AMMI Canada Medical Student Research Proposal

Title: Diagnostic and Prognostic Biomarkers of Host Response to Respiratory Syncytial Virus Respiratory Tract Infection in Ugandan Infants and Children

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Background: Pneumonia was responsible for 700,000 deaths in children under five in 2015, making it the single largest infectious cause of childhood mortality worldwide.[1] An estimated 118,200 of those deaths could be attributed to Respiratory Syncytial Virus (RSV), and 99% of those occurred in low- and middle-income countries.[2,3]

Because there is significant overlap between the clinical syndromes of RSV and bacterial pneumonia and important differences in their treatment, objective diagnostic tests that can rapidly discriminate between infectious aetiologies are beneficial for guiding management.[4] To that end, proteomic signatures of host biomarkers have been shown to provide rapid and actionable clinical information to inform treatment decisions.[5-7] Effective triage and management of pediatric pneumonia also requires a determination of severity that, in many resource-limited settings, is often based on clinical assessment alone.[8] Host biomarker profiles capable of predicting clinical severity and outcomes could augment this assessment and allow for optimal resource allocation and treatment in cases of severe pneumonia.

Several candidate biomarkers of inflammation, pneumocyte injury, and endothelial activation have been examined in pneumonia. C-Reactive Protein (CRP), Chitinase-3-Like Protein 1 (CHI3L1) and Lipocalin-2 (Lipo2) are biomarkers of systemic inflammation. CRP and CHI3L1 have been previously examined in their capacity to distinguish between viral and bacterial pneumonia in children,[9-12] and one study undertaken in Tanzania showed that CRP and CHI3L1 in combination could predict radiographic findings for children with pneumonia when clinical information could not.[13] Similarly, Lipocalin-2 is an early indicator of poor prognosis in childhood pneumococcal pneumonia[14] and has been examined in its ability to distinguish pneumonia etiology and clinical severity in African children.[15] Surfactant Protein (SP)-D is a marker of pneumocyte injury that has been previously identified in both community-acquired pneumonia and RSV Bronchiolitis[16-18]. Endoglin and Intercellular Adhesion Molecule (ICAM)-1 are known markers of endothelial activation, and Endoglin has been shown to predict radiographic findings in Tanzanian children with clinical pneumonia.[13] ICAM1 has been examined in its role in RSV-induced cellular inflammation *in vitro*[19-21], yet little data exists investigating relationships between ICAM1 and clinical presentations of pneumonia.

Objective: The objective of this study is to describe admission characteristics, radiographic and laboratory abnormalities, biomarker profiles and clinical outcomes of children hospitalized with RSV respiratory tract infection in Uganda and compare them to two control groups: (1) children with rhinovirus upper respiratory tract infection; and (2) children with pneumococcal lobar pneumonia.

Methods: Study design and logistical considerations**:** The proposed project is a prospective observational 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 in the study if they: (1) were under 13 years of age; (2) required admission to hospital; and (3) presented with cough or difficulty breathing and signs of lower respiratory tract involvement, including tachypnea, chest indrawing, and/or hypoxemia[22]. Children with clinically suspected tuberculosis were excluded. Children underwent history and physical exam, had blood and nasopharyngeal NP swabs collected, and were followed over the course of hospital admission until discharge, death, or transfer to another facility with frequent monitoring of vital signs. Data

collection, sample collection, transport and storage[23-27] are already completed. Clinical data and samples from 181 patients are now available for analysis in the Hawkes Lab in Edmonton.

Determination of microbiological etiology: NP swabs collected in the field and stored in viral transport medium are available for analysis for all patients. A semi-automated nucleic acid extraction protocol will be used to perform nucleic acid extraction using 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) will be used to identify 33 potential respiratory pathogens in the NP swab. We will use an Applied Biosystems® 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA) to run these samples.

ELISA description: EDTA plasma, collected in the field and stored at -80C until shipment to Canada, is available for analysis for all patients. ELISAs will be used to quantify plasma biomarker levels and will be performed blinded to all associated clinical data. ELISAs will be performed according to the manufacturers' instructions, and the following markers will be quantified: CRP, CHI3L1, Lipo2, SPD, Endoglin and ICAM1. Background signal will be determined from blank wells included on each plate and subtracted from all samples and standards prior to analysis. Samples with optical densities below the lowest detectable standard will be assigned the value of that standard. A 4-parameter logistic regression curve fitted to data will be used to determine biomarker concentrations from the ELISA optical density against a standard curve from manufacturer standards.

Statistical Analysis: Continuous data will be presented as medians (interquartile range) and analyzed non-parametrically. Categorical data will be analyzed using Pearson's χ^2 test. The relationship between continuous variables will be assessed using the Mann-Whitney U test. Time-to-event analysis will be performed using Kaplan-Meier plots and using log-rank testing for differences between factor levels. Cases will be right censored at the time of last encounter.

Ethical considerations: This study has been approved by the School of Biomedical Sciences Research and Ethics Committee (Makerere University, Kampala, Uganda) and the Human Research Ethics Board of the University of Alberta. The Uganda National Council of Science and Technology also approved the study. Accompanying parents or legal guardians provided written informed consent at the time of enrolment.

Significance: The ultimate vision for this research is an inexpensive point-of-care lateral flow immunochromatographic platform for pneumonia biomarkers. Clinically informative biomarkers may improve accuracy of diagnosis and/or prognosis, toward the improved management and improved outcomes of childhood pneumonia in low-resource settings. In healthcare environments where resources are limited, diagnosis based on clinical features can lead to the inappropriate use of antimicrobials in patients with viral illness.[4] Research examining the capacity of biomarkers to indicate pneumonia etiology in African children may have important implications for the prevention of antimicrobial resistance in Sub-Saharan Africa. Triage and risk-stratification based on clinical presentation alone can also lead to inaccurate assessments of severity and inappropriate allocation of scarce resources.[8] Rapid-testing of biomarker profiles may provide physicians with more information on prognosis, thereby promoting increased survival of children with pneumonia and tackling one of the largest global causes of childhood mortality.

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