TITLE

Risk stratification of childhood infection using host markers of immune and endothelial activation: a multi-country prospective cohort study in Asia (Spot Sepsis)

AUTHORS

Arjun Chandna,[1,2] Constantinos Koshiaris,[3,4] Raman Mahajan,[5] Riris Adono Ahmad,[6] Dinh Thi Van Anh,[7] Suy Keang,[1,8] Phung Nguyen The Nguyen,[9] Sayaphet Rattanavong,[10] Khalid Shams,[5] Souphaphone Vannachone,[10] Spot Sepsis Investigator Group,[†] Mikhael Yosia,[5] Naomi Waithira,[2,11] Mohammad Yazid Abdad,[2,11] Janjira Thaipadungpanit,[11,12] Paul Turner,[1,2] Phan Huu Phuc,[7] Dinesh Mondal,[13] Mayfong Mayxay,[2,10,14] Bui Thanh Liem,[9] Elizabeth A Ashley,[2,10] Eggi Arguni,[6] Rafael Perera-Salazar,[3] Melissa Richard-Greenblatt,[2,15,16] Yoel Lubell,[2,11,17*] and Sakib Burza[5,18,19*]

[*]Contributed equally

[†]Members of the Spot Sepsis Investigator Group are listed in the appendix

AFFILIATIONS

1. Cambodia Oxford Medical Research Unit, Angkor Hospital for Children, Cambodia
2. Centre for Tropical Medicine and Global Health, University of Oxford, UK
3. Department of Primary Care Health Sciences, University of Oxford, UK
4. Department of Primary Care and Population Health, University of Nicosia Medical School, Cyprus
5. Médecins Sans Frontières Operational Centre Barcelona, Spain
6. Centre for Tropical Medicine, Universitas Gadjah Mada, Indonesia
7. Viet Nam National Children's Hospital, Viet Nam
8. Angkor Hospital for Children, Cambodia
9. University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam

10. Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit, Mahosot Hospital, Laos
11. Mahidol Oxford Tropical Medicine Research Unit, Mahidol University, Thailand
12. Faculty of Tropical Medicine, Mahidol University, Thailand
13. International Centre for Diarrhoeal Disease Research, Bangladesh
14. Institute for Research and Education Development, University of Health Sciences, Laos
15. Department of Laboratory Medicine and Pathobiology, University of Toronto, Canada
16. Hospital for Sick Children, Canada
17. Amsterdam Institute of Global Health and Development, The Netherlands
18. Clinical Research Department, London School of Hygiene and Tropical Medicine, UK
19. Health in Harmony, United States of America

ABSTRACT

Background

Circulating markers of immune and endothelial activation risk stratify infection syndromes agnostic to disease aetiology. However, their utility in children presenting from the community remains unclear.

Methods

This study recruited children aged 1-59 months presenting with community-acquired acute febrile illnesses to seven hospitals in Bangladesh, Cambodia, Indonesia, Laos, and Viet Nam. Clinical parameters and biomarker concentrations were measured at presentation. The outcome measure was death or receipt of vital organ support within two days of enrolment. Prognostic performance of endothelial (Ang-1, Ang-2, sFlt-1) and immune (CHI3L1, CRP, IP-10, IL-1ra, IL-6, IL-8, IL-10, PCT, sTNFR-1, sTREM-1, suPAR) activation markers, WHO Danger Signs, and two validated severity scores (LqSOFA, SIRS) was compared.

Results

3,423 participants were recruited. 133 met the outcome (weighted prevalence: 0.34%; 95% CI 0.28-0.41). sTREM-1 exhibited highest prognostic accuracy (AUC 0.86; 95% CI 0.82-0.90), outperforming WHO Danger Signs (AUC 0.75; 95% CI 0.70-0.80; $p < 0.001$), LqSOFA (AUC 0.74; 95% CI 0.70-0.78; $p < 0.001$), and SIRS (AUC 0.63; 95% CI 0.58-0.68; $p < 0.001$). Discrimination of immune and endothelial activation markers was particularly strong for children who deteriorated later in the course of their illness. Compared to WHO Danger Signs, an sTREM-1-based triage strategy improved recognition of children at risk of progression to life-threatening infection (sensitivity: 0.80 vs. 0.72), while maintaining comparable specificity (0.81 vs. 0.79).

Conclusions

Measuring circulating markers of immune and endothelial activation may help earlier recognition of febrile children at risk of poor outcomes in resource-constrained community settings.

INTRODUCTION

Whether to refer a febrile child to hospital is a challenging decision facing frontline community healthcare workers globally, particularly in resource limited and conflict affected settings.[1,2] Each day, children who will develop severe disease are missed while referrals of illnesses suitable for community-based management incur avoidable cost for caregivers and health systems.[3,4] In rural locations of many low- and middle-income countries, referral decisions are complex; influenced by poorly functioning health systems, limited referral infrastructure, and geographic, climatic, socioeconomic, and cultural factors.[5-7]

In under-resourced peripheral healthcare settings, the World Health Organization (WHO) recommends certain *Danger Signs* (convulsions, intractable vomiting, lethargy, or prostration), to identify febrile children requiring hospital referral.[8,9] Yet, these suffer from considerable inter-observer variability and lack both sensitivity and specificity.[10,11] Absence of data on children managed in the community setting renders their validity questionable.[12] Better risk stratification tools for common childhood infections are needed.

Circulating markers of immune and endothelial activation have consistently demonstrated ability to risk stratify paediatric fever syndromes agnostic to disease aetiology.[13-15] Elevated concentrations of these markers indicate loss of endothelial integrity and microvascular quiescence that contribute to disease progression, organ dysfunction, and death.[16-18] They may be of particular value for identifying patients whose illness severity is not clinically apparent at presentation and who may be discharged and deteriorate at home.[19] Whether these findings apply at the community setting in Asia is unknown: most research has included only hospitalised children, comprised single-site studies, and been conducted in locations where prevalent causes of infection and host susceptibility patterns differ.[13,20-23]

We report the first multi-country study of markers of immune and endothelial activation in children presenting from the community setting with acute febrile illnesses. Our objective was to determine whether presenting concentrations of these markers predict disease progression, thereby assessing their potential to identify children at risk of severe disease, relative to currently used clinical tools.

METHODS

Study design

Spot Sepsis was a multi-country, prospective, cohort study, which enrolled children (aged > 28 days and < 60 months) presenting with acute febrile illnesses to seven hospitals across Bangladesh, Cambodia, Indonesia, Lao PDR (Laos), and Viet Nam. Sites predominantly serving rural populations and providing a first point of contact with the formal healthcare sector were prioritised (appendix p2-3).

Patients presenting with a febrile illness (axillary temperature ≥ 37.5°C or < 35.5°C and/or history of fever in the preceding 24 hours) of ≤ 14 days duration were eligible for inclusion. Exclusion criteria were prior admission to any health facility during the current illness episode, receipt of > 15 minutes parenteral treatment before screening, presentation within 3 days of routine immunisations, trauma as the reason for attendance, and/or specific known comorbidities (chronic infection, immunosuppression, and/or active cardiorespiratory conditions). Participants could only be enrolled once.

Patients were screened at presentation to the outpatient and emergency departments during daytime working hours. Given high numbers of outpatients, consecutive enrolment of outpatients was not feasible and recruitment was stratified by admission status. Inpatients were enrolled consecutively. Outpatient recruitment was randomised using computer-generated random number tables, with the preceding week's routinely collected hospital attendance data providing the sampling frame (Table S1; appendix p4-5)

Caregivers of all participants provided informed written consent. The study was prospectively registered on ClinicalTrials.gov (NCT04285021) and received ethical approval from the sponsors and ethical review boards in all participating countries (Table S2; appendix p6). MSF maintained a sponsor-investigator role for the study. The Wellcome Trust had no role in study design, data collection, data analysis, data interpretation, writing of the report, or decision to submit for publication.

Data collection

Trained study personnel measured vital signs and anthropometrics, assessed clinical signs (including WHO Danger Signs), and collected venous blood samples and nasopharyngeal swabs at enrolment (Table S3; appendix p7). Demographics and perinatal, past medical, and illness histories were collected via interview with the participant's caregiver and entered onto electronic case record forms using Android tablets via Open Data Kit Collect software. Variable selection was informed by systematic review of the literature.[24] Prioritisation and standardisation followed guidance set out by the Pediatric Sepsis Predictors Standardization working group.[25]

Participants were followed-up on days 2 and 28 after enrolment, with additional follow-up on day 1 and at discharge for inpatients. Participants were provided with routine care by their treating clinician. When feasible, the study supported collection and processing of peripheral blood cultures at the discretion of the clinical team. Study monitoring was conducted by the Clinical Trials Support Group at the Mahidol-Oxford Tropical Medicine Research Unit (MORU) in Bangkok, Thailand.

Selection of biomarkers and comparators

Biomarkers were selected following review of the literature and expert consultation (Table S4; appendix p8). Biomarkers useful for risk stratification in primary care, where the aetiology of infection is typically unknown at the time of assessment, must be predictive across a spectrum of pathogens. Hence, biomarkers with mechanistic links to final common pathways of severe febrile illness and sepsis were prioritised.[16,26-28] Markers of endothelial activation included: angiopoietin-1 (Ang-1); angiopoietin-2 (Ang-2); and soluble fms-like tyrosine kinase-1 (sFlt-1; sVEGFR-1). Markers of immune activation included: chitinase-3-like protein-1 (CHI3L1); C-reactive protein (CRP); interferon-gamma-inducible protein-10 (IP-10; CXCL-10); interleukin-1 receptor antagonist (IL-1ra); interleukin-6 (IL-6); interleukin-8 (IL-8); interleukin-10 (IL-10); procalcitonin (PCT); soluble tumour necrosis factor receptor-1 (sTNFR-

1); soluble triggering receptor expressed on myeloid cells-1 (sTREM-1); and soluble urokinase plasminogen activator receptor (suPAR).

Lactate, glucose, and haemoglobin were included as they are measurable using inexpensive rapid tests, familiar to many clinicians, have prognostic value,[24] and promoted in paediatric sepsis guidelines.[29-31]

In addition to WHO Danger Signs, the Liverpool quick Sequential Organ Failure Assessment (LqSOFA) and Systemic Inflammatory Response Syndrome (SIRS) scores were selected as comparators (Table S5; appendix p9).[32,33] LqSOFA is the most extensively studied age-adapted version of the widely-endorsed qSOFA sepsis screening tool for adults.[34] It was developed specifically for triaging febrile children presenting from the community setting and outperformed other paediatric severity scores during external validation in Asia.[35] Although the Phoenix Sepsis Score has superseded SIRS as the international consensus definition for paediatric sepsis, it does not yet offer a screening tool practicable in resource-limited frontline healthcare settings.[31] Thus, SIRS was included as a widely-recognised paediatric sepsis screening tool.

Laboratory procedures

Venous blood samples and nasopharyngeal swabs were processed immediately. Complete blood counts were performed on site. Peripheral blood cultures were processed at accredited in-country laboratories. Aliquots of whole blood, EDTA-plasma, fluoride-oxalate-plasma, and universal transport medium (UTM) were stored at -20°C or below. Samples were then transported at -80°C to the MORU laboratories in Bangkok, Thailand for further analysis and biobanking.

Biomarker concentrations were quantified in EDTA-plasma using the Simple Plex Ella microfluidic platform (ProteinSimple, San Jose, CA, USA) and suPARnostic ELISA (ViroGates, Denmark), as described in the appendix (Table S6; p10). Lactate (LACT2, Roche Diagnostics,

Germany) and glucose (GLUC3, Roche Diagnostics, Germany) concentrations were quantified in fluoride-oxalate-plasma.

Nucleic acid was extracted from whole blood using the MagNA Pure 24 instrument and Total NA Isolation Kit (Roche Diagnostics, Indianapolis, IN, USA) according to manufacturer instructions. Whole blood viral (chikungunya, dengue, Japanese encephalitis, and Zika) and bacterial (*Leptospira* spp., *Orientia tsutsugamushi*, and *Rickettsia* spp.) targets were detected using laboratory developed real-time polymerase chain reaction (RT-PCR) multiplex assays. Respiratory pathogen targets were detected directly from nasopharyngeal swabs using the FilmArray RP2 panel (BioFire Diagnostics, Salt Lake City, UT, USA), with the exception of Cambodian samples, according to manufacturer protocols. Cambodian respiratory samples were processed for influenza A/B and respiratory syncytial virus (RSV) using the FTD FLU/HRSV assay (Siemens, Germany). All sites used an in-house developed multiplex RT-PCR assay for the detection of SARS-CoV-2 from nasopharyngeal swabs based on the E and N genes as described previously.[36] Molecular targets were restricted to pathogens for which illness causality can be more confidently ascribed.[37]

Outcomes

The outcome measure was development of severe febrile illness within two days of enrolment, defined as death and/or receipt of vital organ support (mechanical and/or non-invasive ventilation and/or inotropic therapy and/or renal replacement therapy).

Prespecified subgroup analyses included children with microbiologically-confirmed infections and different presenting clinical syndromes. Prognostic accuracy of the biomarkers and clinical assessment tools was explored across prediction horizons (< 4 hours, ≥ 4 hours, ≥ 24 hours, and ≥ 48 hours). These secondary analyses were planned to test the hypotheses that immune and endothelial activation markers would predict disease progression across different microbial aetiologies (i.e., were 'pathogen agnostic'), and that value of biomarker measurements would be greatest in children whose illnesses progressed later after the point of presentation.[19,26,28]

Sample size

Spot Sepsis had two main objectives prespecified in the study protocol: to examine the prognostic performance of individual host biomarkers and to develop a clinical prediction model.[38] The methods of Riley et al. were followed to estimate the sample size required to build the clinical prediction model, reported separately, recognising that this would be adequate to evaluate the prognostic performance of individual host biomarkers.[39]

Statistical analyses

Complete case analyses were used as missing data among the primary comparators (immune and endothelial activation markers and WHO Danger Signs) were few. Categorical and continuous variables were summarised using descriptive statistics and compared with the Wilcoxon rank sum test, Pearson's X^2 test, or Fisher's exact test as appropriate. Site-specific outpatient weights were determined by estimating the proportion of all eligible outpatients recruited (Table S1; appendix p4-5). The prognostic accuracy of each biomarker, WHO Danger Signs, and clinical severity scores was quantified using the weighted area under the receiver operating characteristic curve (AUC). Probability weights were applied to adjust for unequal probabilities of selection in the sample, arising due to stratified recruitment. When evaluating combinations of characteristics, such as WHO Danger Signs and sTREM-1, a weighted logistic regression model was used to generate predicted probabilities, which were subsequently used to estimate the AUC.[40]

RESULTS

Study cohort

Between 5 March 2020 and 4 November 2022, 11,947 children were screened, of whom 3,995 were eligible (3,995/11,947; 33.4%) and 3,423 were recruited (572/3,995; 14.3% refusal rate). Eighteen participants were lost to follow-up (18/3,423; 0.5%) and excluded from further analyses (Figure S1; appendix p11).

Median age was 16.8 months (interquartile range [IQR] 8.7-31.0) and 60.0% of the cohort were male (2,029/3,405). Few participants had a known comorbidity (102/3,405; 3.0%). Approximately one in five children were wasted (weight-for-height z-score [WHZ] < -2; 585/3,393; 17.2%) and/or stunted (height-for-age z-score [HAZ] < -2; 664/3,401; 19.5%), and half of these were severely malnourished (WHZ and/or HAZ < -3). Median duration of illness prior to presentation was 3 days (IQR 2-4). The majority of children (2,333/3,405; 68.5%) lived within an hour of the hospital. 1,342 participants (1,342/3,405; 39.4%) had received care in the community at an earlier point in their illness: none had been admitted and 193 (193/3,405; 5.7%) had received parenteral treatment (Table S7; appendix p12). Table 1 shows presenting clinical data for the cohort. Additional information is provided in the appendix (Tables S8 and S9; p13-18).

Outcomes

133 children met the outcome (133/3,405; 3.9%): 22 deaths and 111 survivors who required vital organ support (Bangladesh, n = 39; Cambodia, n = 36; Viet Nam 1, n = 32; Viet Nam 2, n = 26). The weighted outcome prevalence was 0.34% (95% confidence interval [CI] = 0.28-0.41; appendix p4-5). Young age, age-adjusted tachycardia, abnormal mental status, and bedside signs of poor peripheral perfusion and respiratory compromise were more common in participants who progressed to severe disease (Table 1; Table S8; appendix p13-14). Presence of a WHO Danger Sign at enrolment was associated with meeting the outcome, as

were higher LqSOFA and SIRS scores (Table 1). Presenting plasma concentrations of the biomarkers stratified by outcome status are shown (Figure 1; Table S10; appendix p19).

Risk stratification

The predictive performance of each circulating marker is presented in Figure 2a, alongside the performance of WHO Danger Signs and the clinical severity scores. sTREM-1 showed best prognostic accuracy (AUC 0.86; 95% CI 0.82-0.90), demonstrating superior ability to discriminate children who would progress to severe disease, compared to other circulating markers, WHO Danger Signs (AUC 0.75; 95% CI 0.70-0.80; $p < 0.001$), and the clinical severity scores (LqSOFA: AUC 0.74; 95% CI 0.70-0.78; $p < 0.001$; SIRS: AUC 0.63; 95% CI 0.58-0.68; $p < 0.001$). Combining WHO Danger Signs with sTREM-1 (AUC 0.88; 95% CI 0.85-0.91) did not improve performance over sTREM-1 alone ($p = 0.24$; Figure 2b)

Sensitivity and specificity of WHO Danger Signs for recognising children who would progress to severe disease was 0.72 (95% CI 0.66-0.79) and 0.79 (95% CI 0.76-0.82), respectively. sTREM-1 concentrations selected to provide equivalent sensitivity or specificity, improved classification (Table 2). Using the Youden index to identify a sTREM-1 threshold for triage resulted in a sensitivity of 0.80 (95% CI 0.73-0.85) and specificity of 0.81 (95% CI 0.78-0.83). At the current outcome prevalence (0.34%), compared to using WHO Danger Signs, sTREM-1-based triage would identify one additional child who would progress to life-threatening infection for every ~3,000 children tested, without compromising specificity (increasing false positives).

Prognostication in microbiologically-confirmed infections

A microbiological cause for infection was confirmed in 898 children (898/3,405; 26.4%): 429 RSV, 164 arboviral infections (109 dengue, 47 chikungunya, 8 Zika); 146 influenza (87 influenza A, 59 influenza B); 81 SARS-CoV-2; 59 human metapneumovirus; 19 bacteraemias (Table S11; appendix p20); 9 rickettsial infections (6 *Rickettsia* spp., 3 *Orientia*

tsutsugamushi); 9 pertussis (8 *Bordetella parapertussis*, 1 *Bordetella pertussis*); 4 leptospirosis; 3 *Chlamydia pneumoniae;* and 3 *Mycoplasma pneumoniae*. Thirty four children had co-infections with two pathogens. Full details are provided in the appendix (Table S11; p20). Amongst participants with microbiologically-confirmed infections, prognostic accuracy of the circulating markers, WHO Danger Signs, and LqSOFA was largely unchanged (Table S12; appendix p21), with sTREM-1 providing best discrimination (AUC 0.88; 95% CI 0.83-0.94). Few participants had confirmed bacterial infections (n = 47), precluding comparison of prognostic performance between children with viral and bacterial infections.

Syndrome-specific prognostication

In children whose presentations met WHO-pneumonia criteria (cough and/or difficult breathing with age-adjusted tachypnoea and/or chest indrawing),[8] prognostic accuracy of sTREM-1 (AUC 0.84; 95% CI 0.78-0.94; Table S12; appendix p21) was matched by two markers of endothelial activation, Ang-2 (AUC 0.85; 95% CI 0.79-0.91) and sFlt-1 (AUC 0.84; 95% CI 0.77-0.90). Discrimination of the clinical assessment tools was poorer: WHO Danger Signs (AUC 0.58; 95% CI 0.50-0.65; $p < 0.001$); LqSOFA (AUC 0.72; 95% CI 0.66-0.78; $p < 0.001$); and SIRS (AUC 0.62; 95% CI 0.53-0.70; $p < 0.001$). The remaining outcome events were dispersed across clinical syndromes, precluding additional syndrome-specific analyses. Aggregate results for all non-respiratory presentations are included in the appendix (Table S12; p21).

Prognostication across prediction horizons

Extending the prediction horizon to include all cases of severe febrile illness occurring during follow-up (up to day 28), identified an additional 10 children (2 deaths and 8 survivors who required vital organ support). Data for time-stratified analyses were available for 139/143 (97.2%) children: 56 met the outcome within 4 hours, 83 after more than 4 hours, 42 after more than 24 hours, and 21 after more than 48 hours from enrolment. For most circulating

355 markers there was a trend to improved discrimination at distal prediction horizons (Figure
356 3). WHO Danger Signs performed consistently across prediction horizons. Performance of
357 the clinical severity scores was better for children whose illnesses progressed soon after
358 enrolment. sTREM-1 remained superior to other markers and clinical assessment tools
359 across all horizons, demonstrating an AUC of 0.94 (95% CI 0.89-0.98) for discrimination of
360 children who progressed to severe disease more than 48 hours after presentation.
361
362
363 *Sensitivity analyses*
364
365 sTREM-1 maintained prognostic accuracy and outperformed the clinical assessment tools
366 across sites (AUCs 0.84-0.89; Table S13; appendix p22-23). Sensitivity analyses excluding the
367 northern Viet Nam site (n = 612), which departed from the ideal rural target site profile and
368 where outpatient weighting was derived using different methodology (Tables S1 and S13;
369 appendix p4-5 and p22-23), did not affect the results. Finally, in a sensitivity analysis
370 restricted to children who had not received parenteral treatment at the study site prior to
371 baseline data or sample collection (3,037/3,045; 89.2%), sTREM-1 remained the best
372 prognostic indicator (AUC 0.82; 95% CI 0.76-0.88; Tables S14a and S14b; appendix p24-25).

DISCUSSION

In this large and geographically diverse Asian study investigating circulating markers of immune and endothelial activation for the risk stratification of unselected febrile children presenting from community settings, we found sTREM-1 to be consistently superior to WHO Danger Signs (the current standard of care) and LqSOFA (a validated clinical severity score) across prediction horizons, sites, presenting clinical syndromes, and in participants with microbiologically-confirmed infections.

Previous studies highlight the promising prognostic performance of sTREM-1. [13,20,21,23,41,42] However, these focussed exclusively on hospitalised children. Our results provide the first definitive evaluation at the community level, where need for better triage tools is most urgent.[1,43] We identified sites serving as the first point of presentation for rural populations, enrolled unselected febrile children including outpatients, recruited participants immediately upon presentation, excluded children admitted elsewhere prior to screening, and adopted an analysis strategy ensuring an outcome prevalence reflective of community care settings.[44,45]

Our findings support the evidence that certain circulating markers of endothelial and immune activation are pathogen agnostic, reflecting final common pathways to severe infection.[15,26,28] Thus, they are attractive candidates for risk stratification in primary care, where the cause of infection is typically unknown at the time of triage and recognising which child's illness is likely to progress remains a major clinical challenge. Endothelial dysfunction has been demonstrated in ambulatory children with infection.[46] However, until now it was unclear whether concentrations of these markers would be elevated sufficiently early in the natural history of infection to permit their use for risk stratification at the community level. The results of this study, in conjunction with two previously published smaller community-based studies, suggest that this approach warrants further attention.[19,27]

The results of our study are consistent with a study of 507 febrile adults conducted in Tanzanian outpatient clinics, which reported an AUC for sTREM-1 of 0.87 (95% CI 0.81-0.92) for predicting death within 28 days.[27] In a study of children with pneumonia presenting to a

primary care clinic on the Thailand-Myanmar border, Ang-2 demonstrated best prognostic performance (AUC 0.81; 95% CI 0.74-0.87) to predict supplemental oxygen requirement, whereas sTREM-1 did not show discriminatory value (AUC 0.56; 95% CI 0.49-0.63).[19] In part, this may relate to the focus on pneumonia: Ang-2 was the top-performing marker amongst children with pneumonia in our study, although performance of sTREM-1 also remained comparable. Alternatively, the contrasting findings may be explained by the more proximal endpoint (supplemental oxygen requirement vs. vital organ support and death) or pre-analytical differences in sample matrix or storage conditions, which are known to influence biomarker concentrations.[47]

This is the first multi-country study investigating circulating markers of immune and endothelial activation in childhood infection. Other key strengths include: a study design which maximised relevance for community settings as detailed above; the inclusion of a prespecified panel of biomarkers compiled based on existing literature and underpinned by mechanistic links to sepsis pathophysiology, which lends face validity to the findings; simultaneous quantification of multiple markers in a central laboratory to ensure comparability of findings; and recruitment across seven sites in five countries for over 30 months, which improves geographic and seasonal generalisability.

There are several limitations. Despite steps taken to optimise external validity to community settings, inherent differences between patients presenting to rural hospital outpatient departments and primary care facilities will remain. Amongst children progressing to severe febrile illness, median time to developing severe disease was 6 hours (IQR 2-30), indicating a level of severity at presentation that may not be replicated in some community care settings. Nevertheless, time-stratified analyses demonstrate that discrimination of most circulating markers was strongest in children who progressed to severe disease later; a finding not observed for the comparator clinical assessment tools. This suggests that biomarkers may be of greatest value in patients whose illness severity is not clinically apparent at presentation and who are at risk of being sent home and deteriorating. This is consistent with previous work in childhood pneumonia and has implications for operationalising biomarker tests.[19] Our analyses were structured such that every child would receive a biomarker test. Whilst this may be appropriate in certain settings,[1,48] in others a point-of-care test would likely be

used selectively on children for whom decisions to refer are borderline. Future work must explore different strategies for integrating biomarker testing into patient triage and compare the cost-effectiveness of different approaches.

Our outcome measure was selected as it is unlikely to be influenced by factors other than disease severity. Small amounts of outcome misclassification can substantially impact estimates of predictor performance.[49] Nevertheless, predictors of severe disease may not generalise to more proximal outcomes. Studies in Covid-19 and childhood pneumonia indicate that sTREM-1 concentrations do not predict supplemental oxygen requirement as accurately as they do mortality.[19-21,50,51] This underscores the importance of including a range of outcomes when evaluating the performance of predictors in primary care.

The impact of the Covid-19 pandemic must be considered. The majority of children (2,861/3,405; 84.0%) were tested for SARS-CoV-2 and few (81/2,861; 2.8%) found to be infected. Findings should not be biased towards biomarkers implicated in this specific infection. Nevertheless, health systems and care-seeking pathways were substantially impacted during the pandemic, with both attendance rates and the proportion of patients with severe outcomes generally lower than anticipated based on pre-pandemic baseline data. In particular, no severe outcomes were observed at the Indonesia or Laos sites. It will be important to assess the generalisability of our results in non-pandemic times.

Concentrations of circulating markers of immune and endothelial activation predict disease severity across a spectrum of common childhood infections. We demonstrate that these findings may be applicable at the community level, where need for better risk stratification tools is most urgent. Amongst the markers studied, sTREM-1 holds most potential, demonstrating improved sensitivity and specificity compared to the existing standard of care. Future work should focus on validating these findings, explore different approaches for integrating biomarker testing into patient triage, and assess cost-effectiveness. Priority should be given to biomarkers that are harbingers for disease progression and facilitate earlier recognition of patients in whom illness severity is not yet clinically apparent at presentation. Ultimately, point-of-care tests for the most promising biomarkers must be

468 developed if the clinical utility of biomarker-based triage strategies is to be assessed in

469 definitive randomised controlled trials.

CONTRIBUTORS

AC, CK, RAA, PT, MM, EAA, EA, RPS, MRG, YL, and SB conceptualised the study. AC, DTVA, SK, PNTN, SR, KS, SV, PHP, DM, BTL, and EA acquired the data. AC, RM, and MRG curated the data. AC, CK, RM, and RPS did the formal analysis. YL and SB acquired funding. AC wrote the original draft of the manuscript. AC, CK, RM, RAA, DTVA, SK, PNTN, SR, KS, SV, PT, PHP, DM, MM, BTL, EAA, EA, RPS, MRG, YL, and SB reviewed and edited the manuscript. AC, CK, RM, MRG, YL, and SB verified the underlying data.

DECLARATION OF INTERESTS

All authors declare no competing interests.

DATA SHARING

De-identified, individual participant data from this study will be available to researchers whose proposed purpose of use is approved by the data access committees at *Médecins Sans Frontières* and the Mahidol-Oxford Tropical Medicine Research Unit. Enquiries or requests for the data may be sent to data.sharing@london.msf.org and datasharing@tropmedres.ac. Researchers interested in accessing biobanked samples should contact the corresponding author who will coordinate with the Spot Sepsis Sample Use Committee.

ACKNOWLEDGEMENTS

We are extremely grateful to all the participants and their caregivers, and the laboratory and research staff at the participating sites and MORU laboratories in Bangkok for specimen processing, data management, and study monitoring. We acknowledge the support of the Mérieux Foundation who provided laboratory consumables and equipment for some of the

502 respiratory specimen assays. We also acknowledge the valuable support and guidance of
503 Nicholas J White (MORU) at key points during the study.

504 **TABLE 1. Presenting clinical characteristics, stratified by whether a child progressed to**
505 **develop severe disease within two days of enrolment.**
506

Characteristic	Overall N = 3,405[1]	Non-severe N = 3,272[1]	Severe N = 133[1]	p-value[2]
Demographics and background				
Age (months)	16.8 (8.7, 31.0)	17.3 (9.1, 31.3)	4.9 (2.6, 17.3)	<0.001
Male sex	2,029 / 3,405 (60%)	1,941 / 3,272 (59%)	88 / 133 (66%)	0.11
Known comorbidity	102 / 3,405 (3.0%)	100 / 3,272 (3.1%)	2 / 133 (1.5%)	0.4
Recent admission [a]	429 / 3,394 (13%)	412 / 3,262 (13%)	17 / 132 (13%)	>0.9
Anthropometrics				
Weight-for-age z-score	-0.8 (-1.7, 0.0)	-0.8 (-1.7, 0.1)	-1.1 (-2.5, -0.2)	0.004
Wasted (WHZ < -2) [b,*]	585 / 3,393 (17%)	551 / 3,261 (17%)	34 / 132 (26%)	0.008
Stunted (HAZ < -2) [*]	664 / 3,401 (20%)	629 / 3,268 (19%)	35 / 133 (26%)	0.044
Illness history				
Duration of illness (days)	3.0 (2.0, 4.0)	3.0 (2.0, 4.0)	3.0 (2.0, 5.0)	0.003
Sought care prior to presentation	1,753 / 3,405 (51%)	1,681 / 3,272 (51%)	72 / 133 (54%)	0.5
Travel time to study site ≤ 1 hour	2,777 / 3,405 (82%)	2,682 / 3,272 (82%)	95 / 133 (71%)	0.002
Presenting syndrome				
Upper respiratory tract infection	1,121 / 3,405 (33%)	1,082 / 3,272 (33%)	39 / 133 (29%)	0.4
Lower respiratory tract infection	1,347 / 3,405 (40%)	1,261 / 3,272 (39%)	86 / 133 (65%)	<0.001
Diarrhoeal	646 / 3,405 (19%)	631 / 3,272 (19%)	15 / 133 (11%)	0.021
Neurological	430 / 3,405 (13%)	416 / 3,272 (13%)	14 / 133 (11%)	0.5
No focus	527 / 3,405 (15%)	519 / 3,272 (16%)	8 / 133 (6.0%)	0.002
Severity at presentation				
Any WHO Danger Sign present [*]	1,607 / 3,398 (47%)	1,512 / 3,266 (46%)	95 / 132 (72%)	<0.001
Prostration [c]	240 / 3,405 (7.0%)	192 / 3,272 (5.9%)	48 / 133 (36%)	<0.001
Intractable vomiting [*]	687 / 3,401 (20%)	645 / 3,268 (20%)	42 / 133 (32%)	<0.001
Convulsions [*]	433 / 3,400 (13%)	419 / 3,268 (13%)	14 / 132 (11%)	0.5
Lethargy [d,*]	825 / 3,399 (24%)	764 / 3,267 (23%)	61 / 132 (46%)	<0.001
LqSOFA score [*]				<0.001
0	2,639 / 3,402 (78%)	2,579 / 3,269 (79%)	60 / 133 (45%)	
1	642 / 3,402 (19%)	596 / 3,269 (18%)	46 / 133 (35%)	
2	98 / 3,402 (2.9%)	78 / 3,269 (2.4%)	20 / 133 (15%)	

Characteristic	Overall N = 3,405[1]	Non-severe N = 3,272[1]	Severe N = 133[1]	p-value[2]
3	20 / 3,402 (0.6%)	15 / 3,269 (0.5%)	5 / 133 (3.8%)	
4	3 / 3,402 (<0.1%)	1 / 3,269 (<0.1%)	2 / 133 (1.5%)	
Median LqSOFA score *	0 (0, 0)	0 (0, 0)	1 (0, 1)	<0.001
SIRS score *				0.004
0	223 / 2,827 (7.9%)	222 / 2,707 (8.2%)	1 / 120 (0.8%)	
1	1,042 / 2,827 (37%)	998 / 2,707 (37%)	44 / 120 (37%)	
2	916 / 2,827 (32%)	878 / 2,707 (32%)	38 / 120 (32%)	
3	495 / 2,827 (18%)	471 / 2,707 (17%)	24 / 120 (20%)	
4	151 / 2,827 (5.3%)	138 / 2,707 (5.1%)	13 / 120 (11%)	
Median SIRS score *	2 (1, 2)	2 (1, 2)	2 (1, 3)	0.006
Vital signs				
Heart rate *				
1 to 12 months (bpm)	156.0 (140.0, 172.0)	155.0 (140.0, 170.0)	171.5 (157.5, 186.0)	<0.001
12 to 60 months (bpm)	140.0 (127.0, 158.0)	140.0 (126.0, 157.0)	160.0 (140.0, 177.0)	<0.001
Respiratory rate *				
1 to 12 months (bpm)	45.0 (38.0, 55.0)	44.0 (37.0, 53.0)	59.5 (48.0, 66.0)	<0.001
12 to 60 months (bpm)	36.0 (30.0, 42.0)	36.0 (30.0, 42.0)	39.0 (32.0, 55.0)	0.006
Oxygen saturation (%) *	98.0 (97.0, 99.0)	98.0 (97.0, 99.0)	97.0 (95.0, 98.0)	<0.001
Axillary temperature (°C) *	37.6 (37.0, 38.3)	37.6 (37.0, 38.3)	37.6 (36.9, 38.3)	>0.9
Prolonged capillary refill time [e]	194 / 3,405 (5.7%)	165 / 3,272 (5.0%)	29 / 133 (22%)	<0.001
Not alert [f]	86 / 3,405 (2.5%)	67 / 3,272 (2.0%)	19 / 133 (14%)	<0.001

[1]Median (IQR); n / N (%); [2]Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test

[a] overnight admission in last 6 months; [b] calculated in children 45-120cm; [c] unable to feed, sit, or stand when previously able; [d] abnormally sleepy and/or AVPU < A; [e] capillary refill time > 2 seconds; [f] AVPU < A.

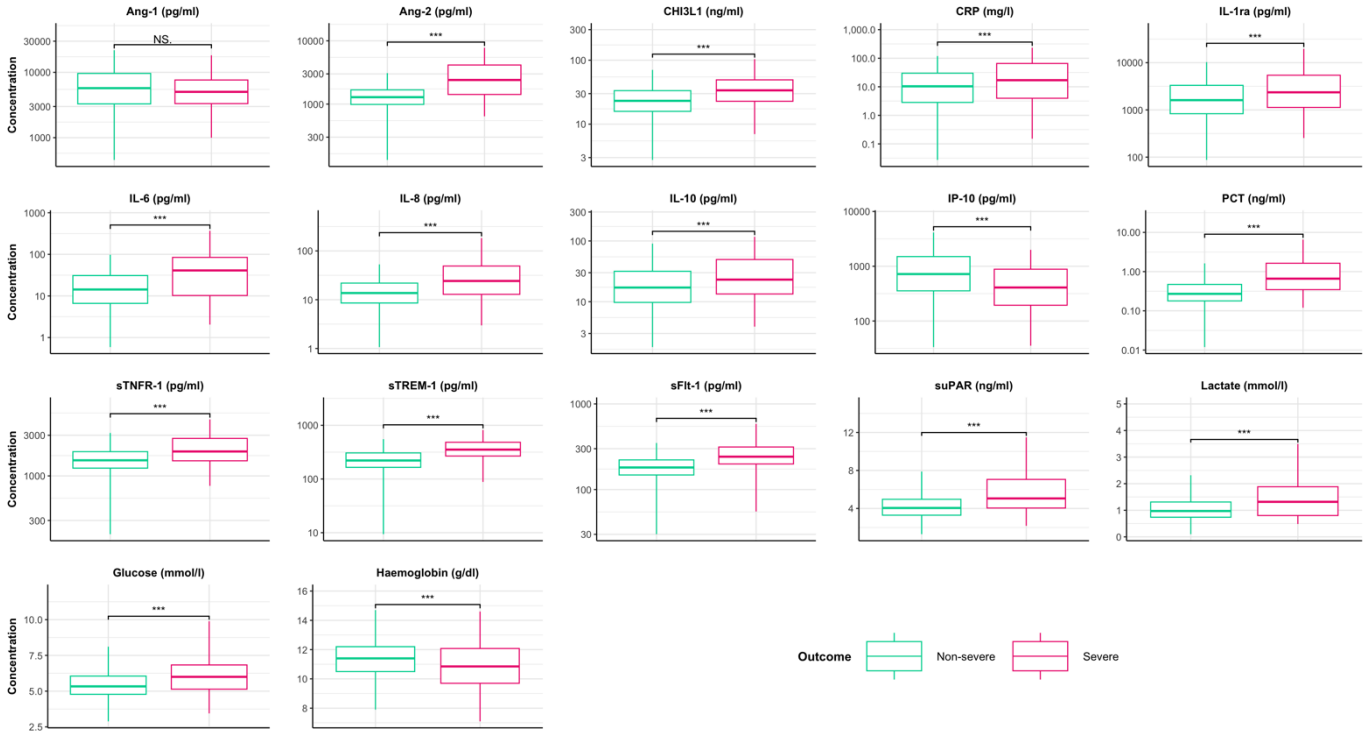
*Missing data: weight-for-height z-score (WHZ), n = 12; height-for-age z-score (LAZ), n = 4; WHO Danger Sign, n = 7; vomiting everything, n = 4; generalised seizures, n = 5; lethargy, n = 6; LqSOFA, n = 3; SIRS, n = 578; heart rate, n = 1; respiratory rate, n = 2; oxygen saturation, n = 205; axillary temperature, n = 1. Missingness for SIRS was greater, as complete blood counts were measured at the discretion of the treating clinical team.

TABLE 2. Sensitivity and specificity of WHO Danger Signs and sTREM-1 for recognition of children who progressed to severe disease within two days of enrolment.

Parameter	Threshold	Method for selection	Sensitivity (95% CI)	Specificity (95% CI)
WHO Danger Signs	Presence/absence	NA	0.72 (0.66-0.79)	0.79 (0.76-0.82)
sTREM-1	279 pg/ml	Fixed at sensitivity of WHO Danger Signs	0.72 (0.65-0.79)	0.83 (0.81-0.86)
sTREM-1	257 pg/ml	Fixed at specificity of WHO Danger Signs	0.80 (0.74-0.86)	0.79 (0.76-0.82)
sTREM-1	261 pg/ml	Youden index	0.80 (0.73-0.85)	0.81 (0.78-0.83)

Recognising that the relative importance of sensitivity and specificity is context dependent, the performance of sTREM-1 was compared to WHO Danger Signs by evaluating specificity at the sTREM-1 concentration that equated to the sensitivity of WHO Danger Signs, and then by evaluating sensitivity at the sTREM-1 concentration that equated to the specificity of WHO Danger Signs. Finally, the Youden index was used to identify the sTREM-1 concentration which maximised accuracy (the optimal trade-off between sensitivity and specificity, assuming both were of equal importance).

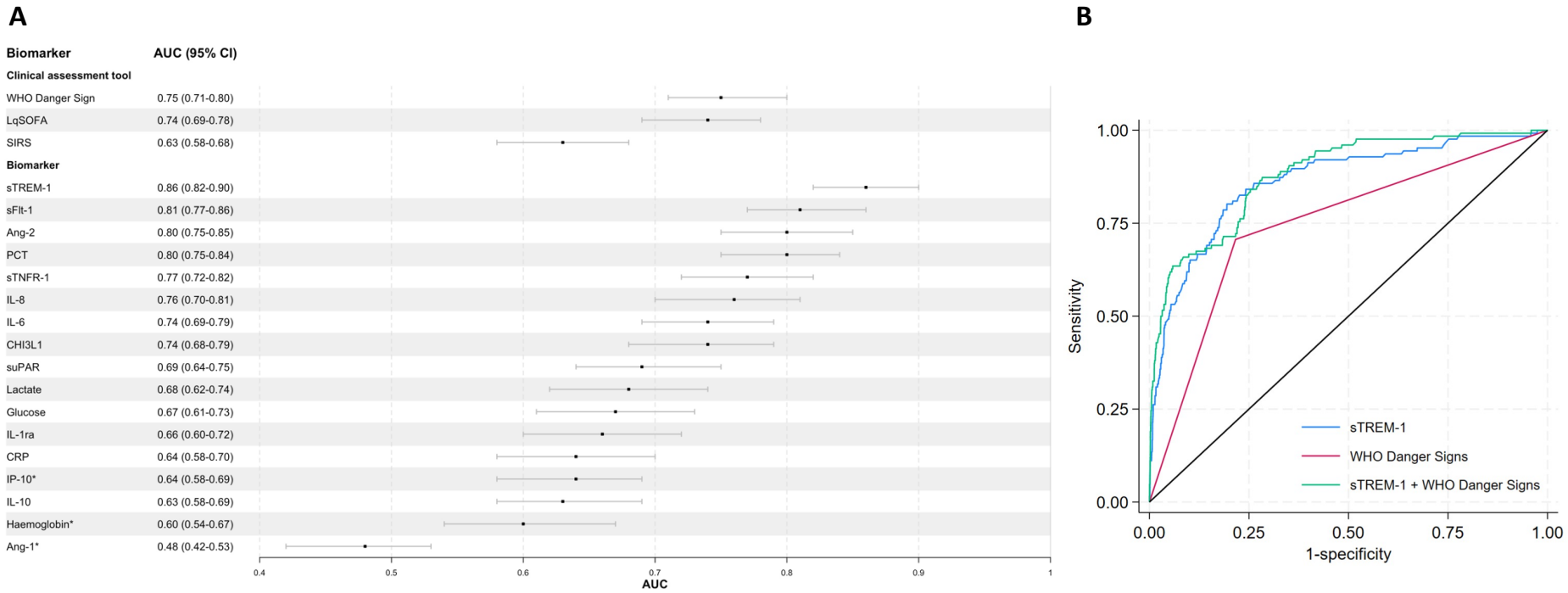
FIGURE 1. Presenting concentrations of circulating markers of endothelial and immune activation, stratified by whether a child progressed to develop severe disease within two days of enrolment.



Box denotes middle 50% of the data, with the median indicated by the solid horizontal line. Upper and lower hinges denote 75th and 25th centile respectively. Whiskers extend from minimum to maximum value (hinge ± 1.5 times the interquartile range). Outliers not plotted to aid clarity.

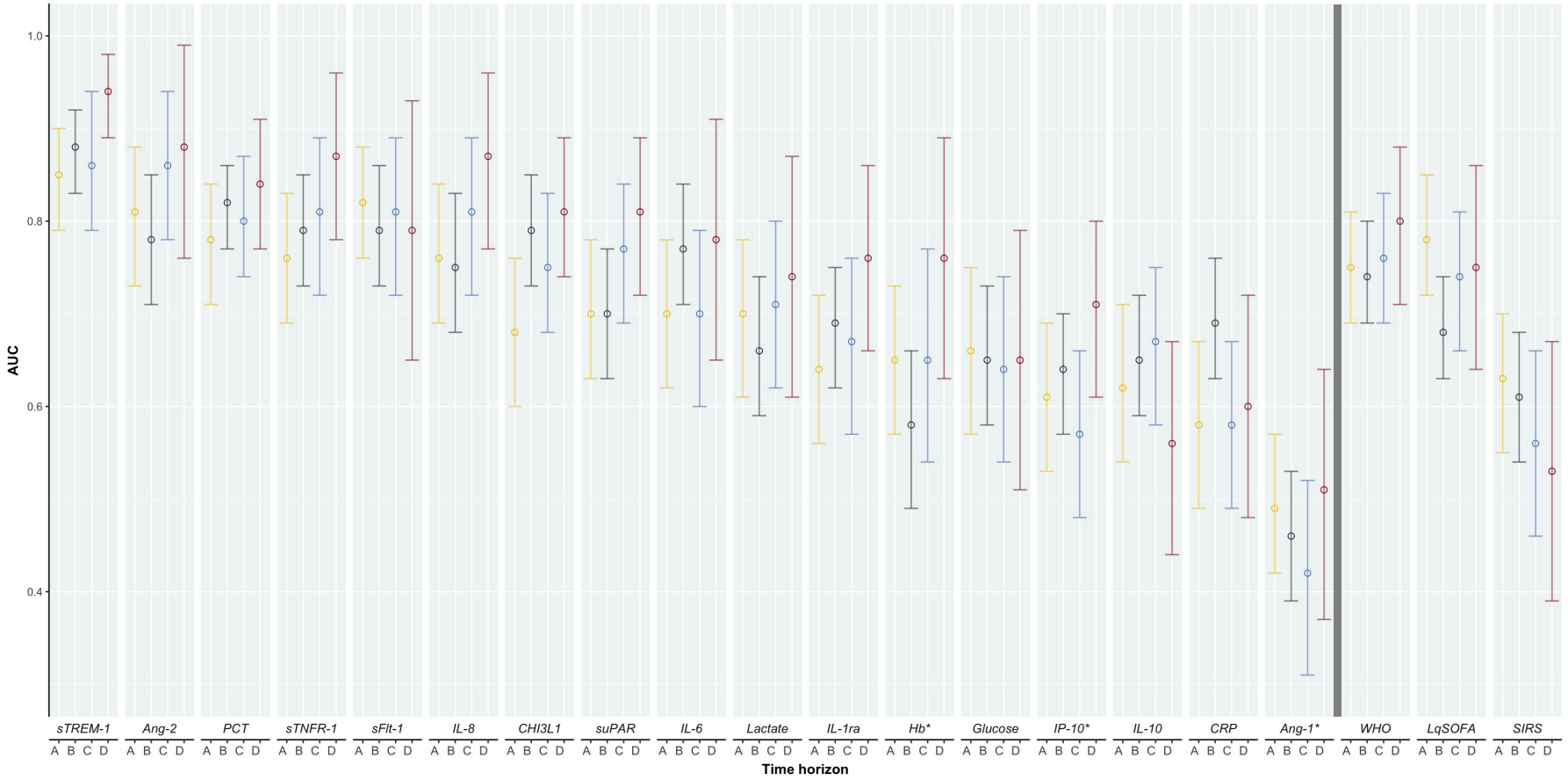
Presenting biomarker concentrations among children who progressed to severe disease (red) compared to children who did not progress to severe disease (green) using Wilcoxon rank sum test; NS = no statistically significant difference, *** = $p < 0.001$.

FIGURE 2. A: Prognostic performance of clinical assessment tools and circulating markers of endothelial and immune activation to predict progression to severe disease within two days of enrolment. B: Prognostic performance of WHO Danger Signs and sTREM-1, alone and in combination, to predict progression to severe disease within two days of enrolment.



A: Solid square = point estimate for AUC; error bars = 95% CI. *Reciprocal concentration used, as lower biomarker concentrations known to be indicative of more severe disease. B: Receiver operating characteristic curves for WHO Danger Signs (red) AUC = 0.75 (95% CI 0.70-0.80); sTREM-1 (blue) AUC = 0.86 (95% CI 0.82-0.90); and sTREM-1 plus WHO Danger Signs (green) AUC = 0.88 (95% CI 0.85-0.91).

FIGURE 3. Prognostic performance of clinical assessment tools and circulating markers of endothelial and immune activation to predict progression to severe disease across different time horizons.



Prediction horizons (events/non-events): A (yellow) = < 4 hours (56/3,319); B (grey) = ≥ 4 hours (83/3,236); C (blue) = ≥ 24 hours (42/3,236); D (red) = ≥ 48 hours (21/3,236). Participants who met the outcome by horizon A are excluded from horizon B, C, and D analyses. Participants who met the outcome prior to 24 or 48 hours are excluded

551 from horizon C and D analyses respectively. Biomarkers presented to the left of solid vertical line, clinical assessment tools presented to the right, both in descending order
552 of mean AUC across prediction horizons. Open circle = point estimate for AUC; error bars = 95% CI.*Reciprocal concentration used, as lower biomarker concentrations
553 known to be indicative of more severe disease.

554 **REFERENCES**

555

556 1. McDonald CR, Weckman A, Richard-Greenblatt M, Leligdowicz A, Kain KC. Integrated
557 fever management: disease severity markers to triage children with malaria and non-
558 malarial febrile illness. *Malar J* 2018; **17**(1): 353.
559 2. Buntinx F, Mant D, Van den Bruel A, Donner-Banzhof N, Dinant GJ. Dealing with low-
560 incidence serious diseases in general practice. *Br J Gen Pract* 2011; **61**(582): 43-6.
561 3. Kruk ME, Gage AD, Arsenault C, et al. High-quality health systems in the Sustainable
562 Development Goals era: time for a revolution. *Lancet Glob Health* 2018; **6**(11): e1196-
563 e252.
564 4. Achan J, Tibenderana J, Kyabayinze D, et al. Case management of severe malaria--a
565 forgotten practice: experiences from health facilities in Uganda. *PLoS One* 2011; **6**(3):
566 e17053.
567 5. Debarre A. Hard to Reach: Providing Healthcare in Armed Conflict: International Peace
568 Institute, 2018.
569 6. Simba DO, Kakoko DC, Warsame M, et al. Understanding caretakers' dilemma in deciding
570 whether or not to adhere with referral advice after pre-referral treatment with rectal
571 artesunate. *Malar J* 2010; **9**(123).
572 7. Hercik C, Cosmas L, Mogeni OD, et al. Health Beliefs and Patient Perspectives of Febrile
573 Illness in Kilombero, Tanzania. *Am J Trop Med Hyg* 2019; **101**(1): 263-70.
574 8. World Health Organization. Integrated Management of Childhood Illnesses. Geneva,
575 Switzerland; 2014.
576 9. World Health Organization. Integrated Community Case Management. Geneva,
577 Switzerland; 2012.
578 10. Izudi J, Anyigu S, Ndungutse D. Adherence to Integrated Management of Childhood
579 Illnesses Guideline in Treating South Sudanese Children with Cough or Difficulty in
580 Breathing. *Int J Pediatr* 2017: 5173416.
581 11. Keitel K, Kilowoko M, Kyungu E, Genton B, D'Acremont V. Performance of prediction
582 rules and guidelines in detecting serious bacterial infections among Tanzanian febrile
583 children. *BMC Infect Dis* 2019; **19**(1): 769.
584 12. Hansoti B, Jenson A, Keefe D, et al. Reliability and validity of pediatric triage tools
585 evaluated in Low resource settings: a systematic review. *BMC Pediatr* 2017; **17**(1): 37.
586 13. Leligdowicz A, Conroy AL, Hawkes M, et al. Risk-stratification of febrile African children
587 at risk of sepsis using sTREM-1 as basis for a rapid triage test. *Nat Commun* 2021; **12**(1):
588 6832.
589 14. Stefanova V, Ngai M, Weckman AM, et al. suPAR as a Prognostic Marker of Ugandan
590 Children at Risk of Severe and Fatal Malaria. *Clin Infect Dis* 2023; **76**(3): e1079-e86.
591 15. Balanza N, Erice C, Ngai M, Varo R, Kain KC, Bassat Q. Host-Based Prognostic Biomarkers
592 to Improve Risk Stratification and Outcome of Febrile Children in Low- and Middle-
593 Income Countries. *Front Pediatr* 2020; **8**: 552083.
594 16. Leligdowicz A, Richard-Greenblatt M, Wright J, Crowley VM, Kain KC. Endothelial
595 Activation: The Ang/Tie Axis in Sepsis. *Front Immunol* 2018; **9**: 838.
596 17. Jolly L, Carrasco K, Salcedo-Magguilli M, et al. sTREM-1 is a specific biomarker of TREM-1
597 pathway activation. *Cell Mol Immunol* 2021; **18**(8): 2054-6.
598 18. Donadello K, Scolletta S, Covajes C, Vincent JL. suPAR as a prognostic biomarker in
599 sepsis. *BMC Med* 2012; **10**(2).

19. Chandna A, Lubell Y, Mwandigha L, et al. Defining the role of host biomarkers in the diagnosis and prognosis of the severity of childhood pneumonia: a prospective cohort study. *Sci Rep* 2023; **13**(1): 12024.
20. Balanza N, Erice C, Ngai M, et al. Prognostic accuracy of biomarkers of immune and endothelial activation in Mozambican children hospitalized with pneumonia. *PLOS Glob Pub Health* 2023; **3**(2): e0001553.
21. Jullien S, Richard-Greenblatt M, Ngai M, et al. Performance of host-response biomarkers to risk-stratify children with pneumonia in Bhutan. *J Infect* 2022; **85**(6): 634-43.
22. Wright SW, Lovelace-Macon L, Hantrakun V, et al. sTREM-1 predicts mortality in hospitalized patients with infection in a tropical, middle-income country. *BMC Med* 2020; **18**(1): 159.
23. Wright JK, Hayford K, Tran V, et al. Biomarkers of endothelial dysfunction predict sepsis mortality in young infants: a matched case-control study. *BMC Pediatr* 2018; **18**(1): 118.
24. Chandna A, Tan R, Carter M, et al. Predictors of disease severity in children presenting from the community with febrile illnesses: a systematic review of prognostic studies. *BMJ Glob Health* 2021; **6**(1).
25. Mawji A, Li E, Chandna A, et al. Common data elements for predictors of pediatric sepsis: A framework to standardize data collection. *PLoS One* 2021; **16**(6): e0253051.
26. Kinasewitz GT, Yan SB, Basson B, et al. Universal changes in biomarkers of coagulation and inflammation occur in patients with severe sepsis, regardless of causative micro-organism. *Crit Care* 2004; **8**(2): R82-90.
27. Richard-Greenblatt M, Boillat-Blanco N, Zhong K, et al. Prognostic Accuracy of Soluble Triggering Receptor Expressed on Myeloid Cells (sTREM-1)-based Algorithms in Febrile Adults Presenting to Tanzanian Outpatient Clinics. *Clin Infect Dis* 2020; **70**(7): 1304-12.
28. Ghosh CC, David S, Zhang R, et al. Gene control of tyrosine kinase TIE2 and vascular manifestations of infections. *Proc Natl Acad Sci U S A* 2016; **113**(9): 2472-7.
29. National Institute for Health and Care Excellence. Suspected sepsis: recognition, diagnosis and early management. United Kingdom, 2024.
30. Khilnani P, Singhi S, Lodha R, et al. Pediatric Sepsis Guidelines: Summary for resource-limited countries. *Indian J Crit Care Med* 2010; **14**(1): 41-52.
31. Sanchez-Pinto LN, Bennett TD, DeWitt PE, et al. Development and Validation of the Phoenix Criteria for Pediatric Sepsis and Septic Shock. *JAMA* 2024; **331**(8): 675-86.
32. Romaine S.T, Potter J, Khanijau A, et al. Accuracy of a Modified qSOFA Score for Predicting Critical Care Admission in Febrile Children. *Pediatrics* 2020; **146**(4): e20200782.
33. Goldstein B, Giroir B, Randolph A, International Consensus Conference on Pediatric S. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. *Pediatr Crit Care Med* 2005; **6**(1): 2-8.
34. Eun S, Kim H, Kim HY, et al. Age-adjusted quick Sequential Organ Failure Assessment score for predicting mortality and disease severity in children with infection: a systematic review and meta-analysis. *Sci Rep* 2021; **11**(1): 21699.
35. Chandna A, Mwandigha L, Koshiaris C, et al. External validation of clinical severity scores to guide referral of paediatric acute respiratory infections in resource-limited primary care settings. *Sci Rep* 2023; **13**(1): 19026.
36. Schilling WHK, Mukaka M, Callery JJ, et al. Evaluation of hydroxychloroquine or chloroquine for the prevention of COVID-19 (COPCOV): A double-blind, randomised, placebo-controlled trial. *PLoS Med* 2024; **21**(9): e1004428.

37. Phommasone K, Xaiyaphet X, Garcia-Rivera JA, et al. A case–control study of the causes of acute respiratory infection among hospitalized patients in Northeastern Laos. *Scientific Reports* 2022; **12**(1).
38. Chandna A, Aderie EM, Ahmad R, et al. Prediction of disease severity in young children presenting with acute febrile illness in resource-limited settings: a protocol for a prospective observational study. *BMJ Open* 2021; **11**(1): e045826.
39. Riley RD, Snell KI, Ensor J, et al. Minimum sample size for developing a multivariable prediction model: PART II - binary and time-to-event outcomes. *Stat Med* 2019; **38**(7): 1276-96.
40. Newson R. Confidence intervals for rank statistics: Somers' D and extensions. *Stata J* 2006; **6**(3): 309-34.
41. Chen HL, Hung CH, Tseng HI, Yang RC. Soluble form of triggering receptor expressed on myeloid cells-1 (sTREM-1) as a diagnostic marker of serious bacterial infection in febrile infants less than three months of age. *Jpn J Infect Dis* 2008; **61**(1): 31-5.
42. Conroy AL, Hawkes M, McDonald CR, et al. Host Biomarkers Are Associated With Response to Therapy and Long-Term Mortality in Pediatric Severe Malaria. *Open Forum Infect Dis* 2016; **3**(3): ofw134.
43. Unitaid and the Foundation for Innovative New Diagnostics (FIND). Biomarkers for acute febrile illness at the point-of-care in low-resource settings. 2021. https://www.finddx.org/wp-content/uploads/2021/05/Meeting-report_Biomarkers-for-acute-febrile-illness-at-the-point-of-care-in-low-resource-settings.pdf (accessed 27 May 2021).
44. Holtman GA, Berger MY, Burger H, et al. Development of practical recommendations for diagnostic accuracy studies in low-prevalence situations. *J Clin Epidemiol* 2019; **114**: 38-48.
45. Leeflang MM, Bossuyt PM, Irwig L. Diagnostic test accuracy may vary with prevalence: implications for evidence-based diagnosis. *J Clin Epidemiol* 2009; **62**(1): 5-12.
46. Charakida M, Donald AE, Terese M, et al. Endothelial dysfunction in childhood infection. *Circulation* 2005; **111**(13): 1660-5.
47. Chandna A, Richard-Greenblatt M, Tustin R, et al. Practical Methods to Permit the Analysis of Host Biomarkers in Resource-Limited Settings. *Am J Trop Med Hyg* 2022; **106**(6): 1765-9.
48. Lubell Y, Chandna A, Smithuis F, et al. Economic considerations support C-reactive protein testing alongside malaria rapid diagnostic tests to guide antimicrobial therapy for patients with febrile illness in settings with low malaria endemicity. *Malar J* 2019; **18**(1): 442.
49. McHugh LC, Snyder K, Yager TD. The effect of uncertainty in patient classification on diagnostic performance estimations. *PLoS One* 2019; **14**(5): e0217146.
50. Van Singer M, Brahier T, Ngai M, et al. COVID-19 risk stratification algorithms based on sTREM-1 and IL-6 in emergency department. *J Allergy Clin Immunol* 2021; **147**(1): 99-106 e4.
51. McDonald CR, Leligdowicz A, Conroy AL, et al. Immune and endothelial activation markers and risk stratification of childhood pneumonia in Uganda: A secondary analysis of a prospective cohort study. *PLoS Med* 2022; **19**(7): e1004057.