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Irving CRC –Research Proposal

Prevalence of diarrhea and gastrointestinal infection in severely malnourished Human Immunodeficiency Virus (HIV) –infected children in Durban, South Africa

1. Study Purpose and Rationale

Background

Severe acute malnutrition (SAM) leads to greater than 1 million preventable deaths per year in the developing world among children under 5 years old [1]. Greater than 60 percent of such fatal cases are reported to be associated with HIV infection [1]. Amongst those who survive, morbidity remains high. SAM puts children at risk for severe infections, electrolyte disorders, micronutrient deficiencies, and increased metabolic complications associated with HAART [2], [7]. Limiting these complications is of additional importance in children because of their tendency to stunt both physical and cognitive development.

Several mechanisms that work either independently or synergistically have been proposed to explain why SAM occurs so commonly in HIV-infected children [3]. One theory highlights malabsorption secondary to an excessive inflammation of the gut epithelium as the primary factor [3]. Damage to the gut mucosa may lead to a protein-losing enteropathy and a malabsorptive state. This may lead to macro and micronutrient malabsorption, as well as malabsorption of ARVs [4]. Such mucosal changes are believed to be due to local HIV infection of the gut or to secondary enteric co-infections, or a combination of both [3]. Enteric co-infections may also cause cell lysis and contribute directly to diarrhea, which can be a source of SAM. However it must be noted that diarrhea is not always a presenting symptoms of SAM.

The prevalence of gastrointestinal co-infections and diarrhea in SAM HIV-infected children has not been described. Though both Enterovirus and the Enteroaggregative E. coli (EAEC) have been reported in the literature for their higher prevalence in HIV-infected children when compared to HIV-negative controls [5], [6]. Interestingly, one proposed pathogenic mechanism of EAEC postulates the release of local mediators of inflammatory cytokines [6]. Though hypothetical, this would support the mechanism above.

Thus, there is a need to characterize the prevalence of enteric co-infections in the SAM HIV-infected population. Further, describing if the presence of such co-infections are associated with the outcomes of impaired nutritional recovery, increased mortality, and poor immunologic reconstitution and virologic response in children being treated for SAM and HIV (with HAART) would add to the presently incomplete body of knowledge guiding treatment in this population. This pilot study may also lead to future avenues of research on the HIV-associated gut-inflammation model.

2. Study Design and Statistical Analysis

Study Aims:

Primary Objective:

1. a. To describe the prevalence of co-infection with Enterovirus or EAEC and diarrhea in severely malnourished HIV-infected children.
   b. To evaluate if low baseline weight, CD4%, viral load, and demographic factors are associated with the presence of co-infection.

2. a. To describe the nutritional recovery at 28 days in severely malnourished HIV-infected children co-infected with Enterovirus or EAEC.
   b. To evaluate if baseline weight, CD4%, viral load, and demographic factors are associated with nutritional recovery at 28 days.

Secondary Objective:

1. a. To describe mortality, immunologic reconstitution, virologic response in children co-infected with Enterovirus or EAEC at 12 and 48 weeks.
   b. To evaluate if demographic factors, baseline CD4%, VL, weight, and other opportunistic infections are associated with mortality, immunologic reconstitution, and virologic response in children infected with Enterovirus or EAEC.
2. To evaluate for a possible IRIS-like phenomenon - if severely malnourished HIV-infected children presenting with diarrhea and co-infection with Enterovirus or EAEC develop worsening diarrhea and elevated levels of systemic inflammation (CRP) after starting ARV therapy verses those not co-infected with Enterovirus or EAEC.

Study Hypotheses:

Primary Hypotheses:

1. Enterovirus and EAEC gastrointestinal co-infection will be prevalent in severely malnourished HIV-infected children with diarrhea.
2. Nutritional recovery at 28 days will be impaired in severely malnourished HIV-infected children co-infected with Enterovirus or EAEC as compared to uninfected children.
3. Younger age, lower baseline weight, lower CD4%, and higher viral load will be associated with poorer nutritional recovery.

Secondary Hypotheses:

   b. There will be increased mortality, impaired immunologic reconstitution, and worse virologic response in children co-infected with Enterovirus or EAEC at 12 weeks and 48 weeks.

c. Differences in diarrhea, demographic factors, baseline CD4%, VL, weight, and other opportunistic infections may affect the mortality, immunologic reconstitution, and virologic response in children infected with Enterovirus or EAEC verses uninfected.
d. Severely malnourished HIV-infected children presenting with diarrhea and co-infection with Enterovirus or EAEC will develop worsening diarrhea and have higher levels of systemic inflammation (CRP) after starting ARV therapy verses those not co-infected with Enterovirus or EAEC because of an IRIS-like phenomenon.

Study Design

Design-

Prospective observational case-series describing the prevalence of gastroinstestinal co-infection with Enterovirus and EAEC in severe-acutely-malnourished HIV-infected children and their nutritional recovery, survival, immunologic reconstitution, virologic response, and systemic inflammation while receiving treatment in a main RCT study. Evaluations will be based on weight-for-heigh z score, %CD4, undetectable viral load, and serum C-reactive protein respectively.

Duration-

Total duration for recruitment to study end: 48 weeks.
Follow-up and endpoints: 1, 2, 3, 4, 12, and 48 weeks.

Statistical Analysis:

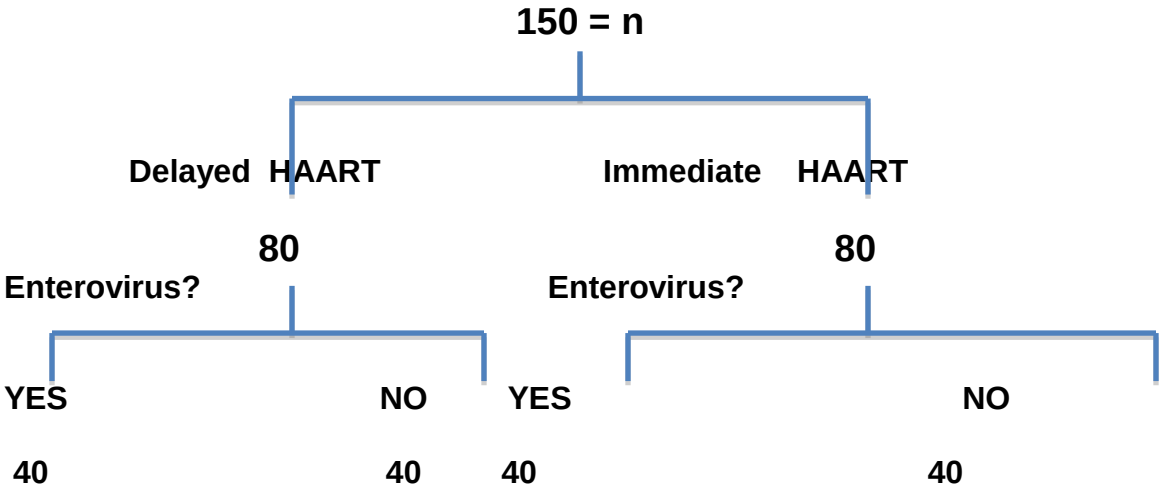
Sample Size and Population: 160 in total.

This proposed study will be a sub-study of a larger randomized control trial comparing immediate initiation of HAART to delayed-initiation of HAART until nutritional recovery in severely malnourished HIV-infected children. The proposed study will draw its participants from this RCT, thus the sample size is confined to 160 participants.

There is currently no data describing the prevalence of gastrointestinal co-infections among severe-acutely-malnourished HIV-infected children, but enterovirus is estimated to be prevalent in 50% of all HIV-infected children in Sub-Saharan Africa.

Power calculation-The power calculation will be based on the estimated prevalence of enterovirus in the HIV-infected population cited in the current literature – 50 percent. In the main RCT, the total 160 participants will be divided into two arms, delayed-ARV and immediate ARV, thus 80 participants will be available in each arm. Therefore, approximately 40 participants in each arm can be expected to be co-infected with enterovirus.

STUDY POPULATION



We expect that children infected with Enterovirus will have a poorer nutritional recovery at 4 weeks. And with this sample size of 40, we will be able to detect a 0.51 difference in weight-for-height (WHZ) z-score at 4 weeks between children with GI co-infection verses those without GI co-infection, at a power of 80%, $\alpha = 0.05$.

Descriptive statistics will be calculated for the clinical and laboratory variables. Chi-sqaured or fisher-exact tests will be used to compare baseline variable of CD4%, VL, demographic factors between groups with and without co-infeciton. Multivariate analyses will be done to evaluate the effect of pathogen co-infection (independent variable) on nutritional recovery, immunologic reconstitution, virologic response, and mortality (dependent variables). Multivariate analyses will also be done to control for confounders- OIs, prophylactic antibiotics, age, gender.

Randomization- For the main RCT, children will be randomized into either "Early HAART" or "Delayed HAART" cohorts.

Cohorts defined-

- Early Initiation of HAART- within 10 days of admission for management of severe acute malnutrition.

- Delayed initiation of HAART- Initiation following nutritional recovery, defined as the first to occur:
    - Reach weight-for-height of -2 z-score **or**
    - Achieving at least 15% weightgain **or**
    - Resolution of edema +return of appetitie **or**
    - 4 weeks post-admission

-Sub-cohorts will be then defined by the presence or absence of Enterovirus or EAEC infection.

Study Procedure

Upon admission for SAM, patient's caregivers will be approached to gauge interest in the study. If interested, further information will be provided after which parent/guardians will be asked to consent for their child's participation prior to enrollment and participation.

At enrollment anthropometrical assessments will be taken (as outlined below). Demographic information will include age, date of admission, discharge, and contents of a to be developed socioeconomic survey. [Such a survey will only be implemented after amendment approval on this studies IRB.] In addition stool samples will be taken (timeline outlined below) for determination of the presence of diarrhea. PCR testing on stool will be carried out for Enterovirus and EAEC. Blood samples will be collected given the outlined time-line as well to determine %CD4, VL, and CRP.

-The <u>primary outcome measure</u> will be:

*** weight-for-height z-score** (nutritional recovery

- The <u>secondary outcome measures</u> will be:
* %CD4 (immunologic reconstitution)
* undetectable viral load (virologic response)_
* mortality – mortality

Table of Study Evaluations

Clinical Measures

	Baseline	1 week	2 weeks	3 weeks	4 weeks	12 weeks	48 weeks
Weight	x	x	x	x	x	x	x
Height/length	x	x	x	x	x	x	x
MUAC	x	x	x	x	x	x	x
Scapular Skin-fold thickness	x	x	x	x	x	x	x
Triceps skin-fold thickness	x	x	x	x	x	x	x
Diarrhea	x	x	x	x	x	x	x
Peripheral Edema	x	x	x	x	x	x	x
OIs	x	x	x	x	x	x	x
Prophylactic Antibiotics	x						
Demographic information	x						

Mortality		x	x	x	x	x	x

Blood Laboratory Measures

	Baseline	1 week	2 weeks	3 weeks	4 weeks	12 weeks	48 weeks
%CD4	x					x	x
VL	x					x	x
CRP	x	x	x	x	x	x	x

Stool laboratory measures

Pathogen	baseline	1 week	2 weeks	Week of nutritional recovery*	4 weeks	48 weeks
Enterovirus	x			x		x
EAEC	x			x		x

*Week of nutritional recovery if in the delayed treatment arm and if occurring before 4 weeks.

Treatment

Under the main RCT-

Nutritional rehabilitation- Per WHO guidelines for the in-patient treatment of severely malnourished children, with lactose-free ready-to-use formula currently provided at King Edward VIII hospital.

Study Drugs

ARV will be used under the MAIN study (described below), but not directly in this proposed observational study.

ARV regimen:

< 3 years	> 3 years
Abacavir	Abacavir
Lamivudine	Lamivudine
Lopinavir/Ritonavir	Efavirenz

Criteria for initiation of treatment :

< 1 years: all infants

1-5 years: CD4 count less than 25% or 750 cell/up (or WHO stage 3/4)

5 years +: CD4 count less than 350 cell/ul (or WHO stage 3 /4) 4

Medical Device

None.

Study Questionnaires

None.

Study Subjects
Inclusion criteria are as follows:

1. 4 months – 12 years old
2. HIV infection as defined by positive ELISA in children over 18 months or a positive DNA PCR in children under 18 months.
3. ART- naive except for PMTCT prophylaxis
4. Eligible for initiation of HAART by South African national treatment guidelines
5. Meet criteria for severe acute malnutrition

Exclusion criteria are as follows:

1. Enrollment in other interventional studies
2. Lack of parent/guardian willing and able to adhere to the study protocol.

Recruitment of subjects
Patients will be admitted to the Kind Edward VIII hospital for severe malnutrition. The pediatric admitting team will gauge interest and seek consent from parent/guardian care-givers to participate in the study after explaining the study, including purpose benefits and risks, in detail.

Confidentiality of Study Data
Medical records, laboratory results, and other relevant records will form case reports that will be kept for each subject. Study participants will not be identified by name on any study document. All forms will be identified by a coded patient information number. Data will be stored and secured on a persona computer, locked and accessible to only the authorized protocol investigators.

Potential conflict of interest
None.

Location
King Edward VIII hospital in Durban, South Africa.

Potential Risks
Minimal blood loss, infection, discomfort resulting from blood draws performed by trained nursing and physician staff.
Potential loss of confidentiality of HIV status, though this is unlikely given the King Edward Hospital has many years of experience keeping protecting the confidentiality of its patients.

Potential Benefits

1. Patients will receive HAART fir length of the study.
2. The information obtained from this study may help further define the management guidelines for SAM HIV-infected children in South Africa.
3. Provide knowledge for better understanding the model of SAM in HIV patients, which may guide future studies.

Alternative Therapies
This study will provide the standard of care. There are no alternative therapies.

Compensation to subjects
Subjects will be provided R150 for any clinic visit that does no coincide with their regular follow-up at the ARV clinic.

Minors as Research Subjects
Minors will be enrolled as research subjects for this study. Parental consent be obtained for all subjects prior to enrollment and participation.

Radiation or Radioactive Substances
None.

References

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[5]Liste MB et al. "Enteric virus infections and diarrhea in healthy and human immunodeficiency virus-infected children" J Clin Microbiol. 2000 Aug;38(8):2873-7.

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