complicated CAP, and its threshold was selected at 1.58 ng/mL using the Youden approach, generating the maximum Youden index of 0.535 for outcome prediction (Figure 5). In the context of the restricted cubic spline analysis, serum AIM2 levels were linearly related to the probability of complicated CAP (P for nonlinearity > 0.05; Figure 6), signifying suitability of linear model in the next statistical analysis. As shown in Table 3, children presenting with complicated CAP, relative to those without such an event, had obviously decreased age and height, as well as held apparently increased serum AIM2 levels, PCIS, CPIS, blood procalcitonin levels, white blood cell counts, and blood C-reactive protein levels (all P<0.05). When all eight significantly different parameters, encompassing age, height, serum AIM2 levels, PCIS, CPIS, blood procalcitonin levels, white blood cell counts and blood C-reactive protein levels, were integrated into the binary logistic regression module, we found that serum AIM2 levels (OR, 6.162; 95% CI, 1.752–21.670; VIF, 2.312; P=0.005), PCIS (OR, 0.907; 95% CI,

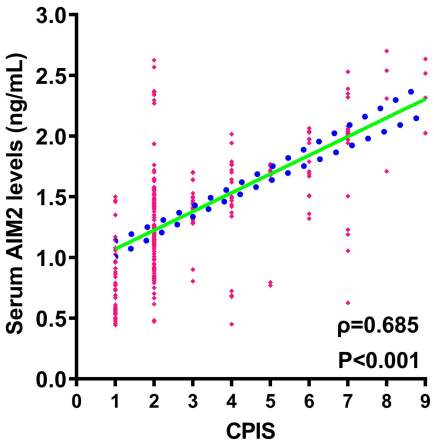


**Figure 3** Relationship between serum absent in melanoma 2 levels and clinical pulmonary infection score after pediatric community-acquired pneumonia. Using Spearman correlation coefficient, serum absent in melanoma 2 levels were intimately positively correlated with the clinical pulmonary infection score of children with community-acquired pneumonia (P<0.001). AIM2 denotes absent in melanoma 2.
**Abbreviation**: CPIS, clinical pulmonary infection score.

0.867–0.949; VIF, 2.419; P=0.001), and CPIS (OR, 1.391; 95% CI, 1.114–1.738; VIF, 2.375; P=0.004) independently predicted complicated CAP. In the subgroup analysis framework, the association between serum AIM2 levels and complicated CAP was not moderated by certain factors, such as age, sex, weight, height, family smoking, preadmission fever duration, and cough duration (all P interaction > 0.05; Figure 7). As for the sensitivity analysis in Figure 8, the

**Table 2** Baseline Features Between Community-Acquired Pneumonia Children with High and Low Serum Absent in Melanoma 2 Levels

| Variables | Serum Absent in Melanoma 2 levels | | P value |
|---|---|---|---|
| | ≥ 1.45 ng/mL | < 1.45 ng/mL | |
| Age (months) | 39.3±28.9 | 46.3±35.3 | 0.056 |
| Gender (male/female) | 91/62 | 84/68 | 0.457 |
| Weight (kg) | 15.9±6.5 | 17.0±9.6 | 0.270 |
| Height (cm) | 99.5±21.8 | 101.1±25.9 | 0.590 |
| Preterm birth | 16 (10.5%) | 10 (6.6%) | 0.225 |
| Family smoking | 36 (23.5%) | 31 (20.4%) | 0.509 |
| Preadmission fever duration (days) | 3 (1–4) | 3 (1–4) | 0.752 |
| Preadmission cough duration (days) | 5 (4–8) | 5 (3–7) | 0.436 |
| Vaccination | 152 (99.3%) | 147 (96.7%) | 0.097 |
| Preadmission antibiotic use | 45 (29.4%) | 41 (27.0%) | 0.636 |
| Pathogens | | | 0.099 |
| Bacterial infection | 17 (11.1%) | 25 (16.4%) | |
| Viral infection | 81 (52.9%) | 76 (50.0%) | |
| Mycoplasma pneumoniae infection | 51 (33.3%) | 40 (26.3%) | |
| Mixed type | 4 (2.6%) | 11 (7.2%) | |
| Heart rate (beats/min) | 114.2±17.0 | 116.0±18.7 | 0.379 |
| Respiration rate (times/min) | 29.1±6.7 | 28.9±6.2 | 0.824 |
| Body temperature (°C) | 38.8±1.5 | 38.4±1.1 | 0.001 |
| Blood white blood cell counts ($\times 10^9$/L) | 12.4 (9.7–15.6) | 11.4 (8.3–12.8) | 0.004 |
| Blood procalcitonin levels (ng/mL) | 0.26 (0.14–0.38) | 0.16 (0.06–0.22) | <0.001 |
| Blood C-reactive protein levels (mg/L) | 28.7 (22.7–42.3) | 23.2 (14.7–32.3) | <0.001 |
| Pediatric critical illness scores | 84 (80–96) | 96 (96–100) | <0.001 |
| Clinical pulmonary infection scores | 4 (2–6) | 2 (1–2) | <0.001 |

**Notes**: Qualitative variables were presented as counts (percentages) and were compared for intergroup differences using chi-square test or Fisher exact test where appropriate. Quantitative variables were summarized as medians (percentiles 25th-75th) or means ± standard deviations as applicable and intergroup comparisons were done using the Mann–Whitney *U*-test or *t* test as suitable.

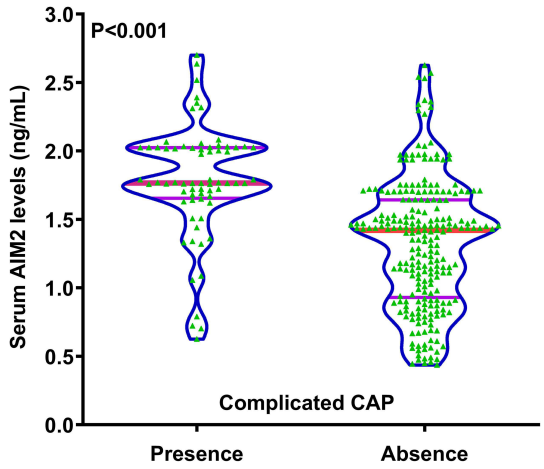


**Figure 4** Differences in serum absent in melanoma 2 levels between children with complicated community-acquired pneumonia and those without such an adverse event. Using the Mann–Whitney *U*-test, serum absent in melanoma 2 levels were substantially higher in children with complicated community-acquired pneumonia than in those not presenting with such an affair (P<0.001). AIM2 signifies absent in melanoma 2.
**Abbreviation**: CAP, community-acquired pneumonia.

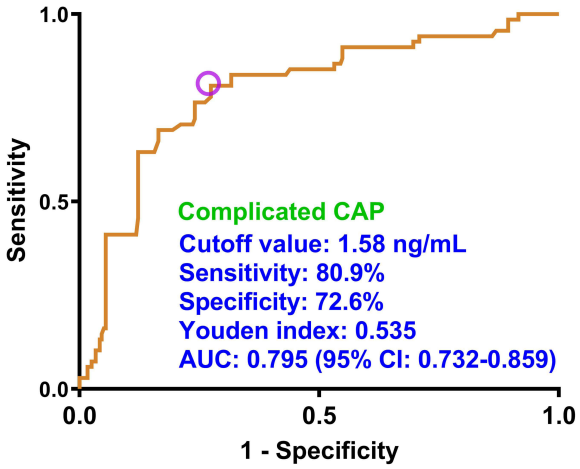


**Figure 5** Receiver operating characteristic curve evaluating discrimination efficiency of serum absent in melanoma 2 levels on complicated community-acquired pneumonia in children. Complicated community-acquired pneumonia was effectively anticipated due to the absence of serum absent in melanoma 2 levels in children. The Youden approach was applied to determine the threshold value of serum absent in melanoma 2 levels to make predictions with medium-to-high sensitivity and specificity. Circle refers to the cutoff value of serum absent in melanoma 2 levels.
**Abbreviation**: CAP, indicates community-acquired pneumonia; AUC, area under the curve; 95% CI, 95% confidence interval.

E-value was 11.8 (95% CI, 2.90, 42.83), denoting enough high E-value versus OR value. In the next step, we modelled a prediction system by integrating the three independent predictors of complicated CAP, namely, serum AIM2, PCIS, and CPIS. The model was pictorially exhibited via the nomogram to instruct clinicians to prognosticate complicated CAP, with higher total scores corresponding to higher risk (Figure 9). In the milieu of the calibration curve analysis, the model had satisfactory goodness of fit, as confirmed by a small mean absolute error at 0.025 (Figure 10). Using the Hosmer-Lemeshow test, P value equaled to 0.235. And, brier score was 0.258. Based on the background of the decision curve analysis, the model presented good clinical validity, as opposed to serum AIM2, PCIS, CPIS, and PCIS combined with CPIS (Figure 11). Under the ROC curve (Figure 12 and Table 4), predictive ability of serum AIM2 resembled those of PCIS and CPIS (both P>0.05); combination of CPIS and PCIS significantly outperformed serum AIM2, PCIS and CPIS (all P<0.05); as well as predictive capability of the model, in which three predictors were integrated, substantially surpassed those of serum AIM2, PCIS, CPIS, and PCIS combined with CPIS (all P<0.05). Also, the conventional biomarkers, that is blood procalcitonin levels, white blood cell counts and blood C-reactive protein levels, were not in


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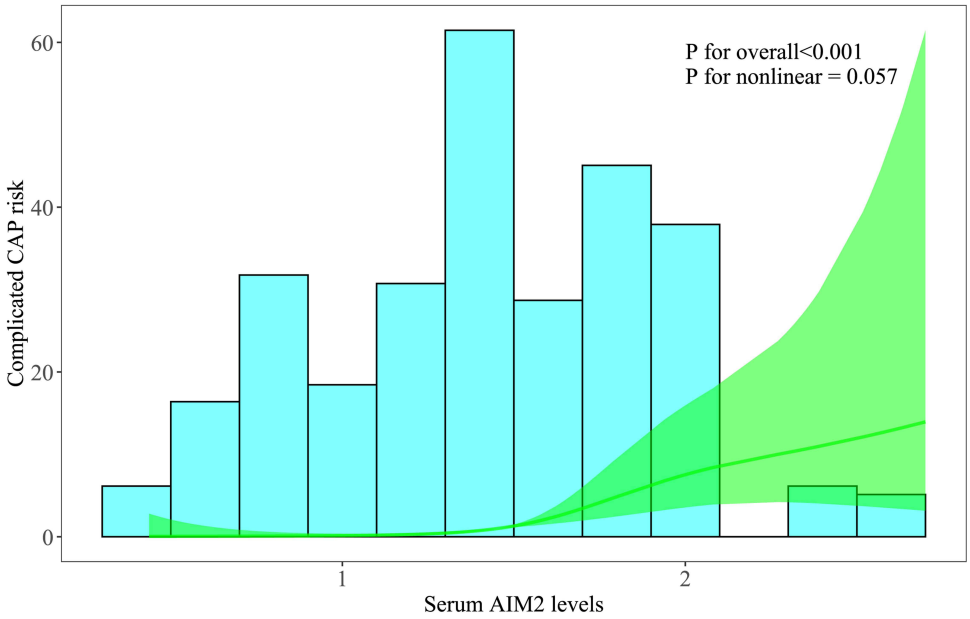


**Figure 6** Restricted cubic spline assessing linear relationship between serum absent in melanoma 2 levels and risk of complicated community-acquired pneumonia in children. Serum absent in melanoma 2 levels were linearly correlated with the likelihood of pediatric complicated community-acquired pneumonia (P for nonlinear > 0.05), indicating that result association could be verified in regression model. AIM2 is indicative of absent in melanoma 2.
**Abbreviation**: CAP, community-acquired pneumonia.

possession of obvious advantages in identifying childhood complicated CAP (all P<0.001; Table 4). Next, the model improvement rate was estimated. As shown in Figure 13, the net reclassification improvement was 0.126 (95% CI, 0.011–0.242) (P=0.032) and the integrated discrimination improvement was 0.066 (95% CI, 0.018–0.114) (P=0.007).

**Table 3** Factors Associated with Complicated Community-Acquired Pneumonia

| Variables | Complicated Pneumonia | | P value |
|---|---|---|---|
| | Presence | Absence | |
| Age (months) | 32.1±21.5 | 45.9±34.4 | 0.002 |
| Gender (male/female) | 41/27 | 134/103 | 0.581 |
| Weight (kg) | 14.8±5.9 | 16.9±8.7 | 0.064 |
| Height (cm) | 95.0±20.3 | 101.8±24.7 | 0.040 |
| Preterm birth | 9 (13.2%) | 17 (7.2%) | 0.115 |
| Family smoking | 12 (17.6%) | 55 (23.2%) | 0.329 |
| Preadmission fever duration (days) | 3 (2–4) | 3 (1–4) | 0.110 |
| Preadmission cough duration (days) | 6 (4–8) | 5 (3–7) | 0.078 |
| Vaccination | 67 (98.5%) | 232 (97.9%) | 0.738 |
| Preadmission antibiotic use | 24 (35.3%) | 62 (26.2%) | 0.140 |
| Pathogens | | | 0.090 |
| Bacterial infection | 7 (10.3%) | 35 (14.8%) | |
| Viral infection | 43 (63.2%) | 114 (48.1%) | |
| Mycoplasma pneumoniae infection | 17 (25.0%) | 74 (31.2%) | |
| Mixed type | 1 (1.5%) | 14 (5.9%) | |
| Heart rate (beats/min) | 114.4±15.9 | 115.3±18.4 | 0.730 |
| Respiration rate (times/min) | 29.1±5.9 | 29.0±6.6 | 0.853 |
| Body temperature (°C) | 38.8±1.4 | 38.5±1.3 | 0.168 |

(*Continued*)

**Table 3** (Continued).

| Variables | Complicated Pneumonia | | P value |
|---|---|---|---|
| | Presence | Absence | |
| Blood white blood cell counts ($\times 10^9$/L) | 15.6 (8.3–17.2) | 11.4 (8.5–12.9) | 0.006 |
| Blood procalcitonin levels (ng/mL) | 0.26 (0.19–0.49) | 0.18 (0.07–0.30) | <0.001 |
| Blood C-reactive protein levels (mg/L) | 28.7 (23.9–44.4) | 25.3 (18.2–34.6) | 0.002 |
| Pediatric critical illness scores | 80 (72–88) | 96 (88–96) | <0.001 |
| Clinical pulmonary infection scores | 6 (4–7) | 2 (2–3) | <0.001 |
| Serum absent in melanoma 2 levels (ng/mL) | 1.77 (1.66–2.02) | 1.42 (0.94–1.64) | <0.001 |

**Notes**: Qualitative variables were presented as counts (percentages) and were compared for intergroup differences using chi-square test or Fisher exact test where appropriate. Quantitative variables were summarized as medians (percentiles 25th-75th) or means ± standard deviations as applicable and intergroup comparisons were done using the Mann–Whitney *U*-test or *t* test as suitable.

## Discussion

To the best of our knowledge, this may be the first study to explore the relationship between serum AIM2 levels, disease severity, and complicated CAP in children diagnosed of CAP. First, a profound increase in serum AIM2 levels after childhood CAP has been demonstrated in comparison to controls. Second, PCIS and CPIS were independent correlates of serum AIM2 levels, whether serum AIM2 was identified as a continuous variable or transformed into a binary variable. Third, serum AIM2, PCIS, and CPIS levels were independently predictive of complicated CAP in children. Finally, the model combining serum AIM2, PCIS, and CPIS showed a good performance in forecasting complicated CAP in children. Taken together, serum AIM2 levels may represent a promising biomarker for estimating CAP severity and predicting complicated CAP in children.

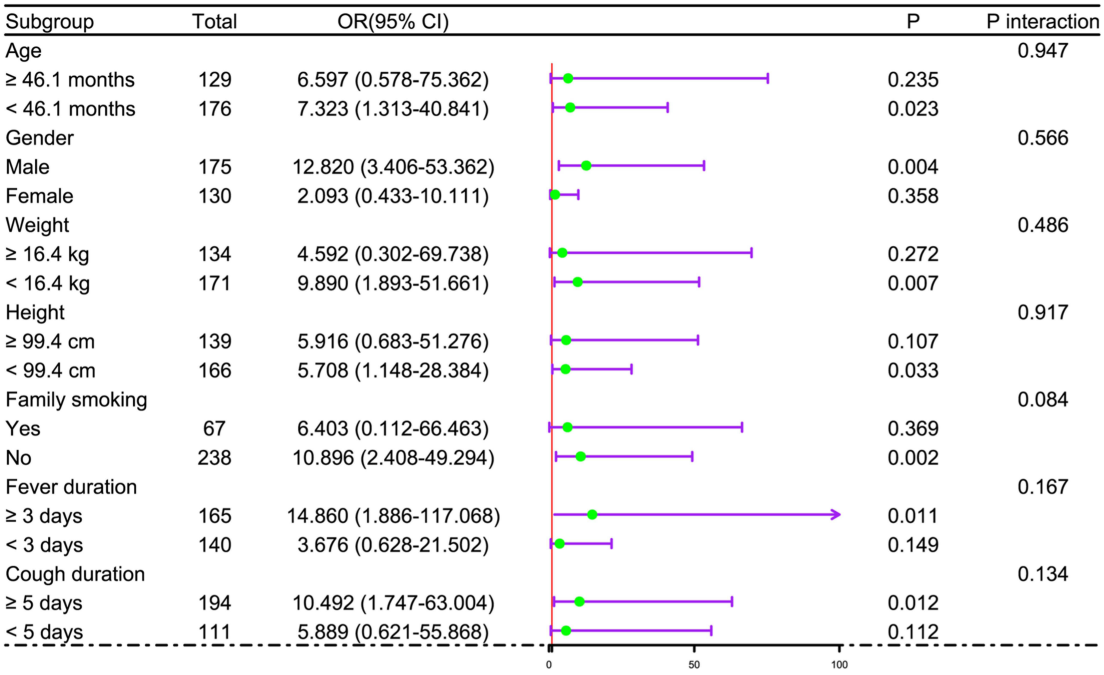


**Figure 7** Subgroup analyses examining interactional effects of some conventional variables on association of serum absent in melanoma 2 levels with childhood complicated community-acquired pneumonia. Age, sex, weight, height, family smoking, pre-admission fever duration, and pre-admission cough duration did not show a markedly moderate relationship between serum absent in melanoma 2 levels and pediatric complicated community-acquired pneumonia (all P interaction > 0.05). OR stands for odds ratio; 95% CI, 95% confidence interval.


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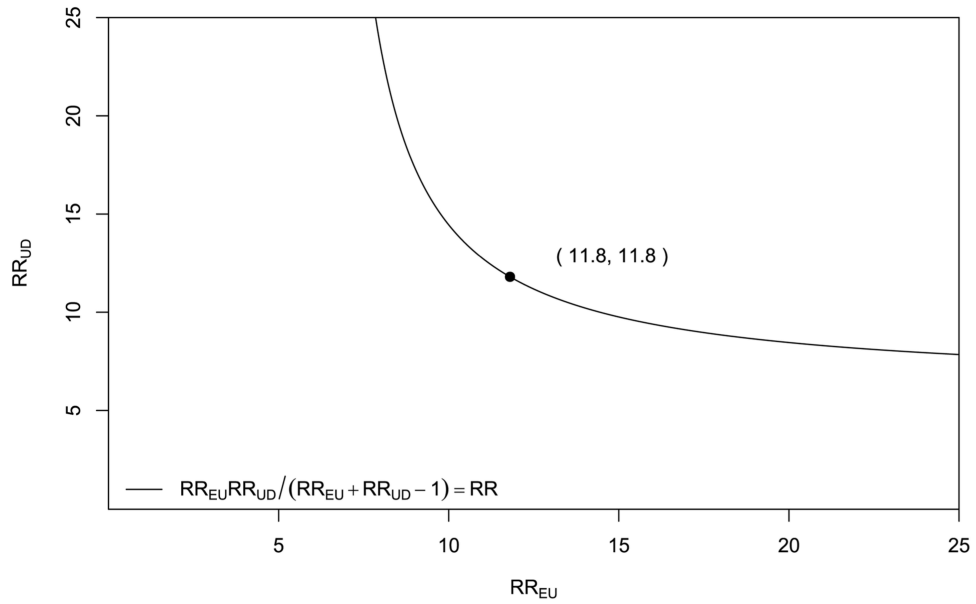


**Figure 8** Diagrammatic sketch showing E-value for expressing robustness of association between serum absent in melanoma 2 levels and childhood complicated community-acquired pneumonia. For sensitivity analysis, the E-value was 11.8 (95% confidence interval, 2.90–42.83) for displaying a robust association of serum absent in melanoma 2 levels with pediatric complicated community-acquired pneumonia.

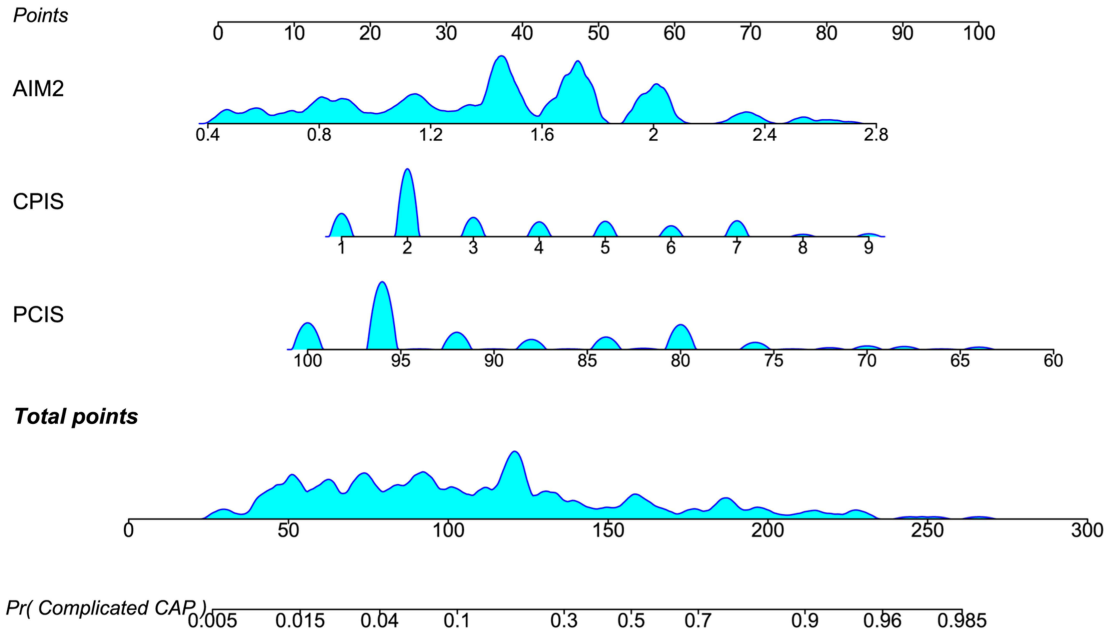


**Figure 9** Nomogram exhibiting model of complicated community-acquired pneumonia in children. The three predictors of complicated community-acquired pneumonia, that is, serum absent in melanoma 2, pediatric critical illness score, and clinical pulmonary infection score, were consolidated to develop a combined model for outcome anticipation in children. The model was visualized via the nomogram, with the summed scores reflecting risk. AIM2 denotes absent in melanoma 2.
**Abbreviations**: PCIS, Pediatric Critical Illness Score; CPIS, Clinical Pulmonary Infection Score; CAP, community-acquired pneumonia.

AIM2 functions as a cytosolic receptor for double-stranded DNA and is extensively involved in inflammasome activation.[24] It is widely expressed in epithelial and immune cells, particularly under infection and stress.[25] AIM2 is upregulated in lung tissues during infections such as tuberculosis and idiopathic pulmonary fibrosis.[26,27] Furthermore, increased expression of AIM2 has been documented in alveolar macrophages and lung epithelial cells in inflammatory

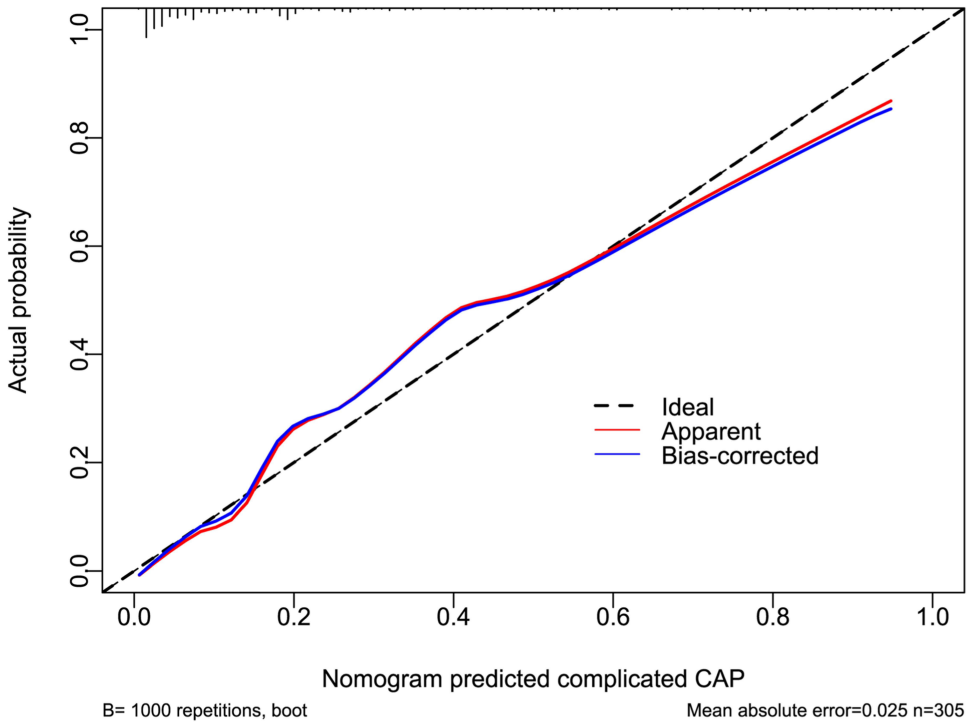


**Figure 10** Calibration curve determining stability of the merged model for forecasting complicated community-acquired pneumonia in children. A model containing serum absent in melanoma 2, pediatric critical illness score, and clinical pulmonary infection score was established to predict complicated pediatric community-acquired pneumonia. In accordance with low mean absolute error at 0.025, the model remained stable for outcome prediction. CAP is indicative of community-acquired pneumonia.

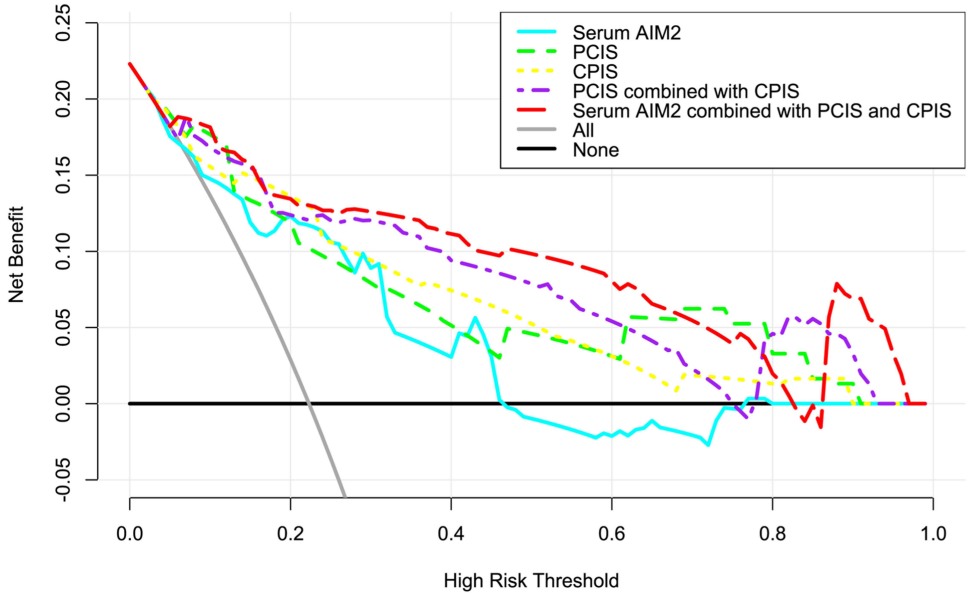


**Figure 11** Decision curve observing validity of the combined model in prognosticating complicated community-acquired pneumonia in children. The model was composed of serum absent in melanoma 2, pediatric critical illness score, and clinical pulmonary infection score. In contrast to serum absent in melanoma 2, pediatric critical illness score, clinical pulmonary infection score, and combination of pediatric critical illness score with clinical pulmonary infection score, the model was demonstrated to benefit the clinical prediction of pediatric complicated community-acquired pneumonia on account of biggest area occupied by the model. AIM2 denotes absent in melanoma 2.
**Abbreviations**: PCIS, Pediatric Critical Illness Score; CPIS, Clinical Pulmonary Infection Score.

and fibrotic lung diseases.[17–20] In adults with acute intracerebral hemorrhage, markedly enhanced admission serum AIM2 levels were strongly associated with a higher risk of stroke-associated pneumonia.[21] Based on our finding that serum AIM2 levels are significantly higher following pediatric CAP, AIM2 may be actively involved in the host immune


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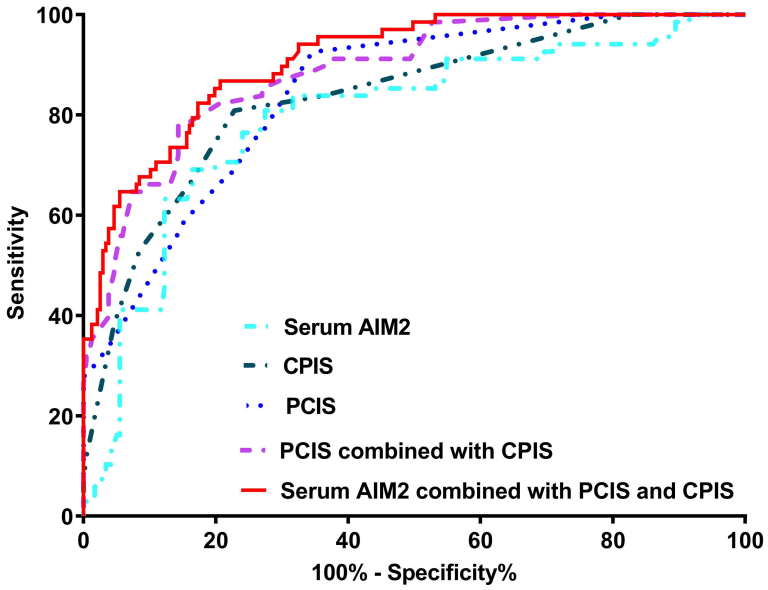


**Figure 12** Receiver operating characteristic curve investigating predictive strength of the model on pediatric complicated community-acquired pneumonia. The model was formed by combining the serum absent in melanoma 2, pediatric critical illness score, and clinical pulmonary infection score. In contrast to serum absent in melanoma 2, pediatric critical illness score, clinical pulmonary infection score, and the combination of pediatric critical illness score with clinical pulmonary infection score, the model was confirmed to possess significantly efficacious prediction ability in childhood complicated community-acquired pneumonia. AIM2 signifies absent in melanoma 2.
**Abbreviations**: PCIS, Pediatric Critical Illness Score; CPIS, Clinical Pulmonary Infection Score.

response to pulmonary tissue injury secondary to childhood CAP. Although it is unclear about detailed mechanisms of AIM2' involvement in CAP or its complications, evidence about inflammasome signaling activation in other diseases implies that AIM2 activation may result in the synthesis of active interleukin-1beta and interleukin-18, thereby inducing pyroptosis, with subsequent participation in pathophysiological processes of pneumonia.[28–30] However, such a hypothesis needs to be demonstrated in future studies.

Compelling data suggest that AIM2 may be a deleterious factor in pulmonary infections,[18,19] and therefore AIM2 may be a potential therapeutic target of CAP and even its complications. On the other hand, it leads to the assumption that serum AIM2 levels may be positively related to CAP severity. CPIS and PCIS are two highly acknowledged severity assessment systems for childhood CAP.[8,9] In this cohort of children with CAP, serum AIM2 levels were strongly associated with CPIS and PCIS in univariate analysis. Using multivariate analysis, serum AIM2 was present in two forms: continuous and binary variables. Finally, it was affirmed that CPIS and PCIS were independently related to serum AIM2 levels in two multivariate modules, namely, the multivariate linear regression model and binary logistic regression

**Table 4** Areas Under Receiver Operating Characteristic Curve for Identifying Complicated Community-Acquired Pneumonia in Children

| Components | AUC (95% CI) | P value | P value | P value |
|---|---|---|---|---|
| Serum AIM2 combined with PCIS and CPIS | 0.908 (0.872–0.944) | | | Reference |
| PCIS combined with CPIS | 0.886 (0.843–0.929) | | Reference | 0.029 |
| Serum AIM2 | 0.795 (0.732–0.859) | Reference | 0.001 | <0.001 |
| PCIS | 0.845 (0.798–0.892) | 0.100 | 0.024 | <0.001 |
| CPIS | 0.834 (0.780–0.888) | 0.221 | 0.001 | <0.001 |
| Blood procalcitonin | 0.649 (0.574–0.724) | <0.001 | <0.001 | <0.001 |
| Blood C-reactive protein | 0.622 (0.548–0.696) | <0.001 | <0.001 | <0.001 |
| Blood leucocyte | 0.610 (0.520–0.700) | <0.001 | <0.001 | <0.001 |

**Note**: The Z test was done for comparing area under curve.
**Abbreviations**: AIM2 means absent in melanoma 2; PCIS, pediatric critical illness score; CPIS, clinical pulmonary infection score; AUC, area under curve; 95% CI, 95% confidence interval.

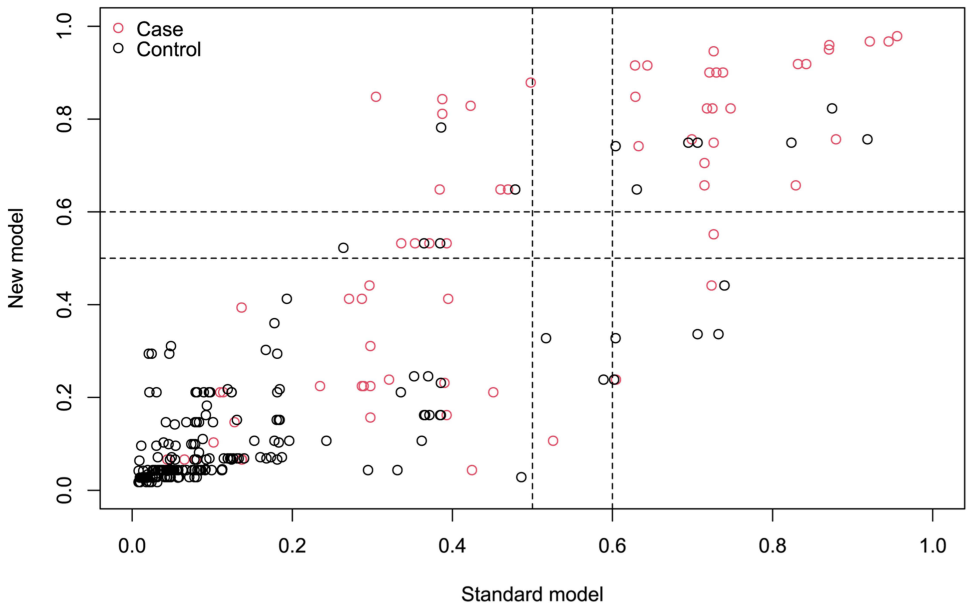


**Figure 13** Plot showing calculation of net reclassification improvement and integrated discrimination improvement. The standard model was composed of the pediatric critical illness and clinical pulmonary infection scores. The new model comprised serum absent in melanoma 2, pediatric critical illness score, and clinical pulmonary infection score. The net reclassification improvement was 0.126 (95% confidence interval, 0.011–0.242) and the integrated discrimination improvement was 0.066 (95% confidence interval, 0.018–0.114), meaning that the combined model may be in possession of markedly higher improvement rate.

model. These data strongly support the notion that serum AIM2 levels are highly correlated with CAP severity in children.

Complicated CAP encompasses one or more of the local or systemic complications of CAP.[10–12] Complicated CAP, which is marked by severe conditions, may massively protract from the disease course, thereby prolonging the length of hospitalization.[10–12] In this study, complicated CAP was identified as the outcome variable of interest. The two CAP severity scaling metrics, CPIS and PCIS, together with serum AIM2, were fully corroborated using multivariate analysis as the three associative factors of pediatric complicated CAP. A restricted cubic spline assessment was initiated in advance to verify the linear relationship between serum AIM2 levels and the possibility of complicated CAP in children. Moreover, the VIF for scaling multicollinearity was less than 10 in the current study, thereby avoiding multicollinearity.[23] Subgroup analysis was performed to investigate the moderating effect, and the association of serum AIM2 levels with complicated CAP was not affected by age, sex, weight, height, or other factors. E-value calculation is a sensitivity analysis modality.[22] The E-value, relative to the OR value, was within the rational range in this cohort of subjects with childhood CAP. This series of statistical measurements ensured the validity and reliability of the results. Therefore, serum AIM2 may be an encouraging biomarker for identifying the risk of childhood complicated CAP.

Early and accurate recognition of the likelihood of pediatric complicated CAP is of the utmost importance in clinical practice.[10–12] Serum AIM2, PCIS, and CPIS levels are three determinants of childhood complicated CAP here. Serum AIM2 levels had a predictive ability comparable to that of PCIS and CPIS. Also, serum AIM2 levels transcended the conventional biomarkers, that is blood procalcitonin levels, white blood cell counts and blood C-reactive protein levels, in terms of identification ability of childhood complicated CAP. The prediction model was composed of independent predictors. As demonstrated by the ROC curve, calibration curve and decision curve, the model was clinically efficient, steady, and beneficial for prognosticating complicated CAP in children. Addition of the Hosmer-Lemeshow test and brier score calculation to statistical analysis further supports the steadiness of the model. Moreover, by estimating the net reclassification improvement and integrated discrimination improvement, the model, as opposed to PCIS combined with CPIS, achieved a significantly elevated improvement rate. Overall, accumulating statistical analyses showed that, from the perspective of additive effects possessed by serum AIM2, serum AIM2 may be an effective predictor of complicated CAP in children.

Several strengths and weaknesses should be mentioned. The strengths are shown below. First, the novelty of our study is pointed out here. To the best of our knowledge, this may be a first series of investigating serum AIM2 in children diseased of CAP and therefore finding that serum AIM2 may be a potential biomarker in relation to severity and complicated CAP in childhood. Second, the clinical values of our study should be elucidated here. In accordance with the cutoff value of serum AIM2 levels, a risk stratification could be done for children with CAP. If serum AIM2 levels are greater than the cutoff value, these diseased children may be at high risk of complicated CAP; so, this group of children should be actively monitored and even admitted into intensive care unit, followed by an aggressive treatment. And, based on numerous statistical methods, the integrated model containing serum AIM2 may be effective in clinical practice of pediatric complicated CAP because the model is able to facilitate risk stratification of complicated CAP in children and assists with aggressive intervention of childhood complicated CAP. The weaknesses are displayed in the following. First, because the risk of overfitting may be existent in model construction in a single-center design lacking external validation, and there are different populations or settings in clinical applications, particularly potential ethnic and environmental differences; these unstable factors possibly lead to difficulty in generalization of model in clinical use. And accordingly, a larger cohort study is warranted to validate effectiveness and stability of the model before the model is applied in prediction of pediatric complicated CAP. Second, even if serum AIM2 alone or the combined model integrating serum AIM2 is demonstrated to be a potential tool for discriminating children at risk of complicated CAP and subsequently instructing clinical treatments, its clinical practicability should be validated in future interventional study.

## Conclusions

In children with CAP, significantly elevated serum AIM2 levels are independently correlated with PCIS and CPIS. Serum AIM2 levels are independent predictors of complicated pediatric CAP. The integrated model containing serum AIM2, PCIS, and CPIS has high clinical effectiveness in forecasting childhood complicated CAP. In summary, serum AIM2 level may be a potential biochemical indicator for pediatric CAP severity appraisal and anticipation of complicated CAP in children; and the combined model incorporating serum AIM2 may be a good tool for risk stratification of pediatric complicated CAP.

## Abbreviations

AIM2, absent in melanoma 2; CAP, community-acquired pneumonia; PCIS, pediatric critical illness score; CPIS, clinical pulmonary infection score; ROC, receiver operating characteristic; AUC, area under the curve; OR, odds ratio; 95% CI, 95% confidence interval.

## Data Sharing Statement

The raw data supporting the conclusions of this study will be provided by the authors without undue retention.

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## Disclosure

The authors declare that they have no competing interests in this work.

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