

Role of Nitric Oxide Scavenging by  
Plasma Hemoglobin and Identification  
of Hemolysis-associated Pulmonary  
Hypertension in Malaria

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# Background

- Malaria is one of the leading infectious causes of death in many of the world's poorest countries.
- Malaria is especially a problem in young children.
- In Mali, one out of every 20 children will not live to adulthood because of malaria.

# Background

- Malaria often leads to massive hemolysis in infected hosts.
- Very little is known about the pathogenic impact of hemolysis on vascular tone and endothelial function or the clinical consequences of hemolysis in malaria

# Background

- Studies in sickle cell disease and other hemoglobinopathies reveal that hemoglobin released into plasma during hemolysis destroys nitric oxide.
- This loss of nitric oxide leads to many problems: inflammation, vasomotor constriction, platelet aggregation, and pulmonary hypertension.

# Background

- Pulmonary hypertension in sickle cell disease is associated with high mortality (40% mortality in 2 years).
- Does hemolysis in malaria lead to pulmonary hypertension?
- Is pulmonary hypertension clinically relevant in malaria?

# Purpose

- This protocol aims to evaluate mechanisms governing interrelationships among malaria, intravascular hemolysis, NO bioavailability, endothelial function, pulmonary hypertension, and evolutionarily-selected host polymorphisms that regulate the host response to hemolysis

# Setting

- Republic of Mali, West Africa
- ~ 12 million people, median age 15
- ~ 2/3 below the poverty line
- ~ 90% Muslim
- ~ 80% illiterate
- Languages: English, French, Bambara

# Primary Objective

- Compare the prevalence and magnitude of pulmonary hypertension in children with severe malarial anemia to healthy controls.
- Pulmonary hypertension = TRV (tricuspid Regurgitant Velocity)  $\geq 2.5$  m/s

# Secondary Objectives

1. Determine degree and spectrum of intravascular hemolysis in malaria
2. Quantify effect of hemolytic rate on nitric oxide bioavailability
3. Determine biological characteristics of hemolysis-associated endothelial dysfunction
4. Determine genetic basis of disease phenotypes relevant to malaria pathogenesis

# Statistics

- Test of null hypothesis that mean TRV is the same in those with severe malarial anemia and healthy controls.
- Setting significance at 0.05, 100 analyzable subjects per treatment group provides 80% power to detect a difference of .2 m/s in TRV (smallest effect that would be clinically significant).
- Need to enroll 125 subjects per group to get usable data on 100.

# Design

- Enroll 125 subjects in each of four groups:
  - A. Healthy controls
  - B. Asymptomatic controls
  - C. Uncomplicated malaria
  - D. Severe malarial anemia
  
- Due to funding propose to enroll groups A and D first.

# Study Site

- *Hopital Gabriel Touré*, a major tertiary center and the national pediatric hospital, Bamako
- History of collaboration with the NIH.
- Subjects: children 1-5 years of age.

# Enrollment

- Parents who bring children to the hospital for suspected malaria will be invited to enroll their sick child, and also to enroll healthy siblings as controls.
- The hospital admits 100-300 children with severe malaria each month during peak season.

# Consent

- Consent (permission) of parent or guardian.
- Consent form in French; orally translated into local language when necessary.
- Parents will provide signature or thumb print to indicate their agreement.
- Children too young to provide assent.

# Payment

- Parents who agree to enroll their child will be given 5,000 CFA (10 US dollars) for initial visit, and another 5,000 for 2<sup>nd</sup> visit.
- This money should cover travel and lodging expenses for parents who do not live near the research site.

# Visits

- Initial evaluation will take about 2-3 hours.
- Follow-up visit after 7-10 days.

# Procedures

- Finger stick to evaluate malaria status
- History and physical
- Echocardiogram to evaluate pulmonary hypertension
- Blood draw for genetic analysis (total blood drawn 9-12 ml)
- Store blood for future research related to malaria
- Repeat after 7-10 days

# Treatment

- All those invited to participate will be treated for malaria free of charge according to the local standard of care (even if they do not enroll).
- We expect that up to 50% of asymptomatic children will be found to be parasitemic; we will treat them at no cost to their parents.

# Genetic Analysis

- Genetic analysis will be limited to genetic regions associated with malaria pathogenesis, protective traits, nitric oxide dysregulation, and pulmonary hypertension.
- Samples stored in Mali and part of samples sent to US and stored for future research.

# IRB Process

- As the IRB, you will review the study and decide whether to approve, approve with stipulations, table, or reject
- How do you want to proceed?

# 8 Ethical Requirements

- 1) Collaborative partnership
- 2) Social Value
- 3) Scientific Validity
- 4) Fair subject selection
- 5) Favorable risk-benefit ratio
- 6) Independent review
- 7) Informed consent
- 8) Respect for human subjects

## 45CFR.46 Protection of Human Subjects (Subpart A, Common Rule)

- To approve research, the IRB will determine:
  - Risks to subjects are minimized and consistent with sound research design
  - Research risks are reasonable in relation to anticipated benefits to subjects and the importance of the knowledge,
  - Subject selection is equitable; cognizant of the needs of vulnerable populations, and
  - Informed consent will be sought from each subject or LAR and appropriately documented.
  - Adequate provisions for monitoring



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