Biomarkers of Systemic Inflammation in Ugandan Infants and Children Hospitalized With Respiratory Syncytial Virus Infection

Sawatzky, Julia BSc*; Soo, Jeremy BSc*; Conroy, Andrea L. PhD†; Bhargava, Ravi MD‡; Namasopo, Sophie MBChB, MPH§; Opoka, Robert O. MBChB, MSc¶; Hawkes, Michael T. MD, PhD‖,**,††

Author Information

*Department of Pediatrics, University of Alberta, Edmonton, Canada

†Ryan White Center for Pediatric Infectious Diseases and Global Health, Indiana University School of Medicine, Indianapolis

‡Department of Radiology and Diagnostic Imaging, University of Alberta, Edmonton, Canada

§Department of Pediatrics, Jinja Regional Referral Hospital

¶Department of Pediatrics and Child Health, Mulago Hospital and Makerere University, Kampala, Uganda

‖Department of Pediatrics

**Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Canada

††Department of Global Health, School of Public Health, University of Alberta, Edmonton, Canada.

#Corresponding author: Michael T. Hawkes, Department of Pediatrics, University of Alberta, 3-588D Edmonton Clinic Health Academy, 11405 87 Ave NW, Edmonton, Alberta, T6G 1C9, Canada, Tel: +1-780-248-5540, Email: mthawkes@ualberta.ca

*current affiliation: Sophie Namasopo, Kabale District Hospital, Kabale, Uganda

---

This is the author's manuscript of the article published in final edited form as:

Sawatzky, J., Soo, J., Conroy, A., Bhargava, R., Namasopo, S., Opoka, R., & Hawkes, M. (2019). Biomarkers of Systemic Inflammation in Ugandan Infants and Children Hospitalized With Respiratory Syncytial Virus Infection. The Pediatric Infectious Disease Journal, 38(8), 854–859. https://doi.org/10.1097/INF.0000000000002343

Background: Optimizing outcomes in Respiratory Syncytial Virus (RSV) pneumonia requires accurate diagnosis and determination of severity that, in resource-limited settings, is often based on clinical assessment alone. We describe host inflammatory biomarkers and clinical outcomes among children hospitalized with RSV lower respiratory tract infection (LRTI) in Uganda and controls with rhinovirus and pneumococcal pneumonia.

Methods: 58 children hospitalized with LRTI were included. We compared 37 patients with RSV, 10 control patients with rhinovirus, and 11 control patients with suspected pneumococcal pneumonia.

Results: Patients in the RSV group had significantly lower levels of C-Reactive Protein (CRP) and Chitinase-3-Like Protein 1 (CHI3L1) than the pneumococcal pneumonia group ($p<0.05$ for both). Among children with RSV, higher admission levels of CRP predicted prolonged time to resolution of tachypnea, tachycardia, and fever. Higher levels of CHI3L1 were associated with higher composite clinical severity scores and predicted prolonged time to resolution of tachypnea and tachycardia, time to wean oxygen, and time to sit. Higher levels of Lipocalin-2 (LCN2) predicted prolonged time to resolution of tachypnea, tachycardia and time to feed. Higher admission levels of all three biomarkers were predictive of a higher total volume of oxygen administered during hospitalization ($p<0.05$ for all comparisons). Of note, CHI3L1 and LCN2 appeared to predict clinical outcomes more accurately than CRP, the inflammatory biomarker most widely used in clinical practice.

Conclusions: Our findings suggest that CHI3L1 and LCN2 may be clinically informative biomarkers in childhood RSV LRTI in low-resource settings.

Introduction

Respiratory Syncytial Virus is a leading cause of lower respiratory tract infection (LRTI) globally. In 2015, RSV resulted in approximately 3.2 million childhood hospital admissions and 118,200 deaths, 99% of which occurred in low- and middle-income countries (2). RSV is an enveloped RNA paramyxovirus that is highly infectious via droplet transmission. The severity of RSV respiratory infections can be attributed largely to the host immune response, which is characterized by neutrophil-mediated inflammation of the airways (1).

There is significant overlap between the clinical manifestations of RSV LRTI and bacterial pneumonia, which may lead to the inappropriate use of antimicrobials in patients with RSV. The current WHO and UNICEF integrated management of childhood illness (IMCI) guidelines for the management of childhood LRTI in low-resource settings include cough, difficulty breathing, tachypnea and chest in-drawing as clinical criteria for the administration of empiric dispersible amoxicillin (3). These criteria are sensitive and will capture most children with bacterial pneumonia; however, a large number of patients meeting these clinical criteria will have RSV infection and will not benefit from antibiotic therapy. Effective management of pediatric LRTI in low resource settings also requires determination of severity and prognosis to guide resource allocation, prioritize the sickest patients, and prevent fatality (4). Although clinical characteristics and outcomes of children with RSV infection have been well documented in resource-intensive settings (5), fewer data are available from low-resource settings where access to viral diagnostics is limited.

Biomarkers of host response to infection may have clinical utility in distinguishing between infectious etiology and determining disease severity in LRTI (6). Proteomic signatures from blood samples have been shown to provide rapid and actionable clinical information capable of guiding treatment decisions (7). Several candidate biomarkers of systemic inflammation have been examined in LRTI, including C-Reactive Protein (CRP) (6), Chitinase-3-Like Protein 1 (CHI3L1) (8) and Lipocalin-2 (LCN2) (9). While some studies have examined these biomarkers in pediatric LRTI in low resource settings (10, 11), none have examined their potential clinical utility in RSV patients or as predictors of clinical course and outcome.

The objective of this study was to describe admission characteristics, radiographic and laboratory abnormalities, inflammatory biomarker levels at admission, clinical course, and outcomes of children hospitalized with RSV LRTI in Uganda. We compared cases with RSV LRTI to two control groups: (1) rhinovirus respiratory tract infection (RTI); and (2) suspected pneumococcal (*Streptococcus pneumoniae, Sp*) lobar pneumonia.

Materials and Methods

Study design and participants

We conducted a prospective study of children hospitalized with signs of pneumonia at two resource-limited hospitals in Uganda: Jinja Regional Referral Hospital and Kambuga District Hospital. Children were included if they: (1) were under 13 years of age; (2) required admission to hospital; and (3) presented with cough or difficulty breathing and one or more of the following signs: tachypnea, chest in-drawing, and/or hypoxemia. Of note, all children included in this study

met the WHO and UNICEF IMCI clinical definition of pneumonia (3). Children with clinically-suspected tuberculosis were excluded.

Enrolled children underwent history and physical exam, had blood and nasopharyngeal (NP) swabs collected, and were followed over the course of admission until discharge, death, or transfer to another facility with frequent monitoring of vital signs. Cases of RSV LRTI were defined as patients meeting the above clinical inclusion criteria, plus detection of RSV from the nasopharynx by multiplex PCR. Two control groups were chosen: (1) rhinovirus RTI, defined as patients meeting the clinical inclusion criteria, plus rhinovirus detected in the nasopharynx and absence of lobar consolidation on CXR; and (2) *Sp* pneumonia, defined as patients meeting the clinical inclusion criteria, plus lobar consolidation on CXR and *Sp* detected in the blood or at high genomic load (>6.9 $\log_{10}$ copies/mL) in the nasopharynx. Of note, high density colonization with *Sp* in the nasopharynx in children with clinical pneumonia, above a threshold of 6.9 $\log_{10}$ copies/mL, has previously been associated with invasive pneumococcal infection (12).

Specimen collection and analysis

Staff were trained in correct procedures for flocked nasopharyngeal (NP) swab collection (FLOQSwabs™, Copan Diagnostics, Murrieta, CA, USA). NP swabs were placed into viral transport medium (UTM™, Universal Transport Medium, Copan Diagnostics, Murrieta, CA, USA) and 1mL aliquots of viral transport medium were stored at -80°C prior to shipment to Canada. A semi-automated nucleic acid extraction protocol was used to perform nucleic acid extraction with a KingFisher™ mL Purification System (Thermo Fisher Scientific Inc, Waltham, MA) and the MagaZorb® Total RNA Mini-Prep Kit (Promega, Madison, WI). Quantitative real-

time PCR (qPCR) with FTDResp33 (Fast-Track Diagnostics, Esch-sur-Alzet, Luxembourg) was used to identify respiratory pathogens in the NP swab. We ran these samples using an Applied Biosystems® 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). To quantify the number of genome copies of *Sp* present in each sample, we used the standard curves relating the rtPCR cycle threshold (CT) to plasmid DNA concentrations provided by the manufacturer. EDTA plasma was collected in the field and stored at -80°C until shipment to Canada. ELISAs were performed according to the manufacturer's instructions and blinded to all associated clinical data in order to quantify the following biomarkers: CRP, CHI3L1, LCN2 (R&D Systems, Minneapolis, MN). Dilutions for CRP ranged from 1:500,000 to 1:5000 to achieve an assay range of 500 to 0.16 µg/ml. Dilutions for CHI3L1 ranged from 1:62.5 to 1:1800 in order to achieve a range of 3600 to 2 nm/ml. Dilutions for LCN2 ranged from 1:20 to 1:200 to achieve a range of 500 to 1.6 ng/ml. Background signal was determined from blank wells on each plate and subtracted from all samples and standards prior to analysis. A 4-parameter logistic regression curve fitted to data was used to determine biomarker concentrations from the ELISA optical density.

Statistical analysis

Continuous data was presented as medians (interquartile range) and analyzed non-parametrically, with relationships assessed using the Mann-Whitney U test. Categorical data were analyzed using Pearson's χ^2 test. Time-to-event analysis was performed using Kaplan-Meier plots and log-rank test for differences between factor levels. Cases were right censored at the time of last encounter. Where multiple *p*-values were calculated, we used the Bonferroni correction for multiple statistical comparisons, to avoid family-wise inflation of type 1 statistical error.

Ethics statement

The study was approved by the School of Biomedical Sciences Research and Ethics Committee (Makerere University, Kampala, Uganda), the Uganda National Council of Science and Technology, and the Human Research Ethics Board of the University of Alberta. Accompanying legal guardians provided informed written consent for participants at the time of enrolment.

Results

Fifty-eight children hospitalized with RTIs between February 25, 2014 and July 3, 2015 were included: 37 patients with RSV LRTI, 11 with rhinovirus RTI, and 10 with *Sp* pneumonia. Table 1 shows clinical characteristics of the study cohort. Patients in the control groups were significantly older and weighed more than the children in the RSV case group. Co-detection of multiple pathogens was common, such that 1 out of 37 patients with RSV LRTI also met inclusion criteria for the rhinovirus control group, and 4 out of 37 also met inclusion criteria for the *Sp* pneumonia control group. We accounted for this overlap in microbial classification in a subsequent sensitivity analysis.

Clinical course and outcomes

Recovery times for clinically relevant endpoints are shown in Table 2. Of note, time to resolution of fever and tachypnea and time to feed were significantly prolonged in patients with *Sp* pneumonia compared to those with RSV LRTI.

Host inflammatory biomarkers distinguish RSV from suspected pneumococcal pneumonia

Table 3 and Figure 1 show plasma concentrations of several host biomarkers. Among patients with RSV LRTI, inflammatory biomarkers were correlated with each other (CHI3L1 with LCN2 $\rho = +0.82$, $p<0.001$; CHI3L1 with CRP $\rho = +0.62$, $p <0.001$; LCN2 with CRP, $\rho= +0.75$, $p <0.001$). Markers of systemic inflammation (CRP, CHI3L1 and LCN2) were significantly higher in the *Sp* pneumonia group than the RSV LRTI and rhinovirus RTI groups. Differences in CRP, CHI3L1, but not LCN2, remained statistically significant following correction for multiple comparisons. In linear regression models adjusting for the potentially confounding effect of age, *Sp* pneumonia remained independently associated with higher CRP ($p=0.002$) and CHI3L1 ($p=0.034$), relative to RSV LRTI. Some patients had multiple organisms detected simultaneously in the NP, such that one patient in the RSV LRTI group also fulfilled criteria for rhinovirus RTI and four patients in the RSV LRTI group also fulfilled criteria for *Sp* pneumonia. We performed a sensitivity analysis, excluding those patients with overlapping microbial classifications, and found that differences in CRP and CHI3L1 between patients with RSV LRTI and *Sp* pneumonia remained statistically significant ($p<0.01$ for both comparisons).

Predictive value of inflammatory biomarkers among children with RSV LRTI

Higher levels of CHI3L1 were associated with higher composite clinical severity scores (RISC), $r= 0.41$, $p =0.019$). Admission biomarker levels directly correlated with recovery times within the RSV LRTI group (n=37): higher admission levels of CHI3L1 were associated with prolonged time to resolution of tachypnea and tachycardia, time to wean oxygen, and time to sit ($p<0.05$ for all comparisons). Similarly, higher admission levels of LCN2 at admission were associated with prolonged time to resolution of tachypnea, tachycardia and time to feed. In contrast, higher admission CRP levels were not significantly associated with any of these endpoints. Higher

admission levels of CHI3L1, LCN2 and CRP, however, were all predictive of a higher total volume of oxygen administered over the course of hospitalization ($p<0.05$ for all comparisons). Prognostic biomarkers are illustrated in Figure 2, with the cohort dichotomized according to median admission biomarker values (CHI3L1, 32 ng/mL; LCN2, 94 ng/mL; and CRP, 18 μg/mL). The biomarkers outperformed clinical variables, including tachypnea and RISC score, as predictors of recovery times. For full results see Table, Supplementary Digital Content.

Discussion

In sub-Saharan Africa, RSV is commonly detected in children with severe acute respiratory illness (13) and is a significant predictor of hospitalization (14). This study provided a unique examination of clinical features and host inflammatory biomarkers in Ugandan children hospitalized with RSV LRTI. Noteworthy strengths of this study include the use of a sensitive molecular virology assay and host biomarker measurement in patients from a low-resource setting where such laboratory data are not usually available, as well as detailed longitudinal follow-up of hospitalized children. Our main findings included: (1) RSV-infected children had significantly lower CRP and CHI3L1 levels compared to a *Sp* pneumonia control group; and (2) admission levels of CHI3L1 and LCN2 were predictive of clinical outcomes in children with RSV LRTI.

The clinical characteristics of our cohort were broadly consistent with prior studies of children with RSV LRTI; however, children in our study had evidence of greater disease severity, including higher respiratory rates, lower oxygen saturation, and a higher proportion with chest

in-drawing (15-17). Control children with *Sp* pneumonia were more frequently lethargic and tachycardic.

CRP is an acute phase protein produced in response to inflammatory cytokines and is widely used as a biomarker of systemic inflammation in clinical practice (18). We found that CRP was lower in patients with RSV LRTI compared to those with suspected *Sp* pneumonia, consistent with previous reports that demonstrated the diagnostic utility of CRP as a marker of serious bacterial infection (6).

We found that admission levels of CHI3L1 were lower in children with RSV LRTI than *Sp* pneumonia, but similar between RSV LRTI and rhinovirus RTI. Furthermore, among children with RSV LRTI, higher levels of CHI3L1 and LCN2 predicted longer recovery times. CHI3L1 is a secreted glycoprotein expressed by immune and respiratory epithelial cells that promotes bacterial clearance and augments host tolerance to infection in pneumococcal pneumonia (8). In past studies, CHI3L1 was elevated in RSV respiratory infection (20) and higher CHI3L1was associated with radiographic consolidation in African children with clinical signs of pneumonia (11). LCN2 is a secreted transport protein produced by neutrophils in the respiratory mucosa that inhibits bacterial uptake of iron and propagates inflammation via IL-8 mediated induction of neutrophils (21). In past studies, LCN2 distinguished between pneumonia etiology (9), was associated with pneumonia severity (9), and predicted poor outcomes in childhood pneumococcal pneumonia (10). Our findings build on these observations, suggesting that CHI3L1 and LCN2 are clinically informative biomarkers in childhood RSV LRTI in a low-resource setting. They may have utility as diagnostic markers to distinguish viral (RSV and

rhinovirus) RTI from *Sp* pneumonia and/or prognostic markers of prolonged recovery time in children with RSV LRTI.

Several factors limit the validity and generalizability of these data. The statistical power of this study was restricted by a small sample size. Diagnostic workup was incomplete, since we did not have access to blood culture or lung aspirates at our low-resource hospital. Further studies would be needed to determine the clinical utility of these biomarkers used in real-time for pneumonia triage and management. Co-detection of multiple pathogens complicated the comparison between RSV LRTI cases and controls; however, we performed sensitivity analyses and demonstrated that our conclusions were robust to exclusion of patients with multiple co-detected micro-organisms.

Findings from the current study may have several implications. Protein biomarkers that could be adapted to a rapid test platform might be used to discriminate bacterial from viral etiology in order to help guide responsible antibiotic usage. Analogous to the HRP2 rapid diagnostic test for malaria, such a test could be inexpensive and achieve rapid penetration to clinical settings in low-resource settings. In Sub-Saharan Africa, the combination of a high burden of communicable diseases and lack of diagnostic capacity is leading to increased rates of antimicrobial resistance (22), which may be combatted by limiting the use of antimicrobials for patients with viral illness (23). Secondly, prognostic biomarkers such as CHI3L1 and LCN2 may help to guide resource allocation and supportive management decisions. This finding is relevant for resource-scarce healthcare settings where triage to high-dependency units should prioritize cases of the greatest severity in order to prevent fatality. Finally, our study shows that high levels of CHI3L1 and LCN2 are associated with either bacterial infection or more severe viral infection, but are not elevated in mild viral infection. In clinical practice, low levels of these biomarkers could be used

to rule out serious infection and identify children with mild viral illness who could be managed as outpatients without antibiotics. Further studies to examine the utility of CHI3L1 and LCN2 in guiding clinical decision-making are warranted.

References

1. Shi T, McAllister DA, O'Brien KL, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. *Lancet*. 2017;390:946-958.

2. Sricharoenchai S. PE, Pasini FL., Sanicas M. Epidemiology of Respiratory Syncytial Virus Lower Respiratory Tract Infections (RSV-LRTI) In Children In Developing Countries. *Journal of Tropical Diseases & Public Health*. 2016;4:212.

3. Izadnegahdar R, Cohen AL, Klugman KP, Qazi SA. Childhood pneumonia in developing countries. *Lancet Respir Med*. 2013;1:574-584.

4. Reed C, Madhi SA, Klugman KP, et al. Development of the Respiratory Index of Severity in Children (RISC) score among young children with respiratory infections in South Africa. *PLoS One*. 2012;7:e27793.

5. Kimberlin DW BM, Jackson, MA, Long SS Respiratory Syncytial Virus. In: Red Book: 2015 Report on the Committee on Infectious Diseases te, ed. Elk Grove Village, IL: American Academy of Pediatrics; 2015.

6. Higdon MM, Le T, O'Brien KL, et al. Association of C-Reactive Protein With Bacterial and Respiratory Syncytial Virus-Associated Pneumonia Among Children Aged <5 Years in the PERCH Study. *Clin Infect Dis*. 2017;64:S378-S386.

7. van Houten CB, de Groot JAH, Klein A, et al. A host-protein based assay to differentiate between bacterial and viral infections in preschool children (OPPORTUNITY): a double-blind, multicentre, validation study. *Lancet Infect Dis*. 2017;17:431-440.

8. Dela Cruz CS, Liu W, He CH, et al. Chitinase 3-like-1 promotes Streptococcus pneumoniae killing and augments host tolerance to lung antibacterial responses. *Cell Host Microbe*. 2012;12:34-46.

9. Warszawska JM, Gawish R, Sharif O, et al. Lipocalin 2 deactivates macrophages and worsens pneumococcal pneumonia outcomes. *J Clin Invest*. 2013;123:3363-3372.

10. Huang H, Ideh RC, Gitau E, et al. Discovery and validation of biomarkers to guide clinical management of pneumonia in African children. *Clin Infect Dis*. 2014;58:1707-1715.

11. Erdman LK, D'Acremont V, Hayford K, et al. Biomarkers of Host Response Predict Primary End-Point Radiological Pneumonia in Tanzanian Children with Clinical Pneumonia: A Prospective Cohort Study. *PLoS One*. 2015;10:e0137592.

12. Baggett HC, Watson NL, Deloria Knoll M, et al. Density of Upper Respiratory Colonization With Streptococcus pneumoniae and Its Role in the Diagnosis of Pneumococcal Pneumonia Among Children Aged <5 Years in the PERCH Study. *Clin Infect Dis*. 2017;64:S317-S327.

13. Simusika P, Bateman AC, Theo A, et al. Identification of viral and bacterial pathogens from hospitalized children with severe acute respiratory illness in Lusaka, Zambia, 2011-2012: a cross-sectional study. *BMC Infect Dis*. 2015;15:52.

14. Hammitt LL, Kazungu S, Morpeth SC, et al. A preliminary study of pneumonia etiology among hospitalized children in Kenya. *Clin Infect Dis*. 2012;54 Suppl 2:S190-199.

15. Oladokun R, Muloiwa R, Hsiao NY, Valley-Omar Z, Nuttall J, Eley B. Clinical characterisation and phylogeny of respiratory syncytial virus infection in hospitalised children at Red Cross War Memorial Children's Hospital, Cape Town. *BMC Infect Dis*. 2016;16:236.

16. Zar HJ, Barnett W, Stadler A, Gardner-Lubbe S, Myer L, Nicol MP. Aetiology of childhood pneumonia in a well vaccinated South African birth cohort: a nested case-control study of the Drakenstein Child Health Study. *Lancet Respir Med*. 2016;4:463-472.

17. Benet T, Sylla M, Messaoudi M, et al. Etiology and Factors Associated with Pneumonia in Children under 5 Years of Age in Mali: A Prospective Case-Control Study. *PLoS One*. 2015;10:e0145447.

18. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest*. 2003;111:1805-1812.

19. Wang HL, Hsiao PC, Tsai HT, Yeh CB, Yang SF. Usefulness of plasma YKL-40 in management of community-acquired pneumonia severity in patients. *Int J Mol Sci*. 2013;14:22817-22825.

20. James A. Serum YKL-40 is elevated in children with pneumonia and RSV infection. *European Respiratory Journal*. 2014;44.

21. Bachman MA, Miller VL, Weiser JN. Mucosal lipocalin 2 has pro-inflammatory and iron-sequestering effects in response to bacterial enterobactin. *PLoS Pathog*. 2009;5:e1000622.

22. Williams PCM, Isaacs D, Berkley JA. Antimicrobial resistance among children in sub-Saharan Africa. *Lancet Infect Dis*. 2017.

23. Blomberg B. Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: a prospective cohort study. *BMC Infectious Diseases*. 2007.

24. Leth-Larsen R, Nordenbaek C, Tornoe I, et al. Surfactant protein D (SP-D) serum levels in patients with community-acquired pneumonia. *Clin Immunol*. 2003;108:29-37.

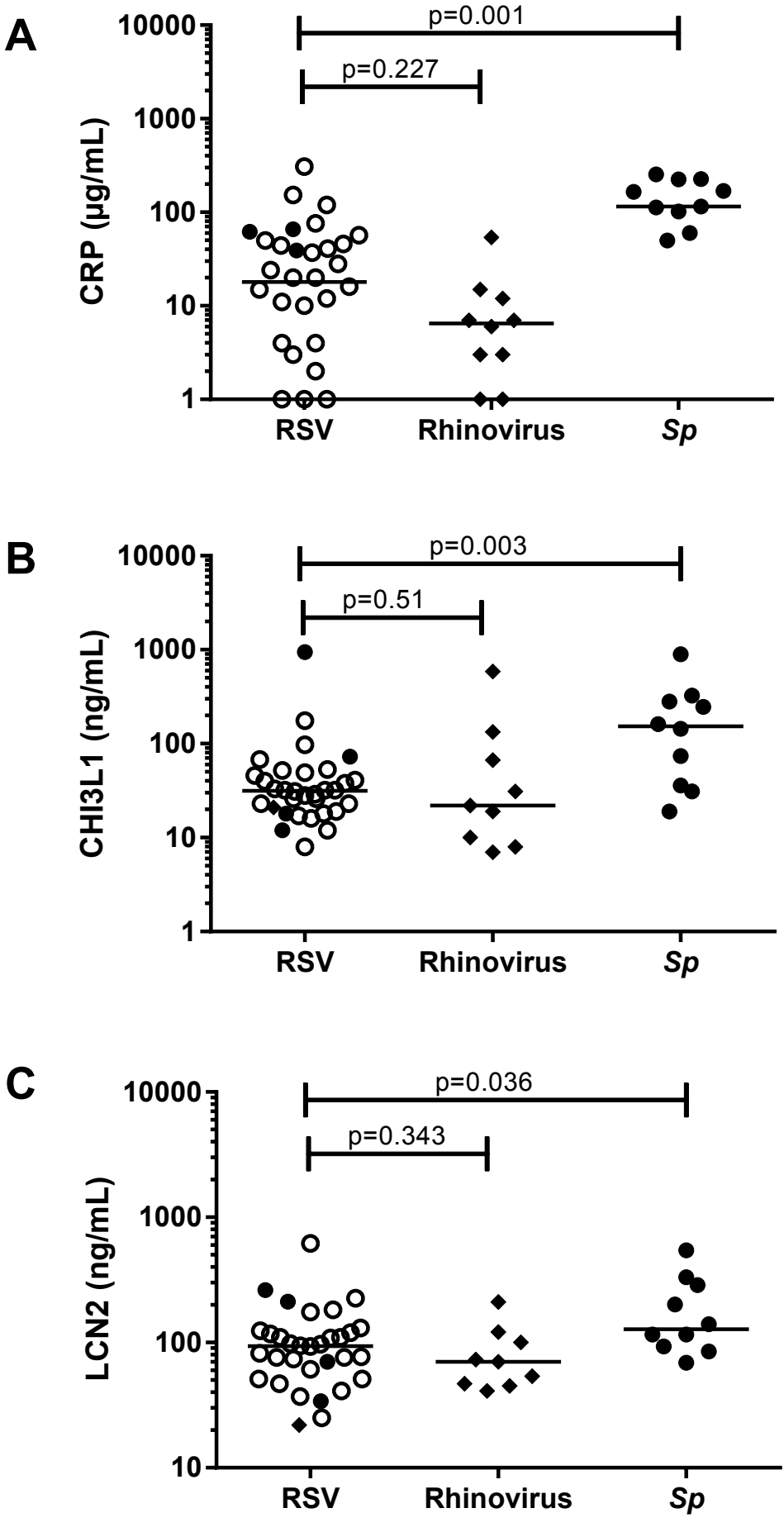
25. Fleming S, Thompson M, Stevens R, et al. Normal ranges of heart rate and respiratory rate in children from birth to 18 years of age: a systematic review of observational studies. *Lancet*. 2011;377:1011-1018.

26. Cherian T, Mulholland EK, Carlin JB, et al. Standardized interpretation of paediatric chest radiographs for the diagnosis of pneumonia in epidemiological studies. *Bull World Health Organ*. 2005;83:353-359.

Figure Legends

Figure 1: Levels of host inflammatory biomarkers in 58 African children hospitalized with respiratory tract infection. CRP **(A)**, CHI3L1 **(B),** and LCN2 **(C)** were similar in the RSV LRTI group (closed diamonds) compared to the rhinovirus RTI control group (open circles), but significantly lower than the *Sp* pneumonia control group (closed circles). Co-detection of rhinovirus or *Sp* in the RSV group is indicated by marker shape.

Figure 2: Time to resolution of tachypnea and total oxygen volume consumed in 37 African children hospitalized with RSV LRTI, dichotomized by median admission biomarker levels. CRP levels at admission did not correlate significantly with time to resolution of tachypnea or total oxygen volume consumed **(A)**; Children with admission CHI3L1 >32ng/mL **(B)** and LCN2 >94ng/mL **(C)** remained tachypneic longer and consumed more oxygen than children with lower levels of CHI3L1 or LCN2.


A
10000
1000
100
10
1
CRP (µg/mL)
p=0.001
p=0.227
RSV
Rhinovirus
Sp
B
10000
1000
100
10
1
CHI3L1 (ng/mL)
p=0.003
p=0.51
RSV
Rhinovirus
Sp
C
10000
1000
100
10
LCN2 (ng/mL)
p=0.036
p=0.343
RSV
Rhinovirus
Sp

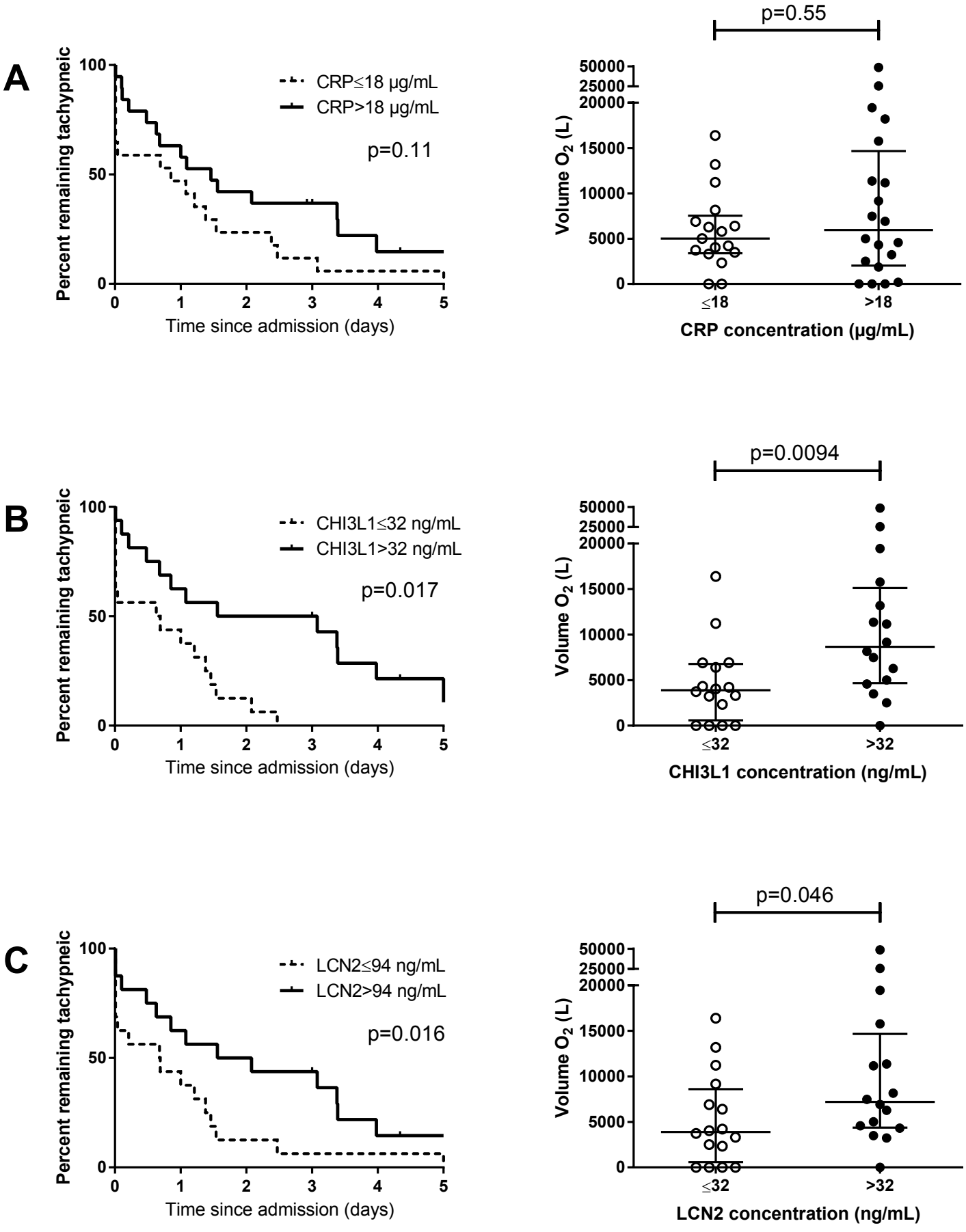

A
Percent remaining tachypneic
100
50
0
0
1
2
3
4
5
Time since admission (days)
CRP≤18 µg/mL
CRP>18 µg/mL
p=0.11
p=0.55
Volume O2 (L)
50000
25000
20000
15000
10000
5000
0
≤18
>18
CRP concentration (µg/mL)
B
Percent remaining tachypneic
Time since admission (days)
CHI3L1≤32 ng/mL
CHI3L1>32 ng/mL
p=0.017
p=0.0094
Volume O2 (L)
≤32
>32
CHI3L1 concentration (ng/mL)
C
Percent remaining tachypneic
Time since admission (days)
LCN2≤94 ng/mL
LCN2>94 ng/mL
p=0.016
p=0.046
Volume O2 (L)
≤32
>32
LCN2 concentration (ng/mL)

Table: Times to clinical endpoint in days measured in 58 African children hospitalized with respiratory tract infection, dichotomized by median biomarker values. Clinical markers (tachypnea and RISC) are given as comparators

CRP			
	≤18ug/mL	>18ug/mL	P-value
Time to resolution of tachypnea	0.85 (0-2.2)[1]	1.5 (0.28-2.6)[1]	0.11
Time to resolution of tachycardia	0.30 (0-0.67)	0.54 (0.17-0.90)	0.47
Time to resolution of fever	0.1 (0-0.22)	0.26 (0-0.52)	0.33
Time to wean oxygen	3.0 (1.9-4.0)	4.3 (1.8-6.9)	0.39
Time to breastfeed or eat	0.09 (0-0.18)	0.03 (0-0.07)	0.13
Time to sit	0.32 (0-0.83)	0.41 (0.043-0.77)	0.896
CHI3L1			
	≤32ug/mL	>32ug/mL	P-value
Time to resolution of tachypnea	0.70 (0-2.1)[1]	1.5 (0-4.5)[1]	**0.017***
Time to resolution of tachycardia	0.017 (0-0.18)	1.0 (0-3.0)	**<0.001***
Time to resolution of fever	0.12 (0.03-0.22)	0.27 (0-0.62)	0.486
Time to wean oxygen	2.2 (0.79-3.7)[1]	4.7 (3.4-6.0)[1]	**0.009***
Time to breastfeed or eat	0.02 (0-0.07)	0.02 (0-0.17)	0.297
Time to sit	0.036 (0-0.25)	0.62 (0-2.0)	**0.018***
LCN2			
	≤95ng/mL	>94ng/mL	
Time to resolution of tachypnea	0.68 (0.45-1.6)[1]	2.1 (2.3-4.5)[1]	**0.016***

Time to resolution of tachycardia	0.039 (0-0.93)	0.84 (0.34-1.3)	**0.001***
Time to resolution of fever	0.097 (0-0.2)	0.28 (0-0.58)	0.16
Time to wean oxygen	2.9 (1.6-4.2)	4.5 (2.3-6.7)	0.378
Time to breastfeed or eat	0[2]	0.098 (0-0.50)	**0.025***
Time to sit	0.11 (0-0.51)	0.57 (0-2.0)	0.10
Respiratory Rate for Age[3]			
	No Tachypnea	Tachypnea	P-value
Time to resolution of tachypnea	0	2.87 (0.0-19.88)	0.147
Time to resolution of tachycardia	0.31 (0.00-1.9)	1.03 (0.00-19.74)	0.468
Time to resolution of fever	0.68 (0.0-0.54)	0.69 (0.0-19.74)	0.147
Time to wean oxygen	2.8 (0.0-7.54)	3.29 (0.0-16.88)	1.0
Time to breastfeed or eat	0.079 (0.0-0.71)	0.12 (0.0-0.92)	0.493
Time to sit	0[2]	0.33 (0.0-2.03)	0.344
RISC Score			
	≤3	>3	P-value
Time to resolution of tachypnea	2.46 (0.0-19.88)	2.75 (0.0-10.09)	0.777
Time to resolution of tachycardia	0.97 (0-19.74)	0.82 (0-4.8)	0.777
Time to resolution of fever	0.78 (0-19.74)	0.18 (0-1.28)	0.777
Time to wean oxygen	3.24 (0-16.88)	3.16 (0-12.72)	0.777
Time to breastfeed or eat	0.058 (0-0.71)	0.23 (0-0.92)	0.065
Time to sit	0.25 (0-2.03)	0.41 (0-1.34)	0.844

* Indicates a statistically significant difference ($p<0.05$) between groups.

Times to endpoint are in days and expressed as mean (range), unless otherwise indicated

[1]Indicates time to endpoint expressed in median (IQR)

[2]All patients feeding from time of admission

[3]Respiratory rate>99th percentile for age{Fleming, 2011 #1}.