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Performance of host-response biomarkers to risk-stratify children with pneumonia in Bhutan

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SUMMARY

Pneumonia is the leading cause of post-neonatal death amongst children under five years of age; however, there is no simple triage tool to identify children at risk of progressing to severe and fatal disease. Such a tool could assist for early referral and prioritization of care to improve outcomes and enhance allocation of scarce resources. We compared the performance of inflammatory and endothelial activation markers in addition to clinical signs or scoring scales to risk-stratify children hospitalized with pneumonia at the national referral hospital of Bhutan with the goal of predicting clinical outcome. Of 118 children, 31 evolved to a poor prognosis, defined as either mortality, admission in the paediatric intensive care unit, requirement of chest drainage or requirement of more than five days of oxygen therapy. Soluble triggering receptor expressed on myeloid cells 1 (sTREM-1) was the best performing biomarker and performed better than clinical parameters. sTREM-1 levels upon admission had good predictive accuracy to identify children with pneumonia at risk of poor prognosis. Our findings confirm that immune and endothelial activation markers could be proactively used at first encounter as risk-stratification and clinical decision-making tools in children with pneumonia; however, further external validation is needed.

Background

Pneumonia is the leading infectious cause of preventable deaths amongst children under five years of age,[1] causing an estimated 740,000 deaths annually, or 13.9% of all global deaths in this age group.[2] Every year, up to 226 million children in this age group are diagnosed with pneumonia.[3] While most children will have self-limited disease, a small proportion of them will progress to severe disease and fatal outcome.[4] Early recognition of children with severe pneumonia enables more aggressive referral and treatment, leading to reduced mortality.[5] Thus, there is a need for early identification of children at risk of progressing to severe disease, particularly at the moment of first contact with the healthcare system. At a primary health care level, a simple triage tool that would discriminate children at risk of severe pneumonia from those with self-limited disease could assist decision-making for early referral to a higher healthcare level, particularly in resource-limited

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settings. In addition, the identification of high-risk children will aid prioritization of care in busy healthcare centres and guide rational allocation of scarce resources.

Clinical signs and simple laboratory testing have been combined to generate clinical severity scores to improve early detection of children with fever or respiratory symptoms at risk of poor outcomes.[6–8] However, most of these severity scores involve the measurement of vital signs (e.g., temperature, respiratory rate, or blood pressure), the assessment of clinical signs (e.g., recognising chest indrawing), or the interpretation of laboratory parameters that require trained healthcare workers. Furthermore, risk scores are validated and routinely used in adults with pneumonia, but none have been widely implemented for childhood pneumonia.[8] Therefore, the unresolved need for a simple severity assessment for children with respiratory symptoms may require an innovative approach to currently proposed clinical strategies.[9]

Specific markers of host response including those associated with immune and endothelial activation, have been previously implicated in the pathogenesis of severe infections, irrespective of their underlying aetiology ("pathogen-agnostic").[5,10,11] The quantification of such biomarkers may enable risk stratification and guide clinical decision-making regarding the need for early triage, referral, hospitalization, and admission to intensive care units.[12] Quantifying these markers at clinical presentation has been shown to be useful in predicting severity and outcomes in adults and children with life-threatening infections, including pneumonia, severe malaria, COVID-19, haemorrhagic fevers, or sepsis.[13–20] However, they have not been widely evaluated in low- and middle-income countries, and their prognostic utility in childhood pneumonia has not been validated vis-à-vis standard risk-stratification clinical algorithms.[12,18]

The Respiratory Infections in Bhutanese Children (RIBhuC) study recruited Bhutanese children aged 2 to 59 months hospitalized with clinical pneumonia. Here we assessed the performance of inflammatory, immune, and endothelial activation markers alone or in addition to clinical signs or scoring scales to risk-stratify children hospitalized with pneumonia and predict their outcome.

Methods

Study design

The RIBhuC study was prospectively conducted during 12 consecutive months at the Jigme Dorji Wangchuck National Referral Hospital (JDWNRH) in Thimphu, Bhutan.[21] Briefly, the paediatric department at JDWNRH consists of 38 beds, including five beds in the paediatric intensive care unit. All children aged 2 to 59 months admitted at JDWNRH and fulfilling the World Health Organization (WHO) criteria for pneumonia or severe pneumonia were recruited.[22] Pneumonia was defined as history of cough or reported breathing difficulty and increased respiratory rate ($\geq$ 50 breaths per minute in children aged 2–11 months or $\geq$ 40 breaths per minute in children aged 12–59 months) or chest indrawing (subcostal and/or intercostal retractions defined as lower chest wall indrawing and supraclavicular and/or suprasternal retractions defined as very severe chest indrawing). Severe pneumonia was defined as history of cough or reported breathing difficulty, and at least one of the following: oxygen saturation $<$ 90%, central cyanosis, severe respiratory distress, or any danger sign (inability to breastfeed or drink, lethargy or reduced level of consciousness, convulsions). We excluded children when the principal reason for admission was a non-respiratory illness or a condition that was not caused by respiratory illness, those admitted in the previous seven days in order to exclude hospital-acquired infection, and children with evidence of a foreign body in the respiratory tract.

For all eligible patients whose parents provided written consent for study participation, we collected demographic and clinical data, biological samples, and a chest radiography on admission. Radiographical endpoints were defined as per WHO radiological criteria.[23] The study protocol was approved by the Research Ethics Board of Health, Ministry of Health, in Thimphu, Bhutan (PO/2016/086) and by the research ethics committee from the Hospital Clínic in Barcelona, Spain (HCB/2017/0741). All methods were performed in accordance with the relevant guidelines and regulations.

Clinical scoring scales and outcomes

We used three simple clinical scoring scales developed for predicting disease severity and mortality in low-resource settings (Table 1). Clinical parameters were assessed upon admission. The Respiratory Index of Severity in Children (RISC) score was developed amongst children 0–24 months hospitalized with respiratory infections.[24] The RISC-Malawi is a modified version, which was developed amongst children $<$ 59 months hospitalized with WHO-defined pneumonia.[6] The Lambaréné Organ Dysfunction Score (LODS) was developed amongst children with severe malaria for identifying those needing referral or close monitoring.[25] Although LODS was not specifically developed for pneumonia, it is a promising prognostic tool used in childhood diseases other than malaria.[7]

The primary outcome was prognosis, defined as "good" if the child survived, did not require admission in the paediatric intensive care unit, did not require supplemental oxygen or only received oxygen therapy for five days or less, and did not present with pleural effusion that required chest drainage; and "poor" if the child died, required care in the paediatric intensive care unit, received oxygen for more than five days, or presented pleural effusion that requested chest drainage. The usual time of duration of hypoxaemia (oxygen saturation $<$ 90% in room air) is 2 to 5 days, therefore we considered longer duration as poor prognosis.[26,27] Clinical decisions such as weaning oxygen and time of discharge were taken by any treating paediatrician working at JDWNRH, unaware of the study outcomes for analysis, and therefore at low risk of introducing performance bias.

Laboratory testing

Blood samples were collected from each participant at time of enrolment and were processed following local standard of care.[21] For measurement of immune and endothelial activation markers, blood (2 mL) was collected in EDTA tube and centrifugated (3000 g for three minutes). Plasma was separated and stored at −80 °C until shipment to the University of Toronto, Canada, for analyte testing. Plasma concentration of interleukin-6 (IL-6), interleukin-8 (IL-8), soluble triggering receptor expressed on myeloid cells 1 (sTREM-1), soluble tumour necrosis factor receptor 1 (sTNFR1), angiopoietin-2 (Angpt-2), soluble fms-like tyrosine kinase-1 (sFlt1), and procalcitonin (PCT) were quantified using a multiplex Luminex platform with reagents from R&D Systems (Minneapolis, MN) as described.[28] C-reactive protein (CRP) was quantified by enzyme-linked immunosorbent assay (R&D DuoSet, Minneapolis, MN). Biomarker concentrations outside of the detection limits were assigned a value of one third below or above the lowest or highest limit in the standard curve, respectively. Erythrocyte sedimentation rate (ESR), and CRP were measured at the study site (JDWNRH). We refer to CRP-study and CRP-ref for differentiating CRP analysed at the study and reference laboratories, respectively. Biomarkers were measured blinded to children clinical characteristics and outcome.

Table 1
Clinical scoring scales.

RISC[a] score		RISC-Malawi score		LODS	
Severity of respiratory signs					
SpO2 ≤90%	3 points	−2 ≤ WAZ < −3 SD[b]	3 points	Prostration[c]	1 point
OR		WAZ ≤ −3 SD[b]	7 points	Blantyre coma score <3	1 point
Chest indrawing	2 points	SpO2 90–92%	2 points	Deep breathing[d]	1 point
Wheezing	−2 points	SpO2 <90%	7 points		
Refusal to feed	1 point	Wheezing	−2 points		
Growth standards		Unconscious at exam	8 points		
WAZ ≤ −3 SD	2 points	Female gender	1 point		
−2 ≤ WAZ < −3 SD	1 point				

Abbreviations: LODS: Lambaréné Organ Dysfunction Score; RISC: respiratory index of severity in children; SD: standard deviations; WAZ: weight-for-age Z-score.
[a] For non-HIV infected children.
[b] Moderate and severe malnutrition were originally assessed with middle-upper arm circumference (MUAC). We substituted these measurements by using WAZ as we did not collect MUAC in our study.
[c] Prostration was defined by not being able to breastfeed, sit, stand, or walk, depending on the age of the child.
[d] Deep breathing is also known as Kussmaul's respiration or 'acidotic' breathing.

Data management and statistical analysis

The statistical associations were assessed using Chi-square, Fisher exact, and Mann-Whitney U tests, as appropriate. Univariable logistic regression models were used to estimate odds ratios of biomarker levels as predictors of prognosis, and multivariable logistic regression models to estimate the degree of association after adjusting for observed confounders. All continuous variables with non-parametric distribution were log transformed for inclusion in logistic regression models. Area under the receiver operating characteristics (AUROC) curve and other performance characteristics (sensitivity, specificity, and likelihood ratios) were calculated to assess the predictive capability, based on each univariable logistic regression model and using cut-off points defined with the Youden's index method (J=max[*sensitivity*+*specificity*-1]). AUROCs were compared using the algorithm suggested by DeLong et al. (1988).[29] Classification and regression tree analyses were performed to create simple algorithms based on risk-stratification. We established the settings of a minimum of 10 cases for parent node and 5 for child node, pruning set with a maximum difference in risk to 0 to prevent over fitting and a maximum level of tree depth of 2.[15,19] We performed subgroup analysis by age groups, as age is a potentially relevant cofounder for clinical signs and biomarker levels. Data analyses and figures were performed with Stata™ v.16.0 (StataCorp, College Station, Texas, USA), SPSS Statistics version 23, and RStudio.[30,31] Statistical significance was set at 0.05.

Results

Of 189 children with clinical pneumonia recruited to the RIBhuC study, 118 (62.4%) had biomarker quantification and were included in the analysis (Supplementary Fig. S1). Our study did not perform additional blood draws outside of clinical care, and therefore children that did not have blood collected at first presentation did not have biomarker analysis performed. The characteristics of children included and excluded from the analysis are summarized in Supplementary Table S1. Except for hypoxaemia, which was more common amongst children included in the analysis ($p = 0.048$), there were no significant differences between children included and excluded from the analysis.

Association of demographic characteristics, clinical signs, and scoring scales with prognosis

Of the 118 children included, 31 evolved to a poor prognosis. Tables 2 and 3 present demographic, clinical, radiological, and laboratory findings collected upon admission, according to prognosis. One-quarter of children with poor prognosis were referred from another healthcare centre. Parental education, employment, and access to care were not associated with prognosis. Amongst children with a poor prognosis, 39.1% (9/23) presented with a normal chest radiograph, while amongst those with a good prognosis, 21.9% (16/73) presented radiological endpoint pneumonia. A positive (and not considered contaminated) blood culture was not associated with prognosis. Hypoxaemia, prolonged capillary refill time, increased respiratory rate, lower chest wall indrawing, very severe chest indrawing, nasal flaring, grunting, rhonchi, prostration, and decreased level of consciousness at presentation were all associated with poor prognosis. The oxygen saturation upon admission was significantly lower amongst children with poor prognosis. An elevated score in any of the four clinical scoring scales (WHO, RISC, RISC-Malawi, and LODS) was also associated with poor prognosis.

Association of host-response biomarkers with prognosis

Overall, results of the routinely ordered laboratory testing, including white blood cells (WBC), platelets, ESR, PCT, and CRP, were not associated with outcome when evaluated using common clinical thresholds (Table 3). Similar results were observed when these laboratory parameters were assessed as continuous variables, with the exception of PCT, which was associated with prognosis (Supplementary Table S2). In contrast, plasma levels of all the immune and endothelial activation factors, except for IL-6, were significantly higher at presentation in children that progressed to severe and fatal infections (Fig. 1 and Supplementary Table S2). After adjusting for selected potential confounding factors, differences remained significant for sTREM-1, sTNFR1, IL-8 and PCT (Supplementary Fig. S2).

Performance of clinical characteristics, scoring scales, and biomarkers at predicting poor prognosis

Of single clinical characteristics, oxygen saturation (AUROC 0.75, 95% confidence interval [CI] 0.63–0.86) and lower chest wall indrawing (AUROC 0.70, 95% CI 0.62–0.79) on admission displayed the best predictive accuracy for prognosis (Fig. 2). Of the clinical scoring scales, RISC presented the best predictive performance (AUROC 0.71, 95% CI 0.61–0.80) and was significantly higher than the WHO severity score at predicting prognosis (AUROC 0.61, 95% CI 0.55–0.67; $P < 0.05$).

The best host-response biomarker for predicting poor prognosis was sTREM-1 (AUROC 0.74, 95%CI 0.63–0.88) (Fig. 2). sTREM-1 performed significantly better than the commonly used inflammatory markers (WBC, ESR, and CRP) and IL-6, but not significantly better

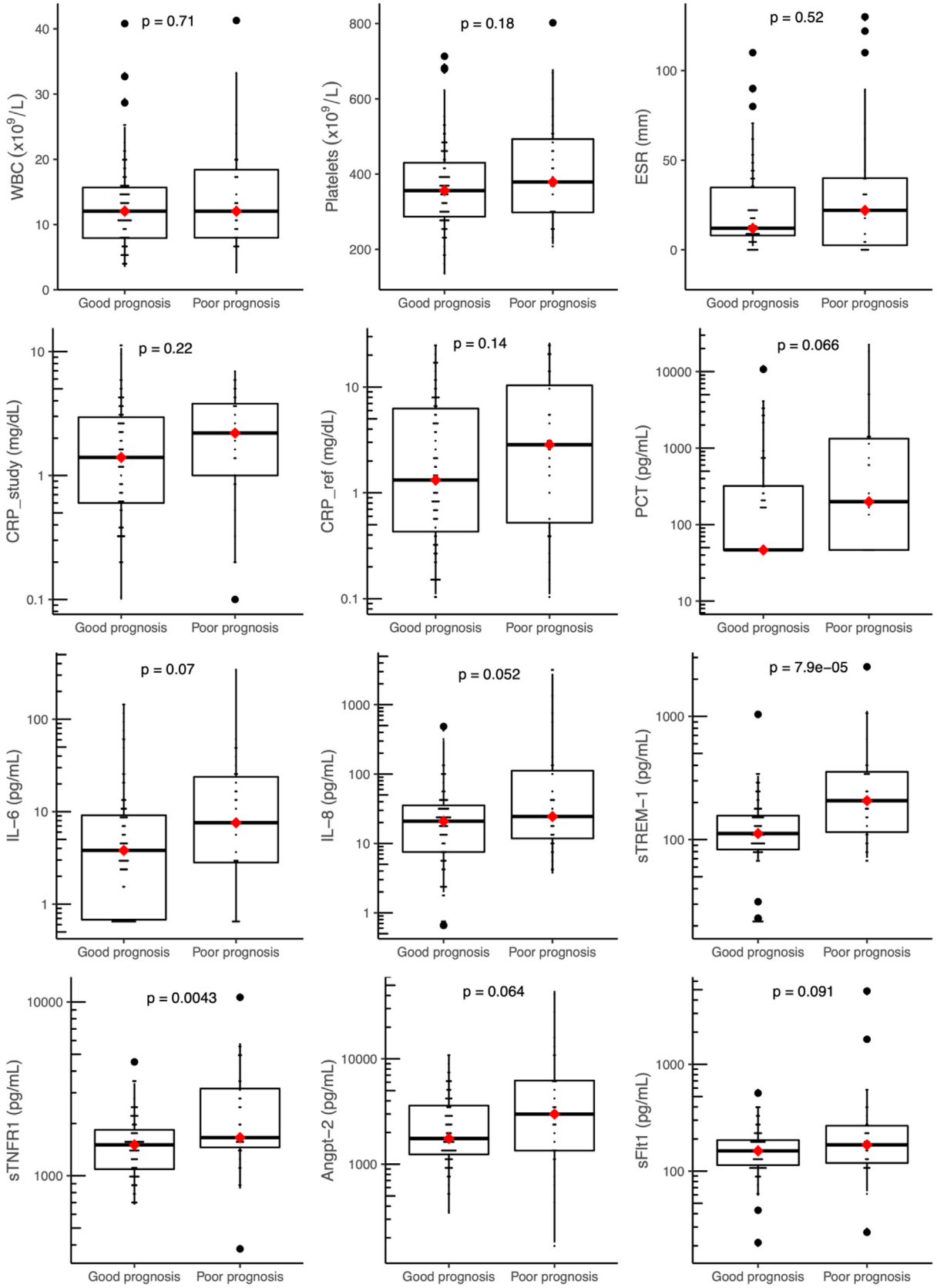


Fig. 1. Performance of host-biomarkers levels according to prognosis.Abbreviations: Angpt-2: angiopoietin-2; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; IL6: interleukin-6; IL8: interleukin-8; PCT: procalcitonin; sFlt1: soluble fms-like tyrosine kinase-1; sTNFR1: soluble tumour necrosis factor receptor 1; sTREM-1: soluble triggering receptor expressed on myeloid cells 1; WBC: white blood cells.Levels of each biomarker is summarized graphically through the median (red dot) and interquartile range (lower and upper side of the box). Good prognosis was defined as survival, no admission in the paediatric intensive care unit, no requirement of oxygen or oxygen therapy for $\leq$ 5 days, and no requirement of chest drainage; while poor prognosis was defined as death and/or admission in the paediatric intensive care unit and/or oxygen therapy for $>$ 5 days and/or required chest drainage. Statistical significance of differences between good and poor outcome for each biomarker level was calculated using the Mann-Whitney U tests, with p-value shown at the top of each biomarker comparison.

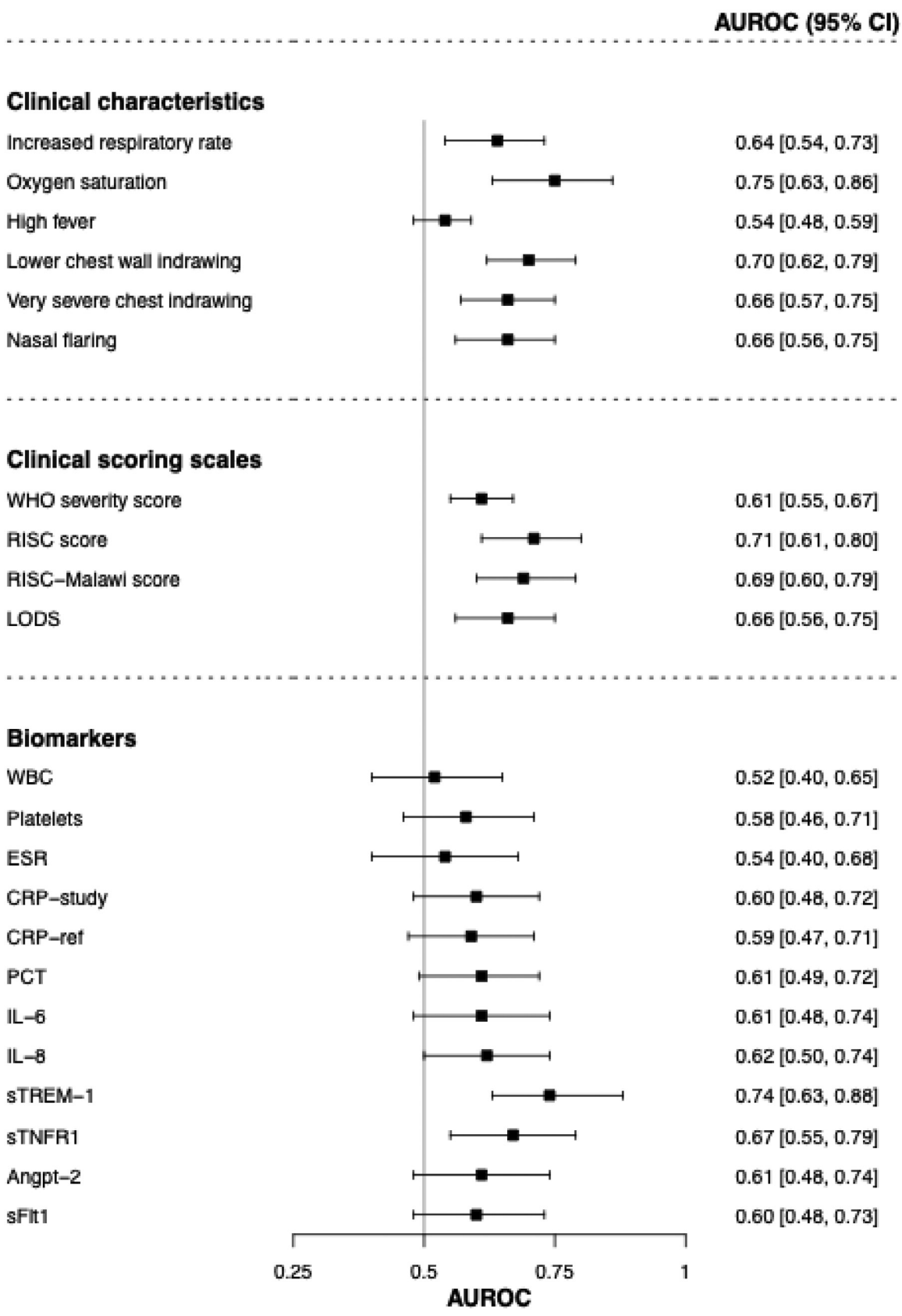


Fig. 2. Prognostic accuracy of clinical characteristics, scoring scales and host-response biomarkers in children with pneumonia.Abbreviations: Angpt-2: angiopoietin-2; AUROC: area under the receiver operating characteristics; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; IL6: interleukin-6; IL8: interleukin-8; LODS: Lambaréné Organ Dysfunction Score; PCT: procalcitonin; RISC: respiratory index of severity in children; sFlt1: soluble fms-like tyrosine kinase-1; sTNFR1: soluble tumour necrosis factor receptor 1; sTREM-1: soluble triggering receptor expressed on myeloid cells 1; WBC: white blood cells; WHO: World health Organization.Nonparametric ROC curves were generated. AUROC was plotted for each variable to illustrate its ability to discriminate between good and poor prognosis. For each variable, AUROC value with the 95% confidence interval in parenthesis are displayed to the right of its plot.

Table 2
Demographic characteristics of participants according to prognosis.

Characteristics	Good prognosis ($N = 87$)	Poor prognosis ($N = 31$)	p-value[a]
Infants (<12 months)	43 (49.4)	19 (61.3)	0.256
Gender, female	44 (50.6)	10 (32.3)	0.079
Immunization status			0.393
Fully	66/84 (78.6)	22 (71.0)	
Partially	18/84 (21.4)	9 (29.0)	
None	0/84 (0)	0 (0)	
Wasting (WAZ ≤ -2SD)[b]	6/87 (6.9)	5/30 (16.7)	0.147
Known case of HIV infection	0 (0)	0 (0)	NA
Exposure to tobacco smoke	12/84 (14.3)	4/30 (13.3)	1.000
Exposure to betel nut (doma)	51/84 (60.7)	23/30 (76.7)	0.116
Exposure to heater with kerosene	7/75 (9.3)	1/29 (3.5)	0.438
Parental education			0.317
Both parents are illiterate	12/84 (14.3)	9/30 (30.0)	
Only one parent has primary education	11/84 (13.1)	4/30 (13.3)	
Both parents have primary education	39/84 (46.4)	11/30 (36.7)	
At least one parent has university education	22/84 (26.2)	6/30 (20.0)	
Both parents unemployed			0.291
Both parents are unemployed	1/81 (1.2)	1/28 (3.6)	
Only one parent is employed	50/81 (61.7)	20/28 (71.4)	
Both parents are employed	30/81 (37.0)	7/28 (25.0)	
Time to access health care facility > 30 min	5/84 (6.0)	3/29 (10.3)	0.421

Abbreviations: NA: not applicable; SD: standard deviations; WAZ: weight-for-age Z-score.
[a] Comparison of proportions using the chi-square or fisher tests.
[b] Nutritional status was based on the WAZ score generated using the 2000 Centers for Disease Control and Prevention Growth Reference[48,49].

than the other immune and endothelial activation markers (AUROC 0.61–0.67) (Supplementary Table S3).

Supplementary Table S4 summarizes additional performance characteristics (sensitivity, specificity and likelihood ratios) of clinical scoring scales and biomarkers.

Top performing biomarkers improve the prognostic performance of clinical characteristics

We assessed the performance of combinations of the best performing clinical signs, scales, and biomarkers. The addition of sTREM-1 significantly improved the prognostic performance of lower chest wall indrawing or the RISC score, but these combinations did not perform better than sTREM-1 alone (Table 4). Taking into consideration that RISC is a clinical scoring scale that includes the assessment of chest indrawing, we concluded that sTREM-1 combined with assessment of lower chest wall indrawing was the most parsimonious prognostic model.

Prognosis performance of clinical characteristics, scoring scales, and biomarkers differ by age groups

We investigated the performance of biomarkers by age groups since inflammatory response varies by age.[32,33] We performed subgroup analyses amongst infants (< 12 months) and older children (≥ 12 months) (Table 5). PCT and IL-6 performed better at predicting poor outcome in children ≥ 12 months compared to infants. The performance of the clinical characteristics and scoring scales did not significantly differ between infants and older children.

The RISC score in infants (AUROC 0.74, 95%CI 0.62–0.87) performed significantly better than WBC, platelets, ESR, CRP-study, CRP-ref, PCT and IL-6 in predicting poor prognosis. In older children, the RISC score (AUROC 0.69, 95%CI 0.56–0.82) performance was similar to all biomarkers. Biomarker levels by age group are reported in Supplementary Table S2.

sTREM-1-based algorithms predict poor prognosis in children with pneumonia

As sTREM-1 demonstrated good prognostic accuracy for children with pneumonia, we examined this marker with top performing clinical characteristics to generate simple algorithms for risk-stratification in community and hospital settings. We performed classification and regression tree analyses to identify optimal cut-off points. We forced the clinical variable to be included in the model first for clinical relevance. Alone, sTREM-1 presented a sensitivity of 35.5% (95% CI 19.2–54.6) and specificity of 98.9% (95% CI 93.8–99.9), and the positive and negative likelihood ratios were 32.27 and 0.65, respectively (Fig. 3). The combination of very severe chest indrawing with sTREM-1 was found to be the best performing combination of sTREM-1 with any clinical characteristics, and increased sensitivity to 61.3% (95% CI 42.2–78.2) with a small decrease in specificity (95.4%; 95% CI 88.6–98.7).

Discussion

Prognostic tools that enable the early identification of children with pneumonia that will progress to severe and potentially fatal disease are currently lacking. Early risk-stratification of children with respiratory symptoms could facilitate triage, early referral, and prioritization of care, and improve outcomes. In the following study, we assessed potential prognostic factors in children hospitalized with WHO-defined pneumonia, including clinical characteristics and a wide range of host-response biomarkers.

We found that several clinical signs upon admission were associated with poor prognosis, including typical clinical indicators of pneumonia such as increased respiratory rate or grunting. However, and in agreement with previous studies, the prognostic performance of single clinical signs would not support clinical decision-making in the field on their own.[12,16,34,35] In addition, the detection of clinical signs depends on the health worker ability to correctly assess them, leading to applicability limitations due to interobserver variability and the need of trained health workers.[34,36]

To improve prognostic performance of single clinical signs, several scoring scales have been developed, combining clinical signs, risk factors, and simple laboratory testing. LODS was initially developed for the risk assessment of children with malaria but was then found to yield good discrimination to predict in-hospital mortality (AUROC 0.86) amongst febrile Ugandan children aged 2–59 months with no malaria.[7,25] In the Bhutanese cohort, LODS was

Table 3
Clinical characteristics of participants according to prognosis.

Characteristics	Good prognosis ($N = 87$)	Poor prognosis ($N = 31$)	p-value[a]
Clinical history for current illness			
Reported duration of illness prior to admission ≥ 5 days	40 (46.0)	16 (51.6)	0.589
Reported duration of fever prior to admission			0.675
No fever	14/86 (16.3)	6/30 (20.0)	
< 5 days	51/86 (59.3)	15/30 (50.0)	
≥ 5 days	21/86 (24.4)	9/30 (30.0)	
Referred from another healthcare centre	6 (6.9)	8 (25.8)	0.005
Started on antibiotics prior to admission	17/85 (20.0)	8 (25.8)	0.501
Clinical characteristics at admission			
Capillary refill > 3 s	0 (0)	4 (12.9)	0.004
Tachycardia for age[b]	25/86 (29.1)	8 (25.8)	0.729
Increased respiratory rate[c]	40 (46.0)	22/30 (73.3)	0.010
SpO2 (median, IQR)[d]	86 (80 to 90)	77 (70 to 84)	0.0002
Hypoxaemia (SpO2 < 90%)	64 (73.6)	30 (96.8)	0.004
Fever (≥37.5 °C, axillar)	38 (43.7)	11 (35.5)	0.427
High fever (> 39 °C, axillar)	2 (2.3)	3 (9.7)	0.113
Lower chest wall indrawing[e]	37/86 (43.0)	26 (83.9)	<0.0001
Very severe chest indrawing[e]	3/86 (3.5)	11 (35.5)	<0.0001
Nasal flaring	12/86 (14.0)	14 (45.2)	<0.0001
Grunting	2 (2.3)	5 (16.1)	0.013
Crackles	46/86 (53.5)	22 (71.0)	0.091
Ronchi	37/86 (43.0)	22 (71.0)	0.008
Wheezing	27/83 (32.5)	5 (16.1)	0.103
Decreased level of consciousness	0 (0)	4 (12.9)	0.004
Prostration	7 (8.1)	12 (38.7)	<0.0001
Seizure	0 (0)	0 (0)	NA
Clinical scoring scales at admission			
Severe WHO pneumonia	65 (74.7)	30 (96.8)	0.007
RISC score (median, IQR)	2 (1 to 3)	3 (3 to 4)	0.0003
RISC-Malawi score (median, IQR)	6 (3 to 8)	7 (7 to 8)	0.0012
LODS (median, IQR)	0 (0 to 0)	0 (0 to 1)	0.0001
Radiological findings			
Endpoint pneumonia	16/73 (21.9)	9/23 (39.1)	0.195
Other infiltrates	15/73 (20.6)	5/23 (21.7)	
Normal	42/73 (57.5)	9/23 (39.1)	
Laboratory findings at admission			
Anaemia (Haemoglobin < 11 g/dL)	23 (26.4)	19 (61.3)	0.001
Leucocytosis[f]	30 (34.5)	11 (35.5)	0.920
Thrombocytosis (> 450 × 10^9 platelets/L)	20 (23.0)	10/29 (34.5)	0.221
High ESR (≥ 50 mm)	12/78 (15.4)	6/30 (20.0)	0.564
High CRP-study (> 4 mg/dL)	11/83 (13.3)	7 (22.6)	0.224
High CRP-ref (> 4 mg/dL)	66 (75.9)	24 (77.4)	0.861
High PCT (≥ 250 pg/mL)	23 (26.4)	14 (45.2)	0.054
Non-contaminated positive bacterial blood culture	5/73 (6.9)	2/27 (7.4)	1.000
Hospital management			
Antibiotic therapy	57 (65.5)	27 (87.1)	0.023
Oxygen therapy	58 (66.7)	31 (100)	<0.001
Hospital stay ≥ 7 days	5 (5.8)	17 (54.8)	<0.001

Abbreviations: CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; IQR: interquartile range; LODS: Lambaréné Organ Dysfunction Score; NA: not applicable; PCT: procalcitonin; RISC: respiratory index of severity in children; WHO: World Health Organization.

[a] Comparison of proportions using the chi-square or fisher tests.

[b] Tachycardia was defined as heart rate > 160/minute for infants ⟨12 months, and ⟩ 150/minute for children ≥ 12 months of age[7].

[c] Increased respiratory rate was defined as >50breaths per minute in children aged 2 to 12 months and >40 breaths per minute in children aged ≥ 12 months.

[d] Peripheral capillary oxygen saturation was measured in room air using Mindray VS-800 Vital Sign Monitor or Biolight BLT M800 Handheld pulse oximeter. Eight children (all of them with poor prognosis including two children with fatal outcome) had SpO2 <90% and were put on oxygen before or at arrival, with missing exact SpO2 value.

[e] Lower chest wall indrawing was defined as subcostal and/or lower intercostal retractions, and very severe chest indrawing was defined as supraclavicular and/or suprasternal retractions.

[f] Leucocytosis was defined as white blood cells greater than 15 × 10^9 cells/L for children aged between 2 and 11 months and greater than 13 × 10^9 cells/L for children aged between 12 and 59 months.

associated with poor prognosis but showed a low sensitivity (38.7; 95% CI 21.8–57.8) and low prognostic performance (AUROC 0.66). Each of the three components of LODS (coma, prostration, and deep breathing) is indicative of severe disease and therefore may have limited utility in the early stages of severe disease.[9] The RISC score was developed specifically for children with respiratory infections and include clinical signs, several of which may have greater utility in early identification of severe pneumonia.[24] RISC demonstrated higher sensitivity (87.1%; 95% CI 70.2–96.4), which is an essential characteristic for a community-based triage tool. However, the RISC score is difficult to determine in low resource settings as it requires anthropometric measurement to assess weight-for-age, a pulse oximeter, the ability to recognize chest indrawing, and auscultation for wheezing. The WHO severity criteria are widely used and rely on their high sensitivity to detect most cases for antibiotic therapy and hospital management.[22] We observed similar findings in our cohort, where all the children progressing to poor prognosis except one were classified as severe pneumonia. In conclusion, we found that clinical scoring scales were significantly associated with poor prognosis and presented high sensitivity at established cut-off points, but specificity was low, leading to a high number of false-positive cases. In addition, they rely on clinical signs, which does not solve the problem of subjectivity and interobserver variability in their assessment.

Table 4
Performance of clinical parameters associated with top predicting biomarker sTREM-1.

	AUROC	
	Clinical parameter	+ sTREM-1
Oxygen saturation	0.75 (0.63 to 0.86)	0.81*
Lower chest wall indrawing	0.70 (0.62 to 0.79)	0.84**
RISC score	0.71 (0.61 to 0.80)	0.82**
sTREM1	0.74 (0.63 to 0.88)	–

Abbreviations: AUROC: area under the receiver operating characteristics; RISC: respiratory index of severity in children; sTREM-1: soluble triggering receptor expressed on myeloid cells 1.
Differences in AUROCs were assessed using the algorithm suggested by DeLong et al. (1988)[29].
* $p < 0.10$ for comparison of AUROC of the clinical parameter alone versus AUROC of the combination of the clinical parameter with sTREM-1.
** $p < 0.05$ for comparison of AUROC of the clinical parameter alone versus AUROC of the combination of the clinical parameter with sTREM-1.

Biomarker concentrations can be measured in the blood, with the benefits of objectivity, accuracy, and reproducibility. Currently, their measurement require training in blood collection and specialized equipment, although the development of rapid diagnostic testing with the best performing biomarkers to conduct with blood drops collected by finger prick could easily overcome these difficulties. WBC, platelets, ESR, and CRP are commonly used in clinical practice as aetiological and prognostic markers. However, studies have consistently shown that these biomarkers are poor prognostic predictors for childhood pneumonia.[24,34,37] In previous studies, levels of immune and endothelial activation markers were associated with disease severity and fatal outcome in life-threatening infections, including pneumonia, sepsis, severe malaria, haemorrhagic fevers, or COVID-19.[11,13,39,14–20,38] In the Bhutanese cohort, IL-8, sTREM-1, sTNFR1, Angpt-2, and sFlt1 were all significantly associated with poor prognosis despite the moderately small size of the cohort and few children with fatal outcome.

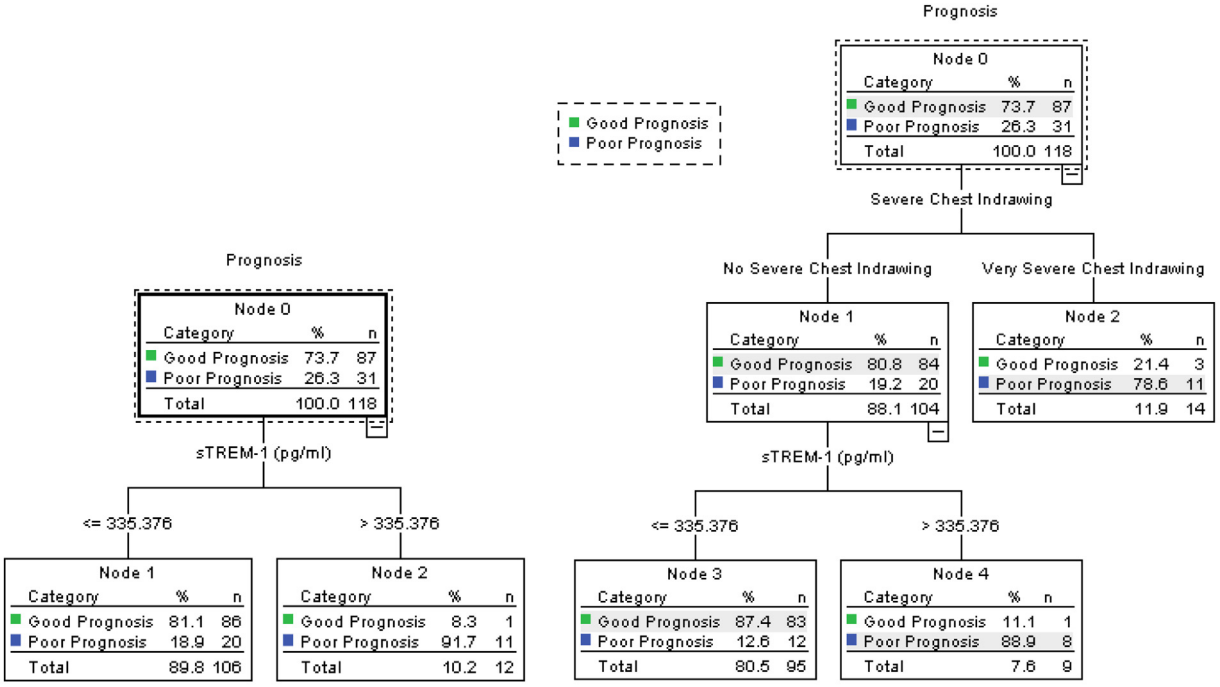
Amongst the immune and endothelial markers analysed in this study for children with pneumonia, sTREM-1 exhibited the highest AUROC (0.74, 95% CI 0.63–0.88). The addition of sTREM-1 significantly improved the prognostic performance of the best performing clinical characteristics such as lower chest wall indrawing. These findings suggest that simple sTREM-1-based algorithms for pneumonia management may represent a strategy to improve care and outcome in children, particularly in resource-limited settings.[40,41] Selection of which biomarker-based model to apply clinically will depend on the primary goal of the triage tool. For example, highly sensitive algorithms with associated low negative likelihood ratio would perform well to correctly classify children at low risk of evolving to poor prognosis. These children could be sent home confidently, while those classified at high risk might require close monitoring to ensure early detection of deterioration of the child. On the other hand, algorithms aiming for higher specificity with associated higher positive likelihood ratio, such as the one based on sTREM-1, perform better at correctly classifying children at risk of poor prognosis and as such, could assist care prioritization decisions. This approach is also useful in the context of the COVID-19 pandemic in any setting, to help improve rationale allocation of resources and decision on patient triage in overburdened hospitals.

This study has several limitations. As there were only three deaths in the cohort, we used a composite primary outcome,

Table 5
Performance of clinical characteristics and biomarkers for identifying children at risk of poor prognosis.

	AUROC (95% CI)		
	All	<12 months	≥12 months
Clinical characteristics			
Increased respiratory rate	0.64 (0.54 to 0.73)	0.63 (0.49 to 0.77)	0.67 (0.56 to 0.78)
Oxygen saturation	0.75 (0.63 to 0.86)	0.79 (0.65 to 0.93)	0.70 (0.50 to 0.89)
Lower chest wall indrawing	0.70 (0.62 to 0.79)	0.74 (0.64 to 0.84)	0.65 (0.51 to 0.80)
Very severe chest indrawing	0.66 (0.57 to 0.75)	0.65 (0.54 to 0.76)	0.69 (0.54 to 0.83)
Clinical scoring scales			
WHO severity score	0.61 (0.55 to 0.67)**	0.66 (0.57 to 0.75)	0.57 (0.52 to 0.62)*
RISC score	0.71 (0.61 to 0.80)	0.74 (0.62 to 0.87)	0.69 (0.56 to 0.82)
RISC-Malawi score	0.69 (0.60 to 0.79)	0.76 (0.65 to 0.88)	0.65 (0.48 to 0.81)
LODS	0.66 (0.56 to 0.75)	0.67 (0.55 to 0.79)	0.63 (0.49 to 0.78)
Acute phase proteins and inflammatory markers			
WBC	0.52 (0.40 to 0.65)**	0.57 (0.40 to 0.74)**	0.62 (0.43 to 0.81)
Platelets	0.58 (0.46 to 0.71)#	0.53 (0.36 to 0.70)**	0.70 (0.50 to 0.89)
ESR	0.54 (0.40 to 0.68)**	0.60 (0.43 to 0.78)**	0.76 (0.59 to 0.93)
CRP-study	0.60 (0.48 to 0.72)	0.57 (0.41 to 0.74)	0.65 (0.49 to 0.82)
CRP-ref	0.59 (0.47 to 0.71)#	0.51 (0.35 to 0.68)**	0.73 (0.56 to 0.89)
PCT	0.61 (0.49 to 0.72)##	0.52 (0.37 to 0.67)**	0.72 (0.55 to 0.90)
Immune activation factors			
IL-6	0.61 (0.48 to 0.74)##	0.50 (0.32 to 0.68)**	0.76 (0.61 to 0.91)
IL-8	0.62 (0.50 to 0.74)	0.62 (0.46 to 0.78)	0.59 (0.39 to 0.79)
sTREM-1	0.74 (0.63 to 0.88)	0.69 (0.52 to 0.85)	0.77 (0.61 to 0.93)
sTNFR1	0.67 (0.55 to 0.79)	0.58 (0.40 to 0.75)	0.77 (0.60 to 0.93)
Endothelial activation factors			
Angpt-2	0.61 (0.48 to 0.74)	0.66 (0.50 to 0.83)	0.54 (0.33 to 0.75)
sFlt1	0.60 (0.48 to 0.73)	0.62 (0.45 to 0.79)	0.55 (0.35 to 0.75)

Abbreviations: Angpt-2: angiopoietin-2; AUROC: area under the receiver operating characteristics; CI: confidence interval; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; IL-6: interleukin-6; IL-8: interleukin-8; LODS: Lambaréné Organ Dysfunction Score; NA: not applicable; PCT: procalcitonin; RISC: respiratory index of severity in children; sFlt1: soluble fms-like tyrosine kinase-1; sTNFR1: soluble tumour necrosis factor receptor 1; sTREM-1: soluble triggering receptor expressed on myeloid cells 1; WBC: white blood cells; WHO: World Health Organization.
Differences in AUROCs were assessed using the algorithm suggested by DeLong et al. (1988)[29].
* $p < 0.10$ for comparison of AUROCs of the RISC score versus each of the other scoring scales and biomarkers.
** $p < 0.05$ for comparison of AUROCs of the RISC score versus each of the other scoring scales and biomarkers.
# $p < 0.10$ for comparison of AUROCs between age groups for each clinical characteristic, clinical scoring scale or biomarker.
$p < 0.05$ for comparison of AUROCs between age groups for each clinical characteristic, clinical scoring scale or biomarker.



	Sensitivity	Specificity	Positive LR	Negative LR
sTREM-1	35.5 (19.2–54.6)	98.9 (93.8–99.9)	32.27	0.65
Very severe chest indrawing + sTREM-1	61.3 (42.2–78.2)	95.4 (88.6–98.7)	13.33	0.41

Fig. 3. Classification and regression tree analysis algorithms to predict poor outcome in children with pneumonia.Abbreviations: LR: likelihood ratio; sTREM-1: soluble triggering receptor expressed on myeloid cells 1.The algorithms were generated for sTREM-1 (left) and very severe chest indrawing and sTREM-1 (right). Good prognosis was defined as survival, no admission in the paediatric intensive care unit, no requirement of oxygen or oxygen therapy for ≤ 5 days, and no requirement of chest drainage; while poor prognosis was defined as death and/or admission in the paediatric intensive care unit and/or oxygen therapy for > 5 days and/or required chest drainage. For all models, the cost of misclassifying a child with poor prognosis was designated as 10 times the cost of misclassifying a child with good prognosis. Classification and regression tree analysis selected the optimal cut-off points. We forced the clinical variable to be included in the model first for clinical relevance. The performance of each of the three algorithms are presented in the table below them.

which limits direct comparison with other studies using mortality as the primary outcome. We did not include other factors known to impact circulating biomarker concentrations such as duration of illness, prior administration of antibiotics, malnutrition, and other comorbidities, which are important considerations in biomarker discovery and validation studies.[42–44] Since pneumonia progresses rapidly, increases or decreases between two measurements of the same biomarker over time (dynamic monitoring) might further help in the risk-stratification of children with this disease.[45] Also, since excess mortality can be observed up to three months after discharge from severe infections, we encourage assessing outcomes including post-discharge mortality to avoid missing late events.[46,47] We did not adjust the statistical analyses for multiple comparisons, due to the exploratory nature of the analysis. Thus, the differences with statistical significance need to be evaluated as such. The size of the clinical cohort and number of outcomes is small, and therefore a potential risk of over fitting models containing more than one predictive variable exists. Further research from additional cohorts is needed to elucidate the best biomarker or combination of biomarkers and which cut-off points to use for risk-stratification of children with pneumonia.

Nonetheless, our study confirms that immune and endothelial activation markers have the potential to become objective risk-stratification tools of children with pneumonia. A biomarker point-of-care tool alone or integrated into a simple clinical algorithm is likely to enhance clinical decision-making (such as triage and prioritization of care) and improve outcomes, in addition to optimizing resource allocation, especially in low- and middle-income countries, where mortality associated with childhood pneumonia remains greatest.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author contributions

SJ, QB, MRG, and KCK conceptualized the work. SJ, TL, and KD collected the data. MN and RS performed the laboratory investigations. SJ run the analyses and drafted the main text. QB, MRG, KCK, and MN substantively revised the manuscript. MRG and MN prepared Figs. 1-3 and S2. All authors reviewed the manuscript.

Declaration of Competing Interest

The authors declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.10.010.

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