Solar Powered Oxygen Delivery: an open label non-inferiority randomized comparison to standard oxygen delivery

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OBJECTIVE

The objective of this proposal is demonstrate that solar energy can be used to concentrate oxygen from ambient air to improve peripheral blood oxygenation in children hospitalized with acute hypoxic respiratory illness in resource-constrained sub-Saharan African settings.

Our specific aims are:

1. *Implement solar oxygen system and collect operational data*
    - To install and monitor the functioning and costs of a solar-driven oxygen concentrator system at a resource-constrained pediatric centre in sub-Saharan Africa.
    - To generate operational data for future scale-up of solar-powered oxygen concentrator system: (a) quantify power requirements of the oxygen concentrator for individual pediatric patients with hypoxic respiratory illness; (b) quantify power provided by solar panels in an operational setting in sub-Saharan Africa.
    - To demonstrate that patients with hypoxic respiratory illness receiving oxygen by solar-powered oxygen concentrators have improved peripheral blood saturation.
    - To measure serum biomarkers of critical pathways of host defense to infection and hypoxia longitudinally over the course of hospitalization among children with hypoxic respiratory illness.
2. *Examine efficacy and cost-effectiveness of solar oxygen delivery relative to conventional oxygen delivery*
    - To compare a solar powered oxygen concentrator system to conventional oxygen delivery system (oxygen in pressurized cylinders) in terms of clinical efficacy, cost-effectiveness, and feasibility.
    - To equip and augment a pneumonia mortality surveillance system at 6 acute care centres across Uganda.
    - To implement solar powered oxygen at 3 intervention sites, comparing pneumonia outcomes at intervention and control sites.

The working hypothesis is that solar energy can be used to drive oxygen concentrators to improve outcomes in pediatric patients with hypoxic respiratory illness. This will be assessed in terms of feasibility in a first proof-of-concept stage at a single pediatric centre. The technology will then be compared to conventional

strategy for oxygen delivery using compressed air cylinders. Finally, extension of the technology to additional centres within a network of sentinel sites with pneumonia mortality surveillance will establish regional generalizability of the technology.

BACKGROUND AND LITERATURE REVIEW

Introduction

Globally, approximately 7.7 million children per year die before the age of 5 years. Infectious diseases account for a large proportion of these deaths, with pneumonia being the leading cause of mortality (2.1 million deaths/year) [1]. Most deaths occur in resource-poor settings in Africa and Asia [2]. Countries in Africa and Asia report 2 to 10 times more children with pneumonia (7 to 40/100 annually) than industrialized countries like the USA [3]. In Uganda alone, child mortality is estimated to be 145,000 deaths per year. Bacterial pneumonia, tuberculosis, sepsis and severe malaria are common infectious etiologies; all of which lead to respiratory distress as a final common pathway. Oxygen (O_2) therapy is essential to support life in these patients. Large gaps remain in the case management of children presenting to African hospitals with respiratory distress, including essential supportive therapies such as supplemental oxygen. In resource-constrained settings, oxygen delivery systems can lead to measurable improvements in survival from childhood pneumonia. A multihospital effectiveness study in Papua New Guinea demonstrated a reduction in mortality from childhood pneumonia from 5.0% to 3.2% (35% reduction in mortality) after implementation of enhanced oxygen delivery system [4]. We propose to investigate a novel strategy for oxygen delivery that could be implemented in remote locations with minimal access to an electrical power supply: solar-powered oxygen (SPO2).

Literature Review

The World Health Organization (WHO) Integrated Management of Childhood Illnesses (IMCI) provides a classification of pneumonia based on clinical features, differentiating 3 risk strata: (1) cough and cold; (2) pneumonia; (3) severe pneumonia or very severe disease [5].

Clinical features of pneumonia in children include fever, respiratory distress, and hypoxemia. Respiratory distress is a useful clinical summary description with good inter-observer consistency among experienced medical practitioners [6]. The following clinical signs may indicate increased work of breathing: sustained nasal flaring; indrawing (recession) of the bony structures of the chest wall (subcostal, intercostal, supraclavicular) on inspiration; tracheal tug; and deep breathing (acidotic or

Kussmaul breathing). Respiratory distress is a sign that one or more serious pathological processes are at play: metabolic acidosis, fluid overload, acute lung injury, and/or co-morbid pneumonitis. Respiratory distress, together with alteration of consciousness, is a strong predictor of mortality in children with severe febrile illness in sub-Saharan Africa [7]. The grim prognostic significance of respiratory distress applies to several disease states, irrespective of microbial etiology, including malaria as well as non-malaria febrile illness [7].

Arterial hypoxemia in pneumonia results from several mechanisms: pulmonary arterial blood flow to consolidated lung resulting in an intrapulmonary shunt, intrapulmonary oxygen consumption, and ventilation-perfusion mismatch [8]. Hypoxemia is a risk factor for mortality in pediatric pneumonia, and was associated with a 5-fold increased risk of death in studies from Kenya and Gambia [9].
In one report from Nepal, the prevalence of hypoxemia (SpO2 < 90%) in 150 children with pneumonia was 39% overall, with increasing rates of hypoxemia across strata of pneumonia severity (100% of very severe, 80% of severe and 17% of pneumonia patients) [10]. General features of respiratory distress were associated with hypoxemia in this study, including chest indrawing, lethargy, grunting, nasal flaring, cyanosis, inability to breastfeed or drink.

Few studies have reported on the use of solar powered oxygen delivery. One online report describes the use of a battery-powered oxygenator in the Gambia that could be adapted to use solar power (http://www.dulas.org.uk). Otherwise, our intervention is to our knowledge the first example of solar powered oxygen delivery.

Among the etiologies of respiratory distress in patients presenting to hospital in sub-Saharan Africa, viral respiratory tract infection, bacterial pneumonia, severe malaria and tuberculosis are important considerations. Viral bronchiolitis is a prevalent respiratory illness among infants and young children. Respiratory syncitial virus, influenza virus, parainfluenza, adenovirus, coronaviruses, and human meta-pneumovirus are common etiologic agents. Bronchiolitis usually begins with upper respiratory tract symptoms, including cough and rhinorrhea. Children are often irritable and feed poorly. Later manifestations include fever, wheeze, tachypnea, use of accessory muscles of respiration, and nasal flaring. Mucous plugging or alveolar involvement (pneumonitis) may result in hypoxemia. Supportive treatment is generally sufficient, although specific antiviral treatment is available for influenza, RSV and adenovirus and may be beneficial in selected patients. Outcomes are generally favourable, although admission to an intensive care unit for respiratory failure is a complication of approximately 1% of RSV hospitalizations.

Bacterial pneumonia is a serious infection of the respiratory tract and a common cause of pediatric mortality. With antibiotic therapy, outcomes are generally favourable, although complications such as parapneumonic effusion, empyema, bronchospasm, and hypoxia contribute to morbidity. The pathogenesis often begins with damage to mucociliary clearance due to viral respiratory tract infection. Damage to terminal bronchioles may lead to air trapping and distal atelectasis. Superimposed bacterial infection on injured airways may lead to inflammation and alveolar exudates. The resulting barrier to oxygen exchange at the level of the alveolar-capillary unit may lead to hypoxemia. Complications of bacterial pneumonia in children include apnea, septicemia, pleural extension, lung abscess, and necrosis of the lung parenchyma. Community-acquired bacterial pneumonia in older infants and children may be caused by a variety of bacterial and viral pathogens, including *Streptococcus pneumoniae*, *Haemophilus influenzae, Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Staphylococcus aureus,* and *Bordetella pertussis*. Based on a review of 8 studies from Africa and South America, bacteria were recovered from lung aspirates in 56% of severely ill children. *Streptococcus pneumoniae* (33%) and *H. influenzae* (21%) were the most frequently identified bacterial pathogens. Of note, use of pneumococcal and *H. influenzae* type b conjugate vaccines could reduce the frequency of childhood pneumonia by one-third [3]. Antibiotic therapy is indicated for children with suspected bacterial pneumonia.

Severe malaria represents another common cause of respiratory distress in the sub-Saharan context. Malaria is the most common diagnosis among hospitalized children in Africa [7] and is associated with a mortality of 10-30%, even with highly effective anti-parasitic medications [11]. In sub-Saharan Africa, cohort studies indicate that 17-29% of patients with severe malaria presenting to hospital emergency facilities have respiratory distress [12]. The presence of deep breathing, characterized by increased inspiratory and expiratory excursion of the chest is generally associated with metabolic acidosis and portends a high mortality [6, 13]. The pathophysiology of respiratory distress in malaria may involve one or more mechanisms, including non-cardiogenic pulmonary edema and acidosis. Pulmonary edema is a well recognized complication of malaria in adults [14], but appears to be less common in children. In children hospitalized with malaria in sub-Saharan Africa, metabolic acidosis and hypovolemia are common presenting signs [15]. Echocardiography reveals severe tachycardia, low stroke volume index, and high inferior vena cava collapsibility index [16]. Thus, impaired tissue perfusion, metabolic acidosis, and Kussmaul breathing, representing an acute pulmonary compensation to systemic metabolic acidosis, appear to account for most cases of respiratory distress in children. Of note, lactic acidosis is a prognostic marker for mortality in children with severe malaria [17], and its association with respiratory distress

represents a final common pathway of decompensated shock, cardiopulmonary insufficiency and impending death. Furthermore, hypoxia related to pulmonary involvement may itself provoke convulsions and deterioration in the level of consciousness and may be the immediate precipitant of death.

Host response to pneumonia

In addition to examining solar-powered oxygen concentrators as a novel oxygen delivery system, we propose to investigate host response in the context of hypoxic respiratory illness. The following literature review focuses on pathways of host response to pneumonia and its causative pathogens.

Inflammation

Excessive pro-inflammatory responses to infection are observed in severe infections such as malaria, pneumonia, and sepsis [18]. The acute-phase reactants C-reactive protein (CRP) and procalcitonin (PCT) are elevated in the setting of bacterial infection [19]. These markers have been used in clinical practice to distinguish between severe bacterial infection and benign causes of fever in children. Other potentially informative inflammatory markers include the 10 kDa interferon gamma-induced protein (IP-10), a chemokine elevated in fatal malaria [20], and soluble triggering receptor expressed on myeloid cells-1 (sTREM-1). The triggering receptor expressed on myeloid cells-1 (TREM-1) mediates pro-inflammatory responses [21]. Inhibition of TREM-1 improves outcome in murine models of sepsis [22]. Membrane density of TREM-1 on peripheral monocytes appears to be increased in patients with uncomplicated malaria cases compared to uninfected individuals [23]. Soluble TREM-1 (sTREM-1) is generated by cleavage of membrane TREM-1 upon myeloid cell activation [24], thereby serving as peripheral blood marker of leucocyte stimulation. Both membrane and sTREM-1 are increased in inflammatory pathologies in humans [25]. We will assess these host proteins among children hospitalized for hypoxic respiratory failure to assess their potential role in pathogenesis.

Endothelial activation

Widespread endothelial activation with capillary leak leads to multi-system organ failure in severe sepsis [26] and malaria[27]. Endothelial activation in the pulmonary vasculature may account in part for impaired gas exchange and hypoxemia in pediatric pneumonia. Following activation by pro-inflammatory stimuli, soluble endothelial cell receptors are released into the circulation via ectodomain shedding or alternative splicing, such that peripheral blood levels of key regulatory proteins reflect the state of whole body endothelial activation. We will examine several critical molecules involved in the regulation of endothelial quiescence and activation.

Intercellular adhesion molecule-1 (ICAM-1) is a cell surface glycoprotein that mediates leukocyte adhesion and trafficking. The soluble form of ICAM-1 (sICAM-1) is increased in sepsis and severe malaria [28, 29]. Likewise, the cell surface receptor *P-selectin* may be shed from the endothelium in severe disease. We hypothesize that elevated levels of sICAM-1 and/or sP-selectin may also predict adverse outcomes in hospitalized African children.

Soluble FMS-like tyrosine kinase-1 (sFlt-1) is generated by alternative splicing of VEGF receptor-1 mRNA and antagonizes the pro-inflammatory and pro-angiogenic effects of VEGF. sFlt-1 is elevated in children with severe malaria as well as sepsis patients [30]. Data from murine models of sepsis suggest that sFlt-1 may play a modulatory role [31], counteracting increased VEGF tissue expression and plasma levels, associated with cerebral malaria [32]. Similarly, *endoglin*, a component of the tumor necrosis factor-beta (TGF-β) receptor complex, participates in inflammatory signaling pathways. The soluble form of the receptor (s-endoglin) is shed from the endothelial surface into the circulation in the setting of critical illness. We hypothesize that sFlt-1 and/or s-endoglin may be clinically informative markers of disease severity in hospitalized African children.

Angiopoietins (Angs) represent a distinct family of vascular growth factors that are increasingly recognized for their role in infectious disease pathogenesis. Four molecules have been characterized, Ang-1, Ang-2, Ang-3, and Ang-4, which signal through the Tie-2 receptor on endothelial cells. Ang-1 is secreted from pericytes within the vascular intima, whereas Ang-2 is stored with von Willebrand factor in Wiebel-Palade bodies. Ang-1 and Ang-2 act antagonistically: Ang-1 promotes stability of the vascular endothelium and Ang-2 promotes permeability of the blood-tissue barrier [26, 33]. Ang-2 also functions as an autocrine regulator by sensitizing the endothelium to the effects of TNF, resulting in increased adhesion receptor expression [34]. Like other endothelial surface molecules, the Tie-2 receptor is released into the circulation in severe illness. Our group has previously described the clinical utility of peripheral blood angiopoietins and sTie-2 for prognosis in sepsis [26] and malaria [27, 35, 36]. We hypothesize that their clinical utility may also be extended to common childhood illnesses requiring hospital admission.

Coagulation

Coagulopathy is a well-recognized manifestation of several severe infectious syndromes such as meningococcemia, sepsis and severe malaria. In addition, regulation of the coagulation cascade is intimately linked to the vascular endothelium, such that alterations in the profile of coagulation proteins also reflect activation and/or dysfunction of the endothelium. Like Ang-2, vWF and its precursor,

vWF propeptide, are stored within WP bodies and released into the circulation in response to inflammatory stimuli. We plan to evaluate vWF and its precursor, vWF propeptide, as potential markers of severe disease in hospitalized African children.

METHODOLOGY

A. Proof-of-concept and operational data

This phase of the project will involve the installation and monitoring of a SPO2 system at Jinja Regional Referral Hospital, Uganda. An existing 4-bed intensive care unit will be refurbished to accommodate a stand-alone “off-the-grid” solar electricity system and an oxygen concentrator capable of delivering up to 10L/min of O_2 to 2 patients simultaneously. The unit also has the capacity to deliver O_2 by cylinder as a failsafe mechanism, and has backup electrical supply including a generator, battery and inverter system.

The electrical specifications of the system (power generated by solar panels and power consumed by oxygen concentrator) will be measured in order to inform prototype design and future scale-up of the technology. Daily and seasonal variations in solar power will be of particular interest in this descriptive phase of the study.

Patients with hypoxic respiratory failure will approached for consent to participate in the study and we will monitor the peripheral blood oxygen saturation before and after administration of supplemental SPO2 among consenting subjects. This phase of the study will be descriptive (case series) in order to demonstrate the clinical efficacy of solar-powered oxygen.

B. Comparison to standard oxygen-delivery sytem: compressed oxygen cylinders

Randomized prospective controlled study. In order to compare the new oxygen delivery system to a conventional method (oxygen in compressed cylinders), we will prospectively enroll children with hypoxic respiratory illness. Children will be randomly assigned to receive oxygen using either solar-powered oxygen or oxygen from compressed air cylinders in a 1:1 ratio. Enrolment will occur at 2 sites: Jinja Regional Referral Hospital and Kambuga District Hospital.

The *a priori* hypothesis is that solar-powered oxygen will be non-inferior to a conventional oxygen delivery system in terms of a clinically meaningful outcome: length of hospital stay.

Secondary study endpoints will be: mortality, duration of supplemental oxygen therapy (time to wean O_2), proportion of patients successfully oxygenated, delivery system failure, cost, system maintenance and convenience.

C. Microbial etiology and biomarkers and critical illness pathways in pediatric pneumonia

Samples obtained in the course of admission may be analysed for evidence of bacterial and viral pathogens. Nasal swabs may be collected at admission for storage and subsequent analysis for viral pathogens. Peripheral blood samples may be analysed using molecular techniques for *Streptococcus pneumoniae*, a common and vaccine-preventable bacterial etiology of childhood pneumonia.

As an additional element of this prospective study, we propose to examine peripheral blood biomarkers of host response to infection and genetic factors, in order to gain insights into pneumonia pathogenesis. This aspect of the study aims to examine activation of critical illness pathways (inflammation, angiogenesis, coagulation and endothelial stability) in pediatric hypoxemic pneumonia over the time course of hospital admission. This will be achieved using several selected peripheral blood proteins, alone and in combination, as quantitative biomarkers of critical pathobiological pathways.

Our prospective study will longitudinally follow peripheral blood levels of key host biomarkers of inflammation, hypoxia, angiogenesis, coagulation and endothelial stability in patients hospitalized with hypoxic respiratory illness. This will yield rich insights into biological pathways activated in disease and their kinetics during recovery from illness. Furthermore, we will link information about clinical outcomes (i.e., mortality and length of admission) to quantitative biomarker levels and their dynamic profile over the course of hospitalization. This information may demonstrate the clinical utility of these biomarkers for patient triage, diagnosis and assignment to advanced therapeutics. Our findings may accelerate the development of new tools for case management that can assist practitioners in resource-constrained African hospitals providing health care to children with hypoxic respiratory illness. New tools for clinical care, in turn, may save lives in resource-constrained settings by focusing efforts toward improved child survival.

Protein biomarkers will be measured using commercially-available enzyme-linked immunosorbent assays (ELISA). Testing for genetic markers of host response to infection may involve micro-array and/or deep sequencing techniques. Relevant biomarkers from critical illness pathways will include the following (although

additional biomarkers may be considered as information becomes available in the literature):

Biomarkers of inflammation

- soluble endoglin
- soluble FMS-like tyrosine kinase-1 (Flt-1)
- C-reactive protein
- procalcitonin
- 10 kDa interferon gamma-induced protein (IP-10)
- soluble triggering receptor expressed on myeloid cells-1 (TREM-1)

Biomarkers of coagulation

- von Willebrand Factor (vWF)
- vWF propeptide

Biomarkers of angiogenesis and endothelial stability

- Angiopoietin-1
- Angiopoietin-2
- soluble Tie-2
- soluble P-selectin
- soluble intercellular adhesion molecule-1 (ICAM-1)

D. Multi-centre prospective evaluation of solar-powered oxygen

This phase of the study will involve roll out of SPO2 delivery systems to intervention sites across Uganda, with a comparison to control sites without SPO2. An inpatient mortality surveillance system is already in place across 6 sentinel sites in Uganda (led by co-investigator Dr. Mpimbaza). The first step will be to augment the capacity of the surveillance sites for pulse oximetry and data capture of key parameters of pneumonia (e.g., respiratory rate). Once the pneumonia mortality surveillance system is operational at all 6 sites and baseline mortality data has been collected, we will implement SPO2 systems at 3 of the 6 sites (intervention sites) and prospectively monitor pneumonia-specific mortality rates at both intervention and control sites. The hypothesis is that introduction of SPO2 systems will result in a lower pneumonia-specific mortality rate at intervention sites, whereas minimal change in the mortality rate will be observed at the control sites. The analysis will be based on a comparison of change (before-after) in pneumonia-specific mortality at intervention sites versus control sites. In the interest of equity, control sites may be offered solar oxygen delivery systems at the end of the data collection period.

Subjects and methods

Patients under 16 years of age admitted to Jinja Regional Referral Hospital, and surveillance sites across Uganda with respiratory illness and hypoxemia.

Study sites

The Jinja Regional Referral Hospital serves 6 districts in mid eastern Uganda. It is also a training hospital for the school of nursing. The hospital has 2 campuses; a wing containing the children unit and larger wing for the rest of the patients as well as the administration and main laboratory. The children's unit is staffed by a clinical team that includes 5 pediatricians, among them Dr. Sophie Namasopo (co-investigator in this proposal). Kambuga District Hopsital, in Kanungu district, has a pediatric ward serviced by clinical officers, a medical officer and four nurses. The 6 surveillance sites are geographically dispersed across Uganda: Jinja, Kambuga, Tororo, Kabale, Apac, and Mubende.

Inclusion criteria

1. Age <13 years
2. IMCI defined pneumonia, severe pneumonia or very severe disease
3. Hypoxemia (SpO2<90%) based on non-invasive pulse oximetry
4. Hospital admission warranted based on clinician judgment
5. Consent to blood sampling and data collection

Exclusion criteria

1. SpO_2 ≥90%
2. Suspected pulmonary tuberculosis
3. Outpatient management
4. Denial of consent to participate in study

The reason for exclusion of pulmonary tuberculosis is for infection control, in order to avoid the risk of transmitting tuberculosis to other patients housed in the same unit.

Outcomes

Primary outcome measure: Length of stay (number of days in hospital from admission to discharge)

Secondary outcome measures:

- Mortality
- Duration of supplemental oxygen therapy (time to wean O_2)
- Proportion of patients successfully oxygenated
- Proportion of cases with oxygen delivery system failure (requiring backup oxygen)

- Cost
- System maintenance
- Convenience of use
- LODS (Lambaréné Organ Dysfunction Score) [48]

Several clinical severity scoring systems have been published including: PRISM (Pediatric Risk of Mortality) [49], PIM (Pediatric Index of Mortality [49], sMODS (simplified Multi-Organ Dysfunction Score), PELOD (paediatric logistic organ dysfunction) [50]. These scoring systems, unlike LODS, make use of laboratory parameters to derive a total prognostic score, which are not available in our resource-limited hospital, as elsewhere in other African settings where child mortality is highest [51].

Sample size

We will enroll a total of 130 hospitalized patients. The primary endpoint is length of stay and the hypothesis tested for the primary analysis is that SPO2 is not inferior to standard oxygen delivery (compressed air cylinders).

This sample size was calculated as follows. A 1:1 ratio of patients will be assigned to SPO2 or control (O_2 from cylinders). We consider a 1 day prolongation of hospital stay to be clinically significant (non-inferiority margin). Using pilot data from a prospective study at Jinja Regional Referral Hospital, 69 patients with hypoxic pneumonia had a mean (standard deviation) length of stay of 2.6 (2.1) days. By standard calculations for normally distributed data, 57 patients in each group will provide 80% power to demonstrate non-inferiority of SPO2 relative to oxygen by cylinder. This sample size was increased by 15% to account for probable non-Gaussian distribution of the data [52].

Statistical Considerations

Primary outcome

Length of stay will be coded as a whole number (continuous variable). Non-parametric tests (Mann-Whitney) will be used to compare the length of stay between groups. The level of significance will be $\alpha=0.05$. The hypothesis test is framed as follows: H_0: the length of stay among patients treated with SPO2 is greater than the length of stay among patients treated with oxygen by compressed air cylinder. The alternative hypothesis, that SPO2 delivery in non-inferior, will be accepted (power=80%) if $p>0.05$ (one-sided), or if the length of stay is significantly shorter in the SPO2 group.

Secondary outcomes

The mortality will be compared between groups using the chi-squared statistic, or Fisher's exact test, as appropriate. Proportion of patients successfully oxygenated and proportion of patients for whom the oxygen delivery system failed will be analysed in a similar manner. The duration of oxygen therapy will be compared using non-parametric methods (Mann-Whitney U-test) and/or Cox-proportional hazard models. Costs will be carefully tabulated (capital investment, ongoing costs, system maintenance, etc.) and summarized for each group of patients. Details of maintenance needs and convenience of use of the systems will be documented and tabulated. The LODS (clinical severity score) will be assessed and recorded serially over the course of hospital admission; comparison between groups will make use of mixed effects linear models to compare the longitudinal time course of recovery in the clinical severity score.

Biomarkers of host response to infection will be measured serially throughout the course of hospital admission. Quantitative peripheral blood levels of these biomarkers with be compared using mixed-effects linear models to compare the longitudinal time course of the change in biomarker levels.

Study Procedures

For pediatric patients presenting to Jinja Regional Referral Hospital in whom admission to hospital is deemed necessary by an attending physician, the parent or guardian will be approached for consent to participate in the study. If granted, the patient will be transferred to a dedicated pneumonia ward, where the patient will be randomized to receive oxygen with the SPO2 system, or with oxygen cylinders (control group). All patients will receive standard care for their underlying disease, including antibiotics for pneumonia, intravenous fluids as necessary, blood transfusion as necessary, antipyretics, and any other medical therapy required. A small volume (1mL) of blood will be withdrawn for processing and storage at admission and daily during the course of hospitalization. A RDT for malaria will be tested at admission, and whole blood lactate level will be performed at the bedside for each of the daily blood draws. Basic demographic and clinical data will be collected from the case admission record, and patients will be followed during their hospital admission. Possible outcomes will include: death, discharge without disability, discharge with disability, abscondment, and loss to follow-up. The length of stay among survivors will be recorded.

Serum samples will be shipped to the collaborating laboratory in Canada for analysis for biomarkers. ELISA-based commercially-available assays for biomarker levels will be used to quantify biomarker levels. In order to measure levels of 13 biomarkers

from a plasma sample of 500uL or less, highly co-ordinated procedures with experienced technicians are required to perform the ELISA. Our laboratory in Canada has established protocols, experienced staff able to perform the testing, as well as equipment and reagents allowing the testing to be done efficiently. While it would be desirable to augment Ugandan capacity for biomarker testing, this would require significant investment of time and resources for training and testing, and may not be feasible in the context of this early study. If biomarkers can be identified that have clinical utility, laboratory capacity for ELISA measurement of levels should be developed or a simplified platform (e.g., lateral flow immunochromatographic test) should be developed.

Pneumonia will be diagnosed clinically, on the basis of WHO (IMCI) criteria. A combination of tachypnea, respiratory distress (nasal flaring, intercostal and/or subcostal indrawing, or cyanosis) and characteristic findings on chest auscultation (asymmetrical air entry, crackles, dullness to percussion) will be used to make a clinical diagnosis of pneumonia. In our setting, chest x-ray is not available on site and radiographic confirmation will not be routinely available.

Safety monitoring

Adverse events will be monitored by study staff and recorded on a log. The relatedness to the study intervention (oxygen) and the severity of the adverse event will be judged by the study personnel. Serious adverse events, defined as those which lead to death, permanent disability, or that prolong hospitalization will be reported in an expedited manner to the SOBSREC and to the UNCST. Other adverse events will be tabulated and presented in an annual report and/or final report. Patients with adverse events will be managed according to Uganda National Guidelines.

Limitations of the study

One limitation of our study is the lack of radiographic confirmation of pneumonia and microbiological confirmation of the infectious etiology in some cases. We will be able to diagnose malaria, based on rapid diagnostic tests; however, bacterial, viral, fungal and other parasitic organisms cannot be routinely identified. We may collect samples (nasal swabs) for viral diagnostics, and we may analyse stored samples for molecular diagnosis of pneumococcus, if resources permit. Additional laboratory testing such as blood culture, sputum culture, viral studies, and a panel of infectious diseases serology would be necessary to definitively diagnose conditions like pneumococcal pneumonia, viral respiratory tract infections, and *Mycoplasma* infection. However, resource limitations preclude an exhaustive battery of diagnostic

tests in our setting and others. Although precise microbiological etiology may not be known, length of stay (primary outcome) as well as mortality (secondary outcome) are clinically meaningful outcomes that can be assessed unambiguously in the context of our study. This study will allow us to demonstrate the non-inferiority of SPO2 for treatment of hypoxic respiratory illness, irrespective of etiology.

ETHICAL CONSIDERATIONS

Risks related to the study

Oxygen therapy is known to be a life-saving intervention, and this study will improve hospital capacity for the treatment of hypoxemic children. While patients will be randomized to one of two possible oxygen delivery systems, there are no risks, and potentially life-saving benefits to receiving oxygen, regardless of delivery modality.

One minimal risk associated with the study relates to drawing blood samples, which will be of low volume and will be collected by experienced practitioners. There is no risk of anemia related to removal of such a small volume of blood. The risk of infection will be minimized by careful decontamination of the puncture site using alcohol. Pain may be minimized using topical anesthetic, according to the practitioner's judgment.

Study participants will be managed at the Jinja Regional Referral Hospital according to national guidelines and local clinical practices. Outcomes assessed in the study will not pose any risk to the study participants (abstracted from the chart record).

Institutional Review Board

The Makerere University (School of Biomedical Sciences) Research and Ethics Committee (SBS-REC), together with the Institutional Review Board (IRB) of the University of Toronto will review this protocol. The Uganda National Council of Science and Technology (UNCST) will also review the study.

No deviation from, or changes to the protocol will be made without prior review and documented approval/favorable opinion from the IRB/independent or institutional ethics committee (IEC) of an amendment, except where necessary to eliminate an immediate hazard(s) to participants, or when the change(s) involve(s) only logistical or administrative aspects of the study (eg, change of monitor(s), change of telephone number[s]).

Subject Confidentiality

Study participant information will be kept strictly confidential. Study data will be accessible only to study personnel. Study data will be stored long-term in files and computer databases in locked offices. Access to databases will be password-restricted, and network security measures will be in place to ensure that information cannot be retrieved by personnel not involved with the study. The study monitor or other authorized representatives may inspect all documents and records required to be maintained by the PI, and the study site will permit access to such records.

Potential benefits of the proposed research to study participants and others:

Current practices at Jinja Regional Referral Hospital for oxygen delivery involve the use of oxygen based on clinical judgment (without the routine use of pulse oximetry). Oxygen concentrators may not be in good repair, or the electrical supply may be inconsistent. Oxygen masks and nasal prongs are available, but of inconsistent quality. This study will substantially improve the care that patients receive with respect to oxygen therapy, with quality-assured devices, well-maintained, consistent power and quality equipment (masks, nasal prongs). Thus participants with hypoxemia will benefit directly from participating in the study with improved delivery of a potentially life-saving therapy. Furthermore, we hope that this study may be an impetus for increased research, which is a potential long-term benefit of the study to the community. If this study documents the clinical utility of SPO2, this would represent an important finding with the potential to accelerate development of clinical tools to promote child survival through improved oxygen therapy across the country and globally.

Consent

Informed consent will be obtained from the caregivers of all children that will participate in the study. The consent process shall be initiated at the time of enrolment into the study and shall continue throughout the child's participation. The consent will be done in the language that the caregiver best understands. If the caregiver provides consent for study participation, the caregiver will be given the consent forms to sign. A copy of the consent form will be given to the caregiver to keep while a duplicate copy will be kept in the patient's file

For those who do not know how to read and write, an independent witness will be present during the informed consent process and will sign the consent form as a witness. The caregivers may withdraw consent at any time throughout the course of the study, and this will be made clear in the informed consent process. All individuals will be informed that there is no requirement to join the study and that standard medical care will remain the same regardless of study enrollment.

If the caregiver chooses not to have their child participate in the study, the case will be turned over to the clinical team on duty for routine care of their condition.

UTILITY OF THE STUDY

New ways to deliver oxygen for children with pneumonia in Africa could improve outcomes and save numerous lives. If this study documents the non-inferiority of SPO2 relative to standard oxygen delivery, this novel method of providing life-saving oxygen could be rolled out across centres in sub-Saharan Africa where oxygen cylinders are not widely available and electrical power is not reliable. Our study incorporates a plan for expansion of the SPO2 systems to several sentinel sites across Uganda, with surveillance for pneumonia mortality that could demonstrate a mortality benefit of SPO2 systems. The potential energy efficiency, low cost and ease of use make solar power an attractive avenue of investigation for use in resource-constrained settings. Proof-of-concept that the sun can be used to drive oxygen delivery could stimulate commercial interest in this technology. The SPO2 system could thus achieve rapid penetration into the most remote or rural settings in sub-Saharan Africa.

CAPACITY BUILDING

This project will build capacity for clinical care of children with pneumonia at Jinja Regional Referral Hospital, Kambuga Hospital, and other sentinel surveillance sites across Uganda. Oxygen therapy is a basic and life-saving intervention that remains sub-optimal in many resource-poor settings. This project will build local capacity for quality assured oxygen delivery. Furthermore, we hope to build capacity for research and innovation at Jinja Hospital and Makerere University. Four study nurses, additional support nursing staff, and two medical officers will be trained in good clinical practices, research methodology and data management. Training will provide added skills including use and maintenance of oxygen concentrators, pulse oximetry, RDT for malaria and point-of-care lactate measurement. Equipment (e.g., Lactate Scout point-of-care instrument for lactate measurement) will remain at Jinja Hospital after the conclusion of the study, providing a valuable tool for improved laboratory diagnostics and patient care. With respect to laboratory skills, a Masters or PhD student may be able to learn ELISA techniques for testing samples in our study, depending on available funding. A Masters or PhD student could also gain skills in clinical epidemiology, by assisting with the data analysis and write-up of this study. Finally, participation in the study design, implementation, analysis and

presentation of data will build capacity among local study team investigators for future research activities.

REFERENCES

1.Bawaskar HS. The world's forgotten children. Lancet 2003;361:1224-5.

2.Black RE, Morris SS, Bryce J. Where and why are 10 million children dying every year? Lancet 2003;361:2226-34.

3.Nascimento-Carvalho CM. Etiology of childhood community acquired pneumonia and its implications for vaccination. Braz J Infect Dis 2001;5:87-97.

4.Duke T, Wandi F, Jonathan M, et al. Improved oxygen systems for childhood pneumonia: a multihospital effectiveness study in Papua New Guinea. Lancet 2008;372:1328-33.

5.Handbook Integrated Management of Childhood Illness. In: World Health Organization; 2005.

6.English M, Waruiru C, Amukoye E, et al. Deep breathing in children with severe malaria: indicator of metabolic acidosis and poor outcome. Am J Trop Med Hyg 1996;55:521-4.

7.Reyburn H, Mbatia R, Drakeley C, et al. Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. BMJ 2004;329:1212.

8.Light RB. Pulmonary pathophysiology of pneumococcal pneumonia. Semin Respir Infect 1999;14:218-26.

9.Onyango FE, Steinhoff MC, Wafula EM, Wariua S, Musia J, Kitonyi J. Hypoxaemia in young Kenyan children with acute lower respiratory infection. BMJ 1993;306:612-5.

10.Basnet S, Adhikari RK, Gurung CK. Hypoxemia in children with pneumonia and its clinical predictors. Indian J Pediatr 2006;73:777-81.

11.Newton CR, Krishna S. Severe falciparum malaria in children: current understanding of pathophysiology and supportive treatment. Pharmacol Ther 1998;79:1-53.

12.Idro R, Aloyo J, Mayende L, Bitarakwate E, John CC, Kivumbi GW. Severe malaria in children in areas with low, moderate and high transmission intensity in Uganda. Trop Med Int Health 2006;11:115-24.

13.Marsh K, Forster D, Waruiru C, et al. Indicators of life-threatening malaria in African children. N Engl J Med 1995;332:1399-404.

14.Taylor WR, Canon V, White NJ. Pulmonary manifestations of malaria : recognition and management. Treat Respir Med 2006;5:419-28.

15.Maitland K, Pamba A, Newton CR, Levin M. Response to volume resuscitation in children with severe malaria. Pediatr Crit Care Med 2003;4:426-31.

16.Yacoub S, Lang HJ, Shebbe M, et al. Cardiac function and hemodynamics in Kenyan children with severe malaria. Crit Care Med;38:940-5.

17.Newton CR, Valim C, Krishna S, et al. The prognostic value of measures of acid/base balance in pediatric falciparum malaria, compared with other clinical and laboratory parameters. Clin Infect Dis 2005;41:948-57.

18.Lyke KE, Burges R, Cissoko Y, et al. Serum levels of the proinflammatory cytokines interleukin-1 beta (IL-1beta), IL-6, IL-8, IL-10, tumor necrosis factor alpha, and IL-12(p70) in Malian children with severe Plasmodium falciparum malaria and matched uncomplicated malaria or healthy controls. Infect Immun 2004;72:5630-7.

19.Chiwakata CB, Manegold C, Bonicke L, Waase I, Julch C, Dietrich M. Procalcitonin as a parameter of disease severity and risk of mortality in patients with Plasmodium falciparum malaria. J Infect Dis 2001;183:1161-4.

20.Armah H, Wilson N, Sarfo B, et al. Cerebrospinal fluid and serum biomarkers of cerebral malaria mortality in Ghanaian children. Malaria Journal 2007;6:147.

21.Bouchon A, Dietrich J, Colonna M. Cutting edge: inflammatory responses can be triggered by TREM-1, a novel receptor expressed on neutrophils and monocytes. J Immunol 2000;164:4991-5.

22.Bouchon A, Facchetti F, Weigand MA, Colonna M. TREM-1 amplifies inflammation and is a crucial mediator of septic shock. Nature 2001;410:1103-7.

23.Chimma P, Roussilhon C, Sratongno P, et al. A distinct peripheral blood monocyte phenotype is associated with parasite inhibitory activity in acute uncomplicated Plasmodium falciparum malaria. PLoS Pathog 2009;5:e1000631.

24.Gomez-Pina V, Soares-Schanoski A, Rodriguez-Rojas A, et al. Metalloproteinases shed TREM-1 ectodomain from lipopolysaccharide-stimulated human monocytes. J Immunol 2007;179:4065-73.

25.Ford JW, McVicar DW. TREM and TREM-like receptors in inflammation and disease. Curr Opin Immunol 2009;21:38-46.

26.Ricciuto DR, dos Santos CC, Hawkes M, et al. Angiopoietin-1 and angiopoietin-2 as clinically informative prognostic biomarkers of morbidity and mortality in severe sepsis. Crit Care Med;39:702-10.

27.Erdman LK, Dhabangi A, Musoke C, et al. Combinations of host biomarkers predict mortality among Ugandan children with severe malaria: a retrospective case-control study. PLoS One;6:e17440.

28.Dietmann A, Helbok R, Lackner P, et al. Endoglin in African children with Plasmodium falciparum malaria: a novel player in severe malaria pathogenesis? J Infect Dis 2009;200:1842-8.

29.Turner GD, Ly VC, Nguyen TH, et al. Systemic endothelial activation occurs in both mild and severe malaria. Correlating dermal microvascular endothelial cell phenotype and soluble cell adhesion molecules with disease severity. Am J Pathol 1998;152:1477-87.

30.Shapiro NI, Yano K, Okada H, et al. A prospective, observational study of soluble FLT-1 and vascular endothelial growth factor in sepsis. Shock 2008;29:452-7.

31.Yano K, Liaw PC, Mullington JM, et al. Vascular endothelial growth factor is an important determinant of sepsis morbidity and mortality. J Exp Med 2006;203:1447-58.

32.Casals-Pascual C, Idro R, Gicheru N, et al. High levels of erythropoietin are associated with protection against neurological sequelae in African children with cerebral malaria. Proc Natl Acad Sci U S A 2008;105:2634-9.

33.Parikh SM, Mammoto T, Schultz A, et al. Excess circulating angiopoietin-2 may contribute to pulmonary vascular leak in sepsis in humans. PLoS Med 2006;3:e46.

34.Fiedler U, Reiss Y, Scharpfenecker M, et al. Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation. Nat Med 2006;12:235-9.

35.Conroy AL, Lafferty EI, Lovegrove FE, et al. Whole blood angiopoietin-1 and -2 levels discriminate cerebral and severe (non-cerebral) malaria from uncomplicated malaria. Malar J 2009;8:295.

36.Conroy AL, Phiri H, Hawkes M, et al. Endothelium-based biomarkers are associated with cerebral malaria in Malawian children: a retrospective case-control study. PLoS One;5:e15291.

37.John CC, Bangirana P, Byarugaba J, et al. Cerebral malaria in children is associated with long-term cognitive impairment. Pediatrics 2008;122:e92-9.

38.John CC, Panoskaltsis-Mortari A, Opoka RO, et al. Cerebrospinal fluid cytokine levels and cognitive impairment in cerebral malaria. Am J Trop Med Hyg 2008;78:198-205.

39.Boivin MJ, Bangirana P, Byarugaba J, et al. Cognitive impairment after cerebral malaria in children: a prospective study. Pediatrics 2007;119:e360-6.

40.John CC, Park GS, Sam-Agudu N, Opoka RO, Boivin MJ. Elevated serum levels of IL-1ra in children with Plasmodium falciparum malaria are associated with increased severity of disease. Cytokine 2008;41:204-8.

41.Opoka RO, Xia Z, Bangirana P, John CC. Inpatient mortality in children with clinically diagnosed malaria as compared with microscopically confirmed malaria. Pediatr Infect Dis J 2008;27:319-24.

42.Opoka RO, Bangirana P, Boivin MJ, John CC, Byarugaba J. Seizure activity and neurological sequelae in Ugandan children who have survived an episode of cerebral malaria. Afr Health Sci 2009;9:75-81.

43.Lovegrove FE, Tangpukdee N, Opoka RO, et al. Serum angiopoietin-1 and -2 levels discriminate cerebral malaria from uncomplicated malaria and predict clinical outcome in African children. PLoS One 2009;4:e4912.

44.Conroy AL, Glover SJ, Hawkes M, et al. Angiopoietin-2 levels are associated with retinopathy and predict mortality in Malawian children with cerebral malaria: a retrospective case-control study*. Crit Care Med;40:952-9.

45.Boggild AK, Krudsood S, Patel SN, et al. Use of peroxisome proliferator-activated receptor gamma agonists as adjunctive treatment for Plasmodium falciparum malaria: a randomized, double-blind, placebo-controlled trial. Clin Infect Dis 2009;49:841-9.

46.Hawkes M, Opoka RO, Namasopo S, et al. Nitric oxide for the adjunctive treatment of severe malaria: Hypothesis and rationale. Med Hypotheses;77:437-44.

47.Hawkes M, Opoka RO, Namasopo S, et al. Inhaled nitric oxide for the adjunctive therapy of severe malaria: Protocol for a randomized controlled trial. Trials;12:176.

48.Helbok R, Kendjo E, Issifou S, et al. The Lambarene Organ Dysfunction Score (LODS) is a simple clinical predictor of fatal malaria in African children. J Infect Dis 2009;200:1834-41.

49.Taori RN, Lahiri KR, Tullu MS. Performance of PRISM (Pediatric Risk of Mortality) score and PIM (Pediatric Index of Mortality) score in a tertiary care pediatric ICU. Indian J Pediatr;77:267-71.

50.Leteurtre S, Martinot A, Duhamel A, et al. Validation of the paediatric logistic organ dysfunction (PELOD) score: prospective, observational, multicentre study. Lancet 2003;362:192-7.

51.Lacroix J, Cotting J. Severity of illness and organ dysfunction scoring in children. Pediatr Crit Care Med 2005;6:S126-34.

52.Hettmannsperger T. Pitman Asymptotic Relative Efficiency In: Statistical Inference based on ranks. New York: Wiley; 1984:71.

APPENDIX 1: EXPERTISE OF THE COLLABORATIVE RESEARCH GROUP

Our collaborative research group is ideally suited to conduct the research study proposed. Our team is currently conducting a randomized controlled trial of nitric oxide and a prospective study of hospitalized febrile children in Jinja, Uganda. Robert Opoka (Ugandan PI of this study) has published numerous reports on the management of febrile children and malaria specifically in Uganda [37-43]. Michael Hawkes (PI, Canada) has field experience with clinical trials in Uganda and has co-authored numerous studies of host biomarkers in sepsis and malaria [26, 27, 36, 43, 44]. Kevin Kain (PI) has led research teams, authored numerous publications ranging from molecular pathogenesis of malaria to randomized clinical trials of novel therapies and has published seminal work on biomarkers for malaria [26, 27, 35, 36, 43-47]. Andrea Conroy has authored numerous publications on host biomarkers in malaria and other febrile syndromes [26, 27, 35, 36, 44], and has extensive field experience in Uganda and other African countries. Dr. Sophie Namasopo has been involved in field trials in Uganda and is a collaborator on the ongoing studies in Jinja [46, 47]. She has a managerial and leadership role at the Jinja Regional Referral Hospital, where the first phases of the study will be conducted. Dr. Arthur Mbimbaza has established a network of surveillance sites across Uganda that are equipped to collect data on inpatient pediatric mortality. Data collected through this surveillance program can be leveraged to monitor mortality at intervention and control sites, following the implementation of SPO2 delivery systems. The combined research expertise of our collaborative team in clinical pediatric infectious diseases, malaria, field trials in resource-constrained settings, and biomarkers of disease processes makes us uniquely qualified to successfully complete this proposed research.