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Health Outcomes, Pathogenesis and Epidemiology of Severe Acute Malnutrition (HOPE-SAM): rationale and methods of a longitudinal observational study

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Health Outcomes, Pathogenesis and Epidemiology of Severe Acute Malnutrition (HOPE-SAM): rationale and methods of a longitudinal observational study

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ABSTRACT

Introduction

Mortality among children hospitalised for complicated severe acute malnutrition (SAM) remains high despite the implementation of WHO guidelines, particularly in settings of high HIV prevalence. Children continue to be at high risk of morbidity, mortality and relapse after discharge from hospital although long-term outcomes are not well documented. Better understanding the pathogenesis of SAM and the factors associated with poor outcomes may inform new therapeutic interventions.

Methods and analysis

The Health Outcomes, Pathogenesis and Epidemiology of Severe Acute Malnutrition (HOPE-SAM) study is a longitudinal observational cohort that aims to evaluate the short- and long-term clinical outcomes of HIV-infected and HIV-uninfected children with complicated SAM, and to identify the risk factors at admission and discharge from hospital that independently predict poor outcomes. Children aged 0-59mo hospitalised for SAM are being enrolled at three tertiary hospitals in Harare, Zimbabwe, and Lusaka, Zambia. Longitudinal mortality, morbidity and nutritional data are being collected at admission, discharge and for 48 weeks post-discharge. Nested laboratory substudies are exploring the role of enteropathy, gut microbiota, metabolomics and cellular immune function in the pathogenesis of SAM using stool, urine and blood collected from participants.

Ethics and dissemination

The study is approved by the local and international institutional review boards in the participating countries (the Joint Research Ethics Committee of the University of Zimbabwe, Medical Research Council of Zimbabwe and University of Zambia Biomedical Research Ethics Committee) and the study sponsor (Queen Mary University of London). Caregivers provide written informed consent for each

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participant. Findings will be disseminated through peer-reviewed journals, conference presentations and to caregivers at face-to-face meetings.

Strengths and limitations of this study

Strengths:

- The primary strength of this study is the rigorous collection of longitudinal data on morbidity, mortality and nutritional status during inpatient care and for 48 weeks after initial admission for SAM.
- Laboratory sub-studies investigating enteropathy, microbiota, metabolomics and immune cell function provide a unique opportunity to understand which pathogenic pathways contribute to SAM and whether these processes normalise with nutritional rehabilitation, capitalising on a well-characterised cohort with appropriate controls.
- This study builds on existing studies of SAM and HIV-SAM prior to the availability of antiretroviral therapy (ART) and will provide the first assessment of longitudinal clinical outcomes in the current ART era.

Potential limitations:

- High loss to follow-up due to participants returning to home settings following hospital discharge. A dedicated clinical study team is in place to maximise follow-up through phone reminders and community visits.
- The clinical heterogeneity of the study participants, including comorbidities such as stunting and co-infections, may make it challenging to identify the specific causes of clinical outcomes. However, the embedded sub-studies will enable multiple pathways to be explored within the same cohort.

INTRODUCTION

Malnutrition underlies almost half of all childhood deaths in developing countries¹. Severe acute malnutrition (SAM) is defined by a weight-for-height Z score <-3, midupper arm circumference <115mm and/or bilateral pitting oedema². Current treatment guidelines distinguish two groups: i) children with uncomplicated SAM who can be managed in the community; and ii) children with complicated SAM, who are hospitalised and undergo resuscitation, stabilisation and nutritional rehabilitation. Inhospital mortality in children with complicated SAM remains high despite the implementation of WHO guidelines³. Furthermore, SAM presents as two major clinical phenotypes: non-oedematous SAM (marasmus), characterised by severe wasting, and oedematous SAM (kwashiorkor), a more complex syndrome characterised by bilateral pitting oedema, steatosis and diarrhea^{4 5}. Despite differing clinical outcomes, treatment protocols are the same for both oedematous and nonoedematous SAM.

A contributory factor to high in-patient mortality is the co-occurrence of HIV infection in around one-third of children hospitalised for SAM in sub-Saharan Africa⁶⁷. While new HIV infections in children have declined⁸, a substantial number of infected children are diagnosed late and present with malnutrition. There is also a growing population of HIV-exposed uninfected (HEU) children who have immune abnormalities, poor growth and higher risk of mortality and infectious morbidity⁹. Hence, HIV has transformed the epidemiology and outcomes of SAM¹⁰. Even with standardised treatment approaches, inpatient deaths are almost four-fold higher among HIV-infected children with SAM (HIV-SAM), compared to HIV-uninfected children with SAM (30.4% vs 8.4%), for reasons that remain unclear¹¹; this mortality is three-fold higher than would be expected from anthropometric parameters alone¹¹. Management of HIV-SAM is particularly challenging because HIV fundamentally alters the clinical presentation of malnutrition and the response to treatment. Children

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with HIV-SAM are more stunted and wasted; have a higher frequency of persistent diarrhoea; tend to have delayed nutritional recovery and have a more complicated clinical course than children with SAM¹¹.

Long-term outcomes of SAM

Following resolution of SAM complications and return of appetite, children are discharged from hospital to continue therapeutic feeds at home. However, emerging data indicate high post-discharge mortality following in-hospital management of SAM¹²⁻¹⁴. Malnutrition together with young age, HIV infection and pneumonia have been associated with higher post-discharge mortality¹⁵. One of the largest prospective studies of growth and mortality in children with SAM (FuSAM), conducted in Malawi from July 2006 to March 2007, collected 12-month outcome data on 87% of 1024 children admitted to the nutrition ward¹². A total of 427 (42%) died and 44% of these deaths occurred after discharge from hospital. Survival was greatest among those who were nutritionally cured upon discharge from outpatient therapeutic feeding centres, defined as two consecutive visits with >80% expected weight-for-height, no oedema and clinically stable. The risk of mortality after hospital discharge was four-fold higher for HIV-infected compared to HIV-uninfected children, but the outcomes among HEU children were not reported. The loss to follow-up was high in the FuSAM study because there was only one follow-up visit, one year after discharge from outpatient-feeding centres.

The impact of SAM appears to persist beyond the first year after discharge from hospital. The ChroSAM study, which followed children with SAM seven years post-discharge, showed that children had poorer growth, body composition and physical function compared to siblings and community controls, which are all indicators of future cardiovascular and metabolic disease¹³.

While anthropometry is used to assess nutritional recovery following discharge from hospital, the pattern and quality of growth recovery in SAM and HIV-SAM are poorly understood. The observation that children treated for SAM have a deficit in lean tissue despite regaining weight suggests that assessing body composition in addition to anthropometry may help to identify children who have not completely recovered and are at potential risk of long-term metabolic diseases¹³. Children with HIV-SAM appear to have potential for catch-up growth since weight-for-age and/or weight-forheight have been shown to normalise with treatment even prior to widespread availability of ART¹⁶. However, the body composition of children with HIV-SAM compared to SAM has not been described. Whether children with SAM (and in particular HIV-SAM) recover fat mass at the expense of lean mass is unknown, but differences in tissue accretion patterns may have implications for survival and longterm metabolic health^{17 18}. There is also a need to consider the effect of SAM on the size of body parts which grow at different rates: relatively shorter legs, for example, are associated with epidemiologic risk of overweight, coronary artery disease, liver dysfunction and diabetes^{19 20}. Taken together, there is clearly an elevated risk of mortality among children with HIV-SAM compared to SAM, and an ongoing mortality risk among all children with SAM that persists after discharge from hospital. There are several gaps in our understanding of the long-term outcomes of SAM and HIV-SAM: (i) causes of death have not been clearly defined; (ii) no studies have systematically and longitudinally collected morbidity and mortality data or documented repeat hospitalisations postdischarge; and, (iii) the long-term outcomes of HIV-infected children with SAM in the era of ART availability are unclear.

Pathogenesis of SAM and HIV-SAM

Better understanding the pathogenesis of SAM may help to explain the high mortality

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of children both during and after hospitalisation and identify new targets for interventions to supplement existing treatment strategies. Consistent evidence that immune mediators are altered in malnutrition²¹ and that systemic and intestinal inflammation are associated with poor outcomes in SAM²², suggest that immune dysfunction contributes to infectious susceptibility²³. Malnutrition is also characterised by a complex derangement in gut microbial²⁴ metabolic,²⁵ immune²⁶ and hormonal pathways, organ dysfunction and micronutrient deficiencies in the context of co-infections, enteropathy and chronic inflammation. Several studies have recently provided insights into these perturbations using new tools^{25 27-29}, including metabolomics and metagenomics, but we still lack a clear understanding of many of the pathogenic pathways driving malnutrition, the interactions between these pathways, and which are the most tractable targets for intervention.

SAM shares several pathological and clinical features with HIV, which may explain clinical outcomes in these co-occuring conditions: 1) both are characterised by intestinal damage, leading to impairment of the mucosal barrier and increased intestinal permeability; 2) both have underlying systemic immune activation; and 3) both are frequently complicated by persistent diarrhoea, pneumonia and sepsis that may plausibly arise due to loss of intestinal barrier function³⁰. Understanding the overlapping impact of HIV and SAM is critical to inform additional interventions to improve outcomes of children with HIV-SAM.

OBJECTIVES OF HOPE-SAM

The Health Outcomes, Pathogenesis and Epidemiology of Severe Acute Malnutrition (HOPE-SAM) study has two primary objectives:

 To describe the short- and long-term clinical outcomes of children with complicated SAM and HIV-SAM, and to identify the risk factors at admission and discharge from hospital that independently predict these outcomes.

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 To better characterise the pathogenesis of SAM and HIV-SAM, through nested laboratory sub-studies evaluating enteropathy, gut microbiota, metabolomics and immune cell function.

STUDY DESIGN

HOPE-SAM is a longitudinal observational cohort study, enrolling between 600-800 children aged 0-59 months admitted with SAM to the tertiary pediatric wards at two sites in Zimbabwe (Parirenyatwa Hospital and Harare Children's Hospital) and one in Zambia (University Teaching Hospital, Lusaka). Both HIV-infected and HIV- uninfected children will be enrolled. All participants with SAM are followed for 48 weeks post-discharge, with longitudinal data collection and blood sampling; a subgroup of 200 children will be recruited to the enteropathy substudy for which they will have the same follow-up procedures but more intensive biological specimen collection including stool (all time-points), urine after lactulose-mannitol (LM) challenge as an assessment of intestinal permeability, and nasogastric aspirate (baseline only). A group of 200 healthy children recruited from the same hospitals, who are well-nourished and matched to children in the enteropathy substudy by age and HIV status, will have data and specimens collected to provide normative data for the laboratory substudies; these healthy controls will not be followed longitudinally. The study overview is shown in **Figure 1**.

The study protocol, data collection forms and standard operating procedures are available as Supplementary Materials.

RECRUITMENT

Screening: Caregivers of all hospitalised children are sensitised about the study. All new admissions aged 0-59 months are screened for SAM, which is defined

according to WHO criteria as any of: weight-for-height Z-score (WHZ) <-3, mid-upper arm circumference <115 mm (if aged 6-59mo) and/or bilateral pitting oedema.

Eligibility for observational cohort: All children with SAM whose caregivers are willing to provide written informed consent and to learn their child's HIV status are offered enrolment. Any children who die prior to study enrolment and those with a known malignancy are ineligible.

Eligibility for enteropathy substudy: Cases (Groups A and C, **Table 1**) are children with SAM aged 6-59 months with a nasogastric tube in place (or due to be placed). Controls (Groups B and D) are children receiving inpatient or outpatient care at the study sites, who are aged 6-59 months (matched to cases within age bands), wellnourished (weight-for-height Z-score >-1) and clinically well (no acute illness or current infections) with known HIV status. Children with underlying chronic gastrointestinal disease or a known malignancy are ineligible.

Table 1: Enteropathy substudy groups

| Children aged 6-59 months | Severe acute ma | Well nourished | |
|---|-------------------------|----------------------|---------------------------------|
| | Oedematous ² | Non-oedematous | WHZ>-1 |
| HIV-infected (HIV PCR+ if <18mo; HIV antibody + if >18mo) | N=50 (Group A-I) | N=50 (Group A-II) | N=100 ³ (Group B) |
| HIV-uninfected (HIV PCR- if <18mo; HIV antibody - if >18mo) | N=50 (Group C-I) | N=50 (Group C-II) | N=100 ⁴ (Group D) |

¹SAM defined according to WHO criteria

²Presence of bilateral pitting pedal oedema.

³Age-matched to group A (within the following age bands: 6-11 months; 12-23 months; 24-59 months). ⁴Age-matched to group C (within the following age bands: 6-11 months; 12-23 months; 24-59 months) Note that children age 0-5 months are excluded from the enteropathy substudy

WHZ: Weight-for-height Z score; MUAC: Mid-upper arm circumference; PCR: polymerase chain reaction.

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Eligibility for other sub-studies: Children enrolled into the enteropathy substudy are also included in the microbiome and metabolome substudies. The immunology substudy comprises all children with SAM providing a blood sample of sufficient volume (>2ml) for cellular assays.

Informed consent procedures: Written informed consent is obtained from the primary caregiver using consent forms translated into local languages; we generally include other family members in the consent process. Illiterate caregivers who have understood a verbal explanation of the study can provide a thumb imprint in the presence of a witness. Assent from children is not sought because all are <5 years old.

STUDY PROCEDURES

Study procedures are outlined in **Table 2**.

Table 2: Summary of procedures in observational SAM cohort

| Assessment (Form | Hospitaliza | ation | Post | | | | |
|---|-----------------------|------------------------|------|----|-----|-----|-----|
| used) | Baseline ¹ | Discharge ² | 2w | 4w | 12w | 24w | 48w |
| Caregiver informed consent to join observational cohort (HOPE-SAM Form_2_Observational Cohort Informed Consent Form and HOPE-SAM Form_6_Specimen Storage and Shipment form) | X | | | | | | |
| Summary checklist (HOPE-SAM Form_3_Study Checklist) | X | | | | | | |
| Locator information ⁴ (HOPE-SAM Form_9_Locator Information) | x | | | | | | |
| Acute admission information (HOPE-SAM | x | | | | | | |

| Form_4_Acute Admission Proforma) | | | | | | | |
|-------------------------------------|-----------------|-------|---------------------------------------|--------|---------|----------|------|
| Baseline data | x | | | | | | + |
| (HOPE-SAM | | | · · · · · · · · · · · · · · · · · · · | | | | |
| Form_5_Baseline | | | | | | | |
| Form) | | | | | | | |
| Daily clinical review ⁵ | Daily during | J | | | | | |
| (HOPE-SAM | hospitalisation | | | | | | |
| Form_7_Daily Follow- up Form) | | | | | | | |
| Blood collection ⁶ | х | Х | | | Х | Х | Х |
| HIV testing ⁷ | х | | | | | | |
| CD4 count and viral load | х | | | | х | х | х |
| (HIV-infected children | | | | | | | |
| only) | | | | | | | _ |
| Full blood count ⁸ | x | X | | | Х | Х | Х |
| Anthropometry | x | x | х | Х | Х | Х | Х |
| Skinfold thickness ⁹ | | x | х | х | Х | Х | X |
| Body composition ¹⁰ | x | x | х | Х | Х | Х | Х |
| Discharge data collection | | x | | | | | |
| (HOPE-SAM | \mathbf{C} | | | | | | |
| Form_8_Discharge Form) | | | | | | | |
| Daily morbidity diary | | | Daily | during | follow- | up perio | nd h |
| (HOPE-SAM | | | carec | | ronom | ap pone | |
| Form_10_Morbidity | | | | , | | | |
| Diary) | | | | | | | |
| Follow-up clinic: history, | | | х | Х | Х | Х | X |
| examination, morbidity | | · · · | | | | | |
| and mortality data | | | | | | | |
| (HOPE-SAM | | | | | | | |
| Form_11_Clinical | | | | | | | |
| Follow-up Form and | | | | | | | |
| HOPE-SAM | | | | | | | |
| Form_12_Nurse | | | | | | | |
| Follow-up Form) | | | | | | | |

¹Children will be enrolled as soon as possible after hospitalisation and will undergo baseline investigations as soon as possible after enrolment. This is to provide a window of opportunity to time collection of research specimens with clinical specimens, and to ensure that the child is clinically stable before undertaking research investigations. ²The discharge procedures will be undertaken on the day of discharge, or as close as possible to

that date.

³Windows will be created around these post-discharge time-points to maximize follow-up for caregivers who miss visits or are unavailable, as follows: 2 weeks (1-3 weeks); 4 weeks (3-5 weeks); 12 weeks (10-14 weeks); 24 weeks (20-28 weeks); 48 weeks (44-52 weeks).

⁴Locator information will updated using HOPE-SAM Form_9_Locator Information at subsequent visits if caregivers have moved or changed contact details.

⁵A clinical review will be undertaken every day between admission and discharge by the study clinician, using HOPE-SAM Form_7_Daily Follow-up Form.

⁶5.4 mL of blood (depending on child weight; amount will not exceed 2 mL/kg total over 2 week period) will be collected by a study nurse into endotoxin-free EDTA tubes. Samples will be used to store whole blood, PBMC and plasma for subsequent measurement of CRP and albumin. Where blood sample volumes allow (≥2mL sample), bacterial binding assays and whole blood stimulations will be conducted and culture supernatants and cells stored for subsequent assessment of immune cell function at each time-point.

Study blood samples will not be collected from children with known haemoglobin <6 g/dL.

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⁷Infant HIV testing is conducted as part of routine clinical practice, but if it has not been undertaken, the study sample will be used to test for HIV, as stated in the informed consent form, since HIV status is required to allocate children to study groups.

⁸Full blood count results will be transcribed from clinical records; if not done by clinical teams, the EDTA sample will be used to measure FBC in clinical laboratories at each site

⁹Skinfold thickness (triceps, subscapular, supra-iliac) and mid-thigh circumference will be measured using Holtain calipers or tape measure.

Baseline procedures:

Baseline data on maternal and household characteristics, the child's past medical history and current illness are collected by a study nurse. Anthropometry, including body composition measured by whole-body (wrist-ankle) bio-electrical impedance analysis (BodyStat 1500MD; BodyStat Ltd., Douglas, Isle of Man), leg length using an electronic knemometer (Zimbabwe only) and triceps, subscapular and supra-iliac skinfold thickness using calipers (Holtain Ltd., Crymych, UK) are undertaken at baseline. Blood (1mL/kg up to 5.4mL maximum) is collected at baseline into an endotoxin-free EDTA tube for all children and, in the enteropathy substudy, additionally into a PAXgene tube (PreAnalytiX GmbH, Hombrechtikon, Switzerland) for subsequent transcriptomic analysis. Blood is not collected from children with severe anaemia (known haemoglobin <6 g/dL). HIV testing is carried out in accordance with national guidelines as part of routine clinical practice; where it has not been done, the child's HIV status is ascertained using a rapid test antibody algorithm for children over 18 months, or HIV DNA PCR for children under 18 months. CD4 count/percentage and viral load are measured in HIV-infected children. Maternal HIV status is documented where available, so that HIV-exposed uninfected children can be identified. Blood samples are sent to research laboratories at each site to conduct whole blood stimulation and bacterial binding assays (as described in the immunology substudy) and to store aliguots of whole blood, peripheral blood cells and plasma at -80°C³¹. In the enteropathy substudy, nasogastric aspirate, stool and urine (after an oral dose of lactulose and mannitol) are also collected. Lactulose and

¹⁰Body composition will be assessed by bioimpedance vector analysis.

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| diary and pre-prepa |
| to complete the diar |
| appointment and co |
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| Follow-up: Children |
| 12, 24 and 48 week |
| clinical assessment |
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nannitol are ingested by the child after fasting and urine is collected over a two-hour period to measure recovery of lactulose and mannitol, a measure of intestinal absorptive capacity and permeability, as previously described³².

Daily procedures: Routine inpatient management is undertaken by ward clinical eams according to local hospital protocols, which are based on WHO guidelines³³. In addition, the HOPE-SAM study clinician at each hospital site collects daily data until discharge on clinical parameters (including daily examination), resolution of acute nfections, nutritional recovery (loss of oedema, restoration of appetite, weight gain), and treatment/nutritional supplements received. Children with HIV-SAM who are ART-naïve start ART according to WHO guidelines³⁴.

Discharge: The clinical team decides when the child is ready to be discharged. Children receive ready-to-use therapeutic feeds (RUTF) to take at home according to local guidelines. At discharge, the study nurse collects data and a repeat blood sample (including full blood count) and undertakes discharge anthropometry, body composition, leg length (Zimbabwe only) and triceps, subscapular and supra-iliac skinfold thickness measurements (**Table 2**). The caregiver is given a daily morbidity diary and pre-prepared stickers corresponding to different illnesses and shown how to complete the diary. The caregiver is provided with the date of the first follow-up appointment and contact details of the study nurse.

Follow-up: Children attend follow-up appointments at dedicated study clinics at 2, 4, 12, 24 and 48 weeks post-discharge. At each visit, the study physician undertakes a clinical assessment and the study nurse captures illness, medication and feeding data. Clinic data are transcribed from handheld medical records if available and the morbidity diary is reviewed and a new diary and stickers supplied. Anthropometry, body composition, leg length (Zimbabwe only) and triceps, subscapular and supra-

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iliac skinfold thicknesses are measured at each visit. Acute illnesses are treated in the study clinic, or the child is referred to hospital if necessary. Children with relapsed malnutrition are provided with nutritional supplements according to local guidelines. Transport reimbursement for clinic attendance is provided to caregivers for each visit.

Blood is collected at weeks 12, 24 and 48 post-discharge into endotoxin-free EDTA tubes to measure full blood count, CD4 count and viral load (HIV-infected group only), conduct whole blood stimulation and bacterial binding assays, and store peripheral blood cells and plasma aliquots for subsequent analysis, including soluble and cellular markers of immune activation, as outlined in **Supplementary Table 1**. Children in the enteropathy substudy have additional stool and urine collection following lactulose-mannitol dosing as shown in **Table 3**.

| Assessment (Form | Hospitaliza | ation | Post | t-discha | arge ³ | | |
|---|-----------------------|------------------------|------|----------|-------------------|-----|-----|
| used) | Baseline ¹ | Discharge ² | 2w | 4w | 12w | 24w | 48w |
| Caregiver informed consent to join observational cohort and enteropathy substudy (HOPE-SAM Form_14_Enteropathy Substudy Case Consent Form and Form_6_Specimen Storage and Shipment form) | x | | | | | | |
| Summary checklist (HOPE-SAM Form_3_Study Checklist) | x | | | | | | |
| Locator information ⁴ (HOPE-SAM Form_9_Locator Information) | x | | | | | | |
| Acute admission information (HOPE-SAM Form_4_Acute | x | | | | | | |

Table 3: Summary of procedures for cases in the enteropathy substudy

| Admission Proforma) | | | | | | | | |
|---|--------------|-----|-------|------|-----|----------|--------|-----|
| Baseline data | х | | | | | | | |
| (HOPE-SAM | | | | | | | | |
| Form_5_Baseline | | | | | | | | |
| Form) | | | | | | | _ | _ |
| Daily clinical review ⁵ | Daily during | | | | | | | |
| (HOPE-SAM | hospitalisat | ion | | | | | | |
| Form_7_Daily Follow- | | | | | | | | |
| up Form) Blood collection ⁶ | x | x | | | | x | × | , |
| | * | × | | | | X | X | |
| HIV testing ⁷ | x | | | | | | | |
| CD4 count and viral | х | | | | | х | x |) |
| load (HIV-infected | | | | | | | | |
| children only) | | | | | | | | |
| Full blood count ⁸ | x | х | | | | Х | х |) |
| Gastric aspirate ⁹ | x | | | | | | | |
| | | | | | | | | |
| Stool collection ¹⁰ | x | x | | | | х | x | |
| Lactulose-mannitol | x | x | | | | х | |) |
| testing ¹¹ | | | | | | | | |
| Anthropometry 12 | х | x | Х | х | | Х | Х | > |
| Skinfold thickness ¹² | | x | Х | Х | | Х | Х |) |
| Body composition ¹³ | x | x | Х | х | | Х | Х |) |
| Discharge data | | x | | | | | | |
| collection | | | | | | | | |
| (HOPE-SAM | | | | | | | | |
| Form_8_Discharge Form) | | | | | | | | |
| Daily morbidity diary | | | Daily | | ing | follow- | | ind |
| (HOPE-SAM | | | care | | - | 1011010- | up pen | lou |
| Form_10_Morbidity | | | Carc | give | 13 | | | |
| Diary) | | | | | | | | |
| Follow-up clinic: history, | | | х | | х | x | x | |
| examination, morbidity | | | ~ | | ~ | ~ | ~ | 1 |
| and mortality data | | | | | | | | |
| (HOPE-SAM | | | | | | | | |
| Form_11_Clinical | | | | | | | | |
| Follow-up Form and | | | | | | | | |
| HOPE-SAM | | | | | | | | |
| Form_12_Nurse | | | | | | | | |
| Follow-up Form) | | | | | | | | |

¹Children will be enrolled within 24h of hospitalization and will undergo baseline investigations within 72h of hospitalization. This is to provide a window of opportunity to time collection of research specimens with clinical specimens, and to ensure that the child is clinically stable before undertaking research investigations.

²The discharge procedures will be undertaken on the day of discharge, or as close as possible to that date.

³Windows will be created around these post-discharge time-points to maximize follow-up for caregivers who miss visits or are unavailable, as follows: 2 weeks (1-3 weeks); 4 weeks (3-5 weeks); 12 weeks (10-14 weeks); 24 weeks (20-28 weeks); 48 weeks (44-52 weeks).

⁴Locator information will updated using **HOPE-SAM Form_9_Locator Information** at subsequent visits if caregivers have moved or changed contact details.

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⁵Daily clinical review will be conducted every day between admission and discharge by the study clinician, using HOPE-SAM Form_7_Daily Follow-up Form

⁶During hospitalisation, 5.4 mL of blood (depending on child weight; amount will not exceed 2 mL/kg total over 2 week period) will be collected by a study nurse into a 2.7 mL endotoxin-free EDTA tube and a 2.7 mL PAXGene tube, for subsequent isolation of RNA and gene expression analysis (see Table 7). After discharge (weeks 12, 24 and 48), 5.4 mL of blood (depending on child weight; amount will not exceed 2 mL/kg total over 2 week period) will be collected by a study nurse into two 2.7 mL endotoxin-free EDTA tubes.

⁷Infant HIV testing is conducted as part of routine clinical practice, but if it has not been undertaken, the study sample will be used to test for HIV (see section 9.4), as stated in the informed consent form, since HIV status is required to allocate children to study groups.

⁸Full blood count results will be transcribed from clinical records; if not done by clinical teams, the EDTA sample will be used to measure FBC in clinical laboratories at each site

A gastric juice sample will be collected at the same time as the blood draw by aspirating the nasogastric tube with a sterile feeding syringe, to test for gastric pH; sterile water or saline will then be instilled and a sample of gastric juice collected for storage for subsequent PCR and culture (section 7.5.2)¹⁰Stool collection will be undertaken at the same time as the blood draw as described in section

7.5.3. ¹¹Lactulose-mannitol testing will be conducted as described in section 7.5.4, with collection of a baseline urine sample, followed by a 2hr urine collection post-LM indestion. This test will be deferred until children are judged to be clinically stable by the study physician during daily reviews. In general, this will be a child in the nutritional rehabilitation phase, who has no cardiorespiratory

compromise. ¹²Skinfold thickness (triceps, subscapular, supra-iliac) and mid-thigh circumference will be measured using Holtain calipers or tape measure as explained in section 7.5.7.

³Body composition will be assessed by bioimpedance vector analysis (see section 7.5.6)

Caregivers are reminded of follow-up visits by phone, and visit completion is tracked

on a dedicated database. If caregivers do not attend follow-up appointments,

attempts are made to contact them by phone and home visits are made if feasible,

particularly for those defaulting the 48-week visit, so that long-term outcome data can

be collected. For post-discharge deaths, a home visit is undertaken by study nurses

where possible to conduct a verbal autopsy. Children who are readmitted to one of

the study sites with relapsed SAM have data collected during the new episode of

hospitalisation. The study ends for each participant at the week 48 visit.

SUBSTUDIES

Several nested substudies will utilise biological specimens to address mechanistic questions related to enteropathy, microbiota, metabolomics and immune function.

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Enteropathy substudy

The gut, which acts as an internal interface between humans and the environment. must contain the nutrient stream and the symbiotic microbiota while allowing molecular intimacy to permit absorption. The mechanism underlying this duality is the integrity of the gastrointestinal barrier; intestinal damage (enteropathy) can impair this critical barrier function. A spectrum of enteropathies affect children in developing countries³⁰. Environmental enteric dysfunction (EED), characterised by small intestinal inflammation, blunted villi and increased intestinal permeability, is almost universal and is morphologically indistinguishable from HIV enteropathy³⁰. Children in resource-poor settings also suffer from frequent diarrhoea, food insecurity and micronutrient deficiencies, which all exacerbate enteropathy³⁰. As a result, a cvcle of intestinal infection, impaired mucosal function and malnutrition commonly arises, which may ultimately precipitate SAM, especially in the context of HIV infection^{35 36}. It is not yet established if the enteropathy seen in children with SAM³⁷, which we here refer to as malnutrition enteropathy³⁷, is qualitatively or quantitatively distinguishable from EED. In addition to local intestinal pathology, enteropathies may cause systemic pathology due to persistent immune activation arising from enteric inflammation and microbial translocation across the damaged gut wall³⁰. It is becoming apparent that chronic inflammation may be particularly deleterious in malnourished individuals²²: in children with SAM, systemic inflammation arising from underlying enteropathy may further increase morbidity and mortality.

We hypothesize that i) the degree of enteropathy during hospitalisation differs between oedematous and non-oedematous SAM and is independently associated with morbidity, mortality and nutritional recovery during hospitalization; ii) the degree of enteropathy at discharge is independently associated with morbidity, mortality and relapse of SAM; and iii) children with HIV-SAM have more severe enteropathy than children with SAM alone, which contributes to their poorer outcomes. BMJ Open: first published as 10.1136/bmjopen-2018-023077 on 1 February 2019. Downloaded from http://bmjopen.bmj.com/ on May 15, 2025 at Department GEZ-LTA Erasmushogeschool . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

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Using stored samples, a longitudinal series of investigations will compare gastric and small intestinal barrier function, using a range of biomarkers to capture the domains of malnutrition enteropathy (**Supplementary Table 2**). To understand better the extra-intestinal consequences of enteropathy, we will first compare the microbial composition of the upper gut and plasma using deep sequencing in a subgroup of children with paired gastric and blood samples. Secondly, we will undertake transcriptomics using PAXGene blood samples to determine i) whether there are differences in gene expression profiles between healthy controls, SAM and HIV-SAM (including comparison of oedematous and non-oedematous types); and ii) whether specific patterns of gene expression are associated with morbidity and mortality in SAM.

Microbiota substudy

Normal assembly of the gut microbiota in early life is critical for many aspects of physiological, neurological and immune development³⁸. Recent evidence suggests that an immature or pathogenic microbiota plays a causative role in the pathogenesis of SAM²⁷. For example, a number of microbial taxa have been identified, including *Faecalibacteium prausnitzii*, which discriminate and predict gut microbiota maturity and child growth³⁹. Other pathogenic microorganisms, including IgA-targeted *Enterobacteriaceae*, are associated with impaired growth and may contribute to SAM⁴⁰. Nutritional rehabilitation with RUTF induces temporary recovery of a disturbed microbiota; however, the microbiota appears to revert back to an immature diseased state following nutritional recovery⁴¹. HIV infection is also associated with a disturbed gut microbiota⁴², which may further compound enteropathy phenotypes. Furthermore, there is some evidence that differences exist in malnutrition enteropathy between oedematous and non-oedematous SAM⁴³; however, few

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studies have investigated differences in the gut microbiota between the two forms of the disease.

We hypothesize that (i) a unique gut microbial signature exists in HIV-SAM, compared with SAM, that is independently associated with morbidity, mortality, nutritional recovery and degree of enteropathy during hospitalisation; (ii) a unique gut microbial signature exists in oedematous compared with non-oedematous SAM; (iii) specific microorganisms or gut microbial diversity indices are independently associated with morbidity, mortality, nutritional recovery and degree of enteropathy during hospitalisation; and (iv) the gut microbiota is partially restored to a healthy state with nutritional rehabilitation but reverts to a dysbiotic state during follow-up, which predicts morbidity, mortality and relapse of SAM.

Using stored stool samples collected at baseline, a cross-sectional investigation will determine differences in the gut microbial composition and predicted function between SAM versus HIV-SAM, oedematous versus non-oedematous SAM, and healthy controls. Gut microbial composition and predicted function will be compared between groups at discharge and at 12, 24 and 48 weeks post-discharge. Briefly, total DNA and/or RNA will be extracted from stool samples and used as template for next-generation sequencing library preparation and for quantitative polymerase chain reaction (qPCR). Whole metagenome shotgun sequencing will be performed using the HiSeq 2500 system. Raw metagenomic sequencing data will be quality-filtered and analysed through a well-validated bioinformatics pipeline using MetaPhlAn⁴⁴ and HUMAnN⁴⁵. The compositional and predicted functional metagenomic data generated will be used to identify signatures of SAM and to investigate associative links between specific gut microbial signatures and clinical outcomes.

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Metabolomics substudy

During SAM, metabolic processes are altered in response to a starved environment, and may plausibly contribute to long-term clinical outcomes. Previous studies suggest that amino acid turnover, lipid metabolism, oxidative stress and other metabolic pathways are disrupted in SAM and may be associated with disease state and clinical outcome^{25 46 47}; however, little is known about how the metabolic phenotype responds to nutritional therapy. It is hypothesised that disturbed gut microbiota composition and function may drive microbial metabolic dysregulation in addition to host-derived dysregulation. Of particular interest are differences in the metabolic phenotype between oedematous and non-oedematous SAM. The 'reductive adaptation' seen in non-oedematous SAM (utilisation of fat and muscle stores) is disrupted in oedematous SAM, which may contribute to differences in clinical outcomes. Specifically, protein turnover, inflammation, oxidative stress and bile acid metabolism are disrupted in oedematous-SAM, which may contribute to comorbidities including diarrhoea, steatosis and enteropathy^{48 49}.

We hypothesize that: (i) a unique plasma and urine metabolic phenotype exists in oedematous compared with non-oedematous SAM during hospitalisation, which is independently associated with morbidity, mortality and nutritional recovery; (ii) the metabolic phenotype is partially restored to a healthy state with nutritional rehabilitation but reverts to a disturbed state during follow-up, which predicts morbidity, mortality and relapse; and (iii) both host-derived and gut microbial-driven metabolic dysregulation underlie clinical outcomes.

Using stored urine and plasma samples collected during hospitalisation, a crosssectional investigation will determine differences in the metabolic phenotype between children with oedematous SAM, non-oedematous SAM and healthy controls. Urine and plasma metabolic phenotypes will be compared between groups at discharge

and 12, 24 and 48 weeks post-discharge. Briefly, global untargeted metabolomic phenotyping will be performed via ¹H nuclear magnetic resonance (NMR) spectroscopy using a 700 MHz Bruker NMR spectrometer to identify metabolic signatures of SAM. Targeted analysis via ultra-performance liquid chromatographymass spectrometry will be performed to examine specific pathways of interest, including tryptophan and bile acid metabolism.

Immunology substudy

Bacterial infections are common among children hospitalised for SAM⁵⁰⁻⁵³ and mortality is driven by a range of species^{51 53-56}, consistent with generalised defects in innate anti-bacterial defence. Increased infectious morbidity and mortality persist after discharge from hospital⁵⁷⁻⁵⁹, suggesting that restoration of anti-bacterial immune responses may lag behind nutritional rehabilitation. A recent randomised trial in children with SAM confirmed that deaths following hospitalisation were predominantly due to bacterial infections but were not prevented by daily co-trimoxazole prophylaxis⁵⁹. Collectively, these observations highlight that children remain vulnerable to infection despite current treatment approaches; targeting persistent immune dysfunction could plausibly reduce infectious mortality after discharge⁶⁰. BMJ Open: first published as 10.1136/bmjopen-2018-023077 on 1 February 2019. Downloaded from http://bmjopen.bmj.com/ on May 15, 2025 at Department GEZ-LTA Erasmushogeschool . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

Multiple innate and adaptive immune mediators are dysregulated in malnutrition⁶⁰⁻⁶². However, few studies have assessed cellular immune function in malnourished children; most existing studies were undertaken decades ago on small crosssectional cohorts without the benefit of recent advances in immunology techniques⁶¹. Immune dysfunction in SAM likely reflects both *intrinsic* defects, whereby immune cells lack capacity to adequately respond to infection, and *extrinsic* defects, where cells have intact anti-bacterial capacity but are chronically modulated by the systemic pro-inflammatory environment which characterises SAM (i.e. heightened proinflammatory cytokines^{50 63} and circulating bacterial antigens^{64 65}). Systemic

inflammation is directly associated with mortality in SAM⁵⁰ and driven by multiple comorbidities, including bacterial translocation from the damaged gut into the blood, sub-clinical infections and metabolic dysregulation^{63 66-68}. The implications of innate immune cell dysfunction for subsequent acquisition of infections and infectious mortality have not been investigated.

We hypothesise that: (i) anti-bacterial functions of innate immune cells are compromised in SAM due to a combination of intrinsic and extrinsic defects; ii) innate immune cell function is independently associated with infectious morbidity and mortality during hospitalisation for SAM; and iii) nutritional rehabilitation only partly restores innate immune cell function, leading to an ongoing risk of bacterial infections post-discharge.

Using blood samples collected at baseline, discharge and 12, 24 and 48 weeks postdischarge, the longitudinal relationship between circulating innate immune cell function and bacterial infections will be assessed. The intrinsic phagocytic capacity, secreted cytokine response and maturation state of innate immune cells after culture with bacterial antigens will be assessed. Plasma concentrations of endotoxin and pro-inflammatory cytokines will be quantified at each time-point and the degree to which these extrinsic factors influence innate immune cell antibacterial function will be assessed via plasma co-culture with innate immune cells from healthy donors. Bacterial infections during hospitalisation will be diagnosed using clinical criteria and blood culture, stool culture and urinalysis where available.

SAMPLE SIZE

Observational study: The observational cohort will recruit as many children with SAM as possible during the period of enrolment (July 2016 to March 2018), estimated at 600-800 children, to provide a robust assessment of outcomes among children

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hospitalised with SAM. Assuming 15% mortality and 15% loss to follow-up, there would be at least 420 evaluable children post-discharge, of whom up to 168 would have HIV-SAM, based on an estimated inpatient HIV prevalence of 40%.

Enteropathy substudy: The sample size was estimated using previously reported values for LM ratios, which remain a widely used non-invasive marker of enteropathy. Comparing 100 versus 100 children with two-sided alpha=0.025 (to allow for two primary comparisons, i.e. HIV-SAM versus SAM, and HIV-SAM versus HIV) provides >80% power to detect differences in mean LM ratio during hospitalisation of at least 0.16 (assuming SD=0.36), a difference which would be clinically relevant given the LM ratios previously reported for well-nourished children (0.42), malnourished children (1.3) and children with persistent diarrhoea (2.85) in the Gambia⁶⁹. It also provides >80% power to detect differences of at least 0.1 in the mean change in LM ratio from enrolment (assuming SD for change=0.23 and 7% missing samples). For inflammatory markers, comparing 100 versus 100 children with two-sided alpha=0.025 provides >80% power to detect differences in mean log₁₀ concentrations of at least 0.44 times their standard deviation, or 2.75-fold differences between groups. Inclusion of healthy controls provides an indication of normal ranges in young African children. SAM groups will be stratified to include approximately 50 children with and without oedematous malnutrition, if possible.

Microbiota and metabolomics substudy

Power calculations are difficult in metagenomics and metabolomic analyses due to the large number of observed outcomes and unknown effect sizes and variance. Previous studies using smaller sample sizes have identified significant taxonomic differences in twin pairs discordant for oedematous-SAM (n=13)²⁴ and metabolic differences between the two forms of SAM (n=40)⁷⁰. These studies suggest that a difference of 50% in metabolites could be expected. Using ANCOVA, setting α =0.05

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and assuming either low (ρ =0.1) or high (ρ =0.7) correlation, the study would require 95-126 subjects to achieve 80% power⁷¹. False discovery rate (FDR) multiple correction testing will be applied to reduce the high-dimensionality of the data and

Immunology substudy

limit false-positives.

Up to 200 children with SAM and 200 healthy controls will be included in a crosssectional analysis of innate immune cell function during hospitalisation. Assuming similar infectious mortality to a recent Kenyan study (15%)⁵⁹, a cohort of 200 provides 80% power to detect associations between immune profiles and infectious mortality at an odds ratio of 1.7 and 2-sided alpha of 0.05. We will aim for 100 children with longitudinal analysis of innate immune cell function at discharge, 12, 24 and 48 weeks post-discharge⁵⁹.

Ĉ.

ANALYSIS

Observational Cohort

The primary comparison will be the clinical and nutritional outcomes of children with SAM compared to HIV-SAM. We will review all deaths and adjudicate clinical diagnoses and causes of death to ensure robust and consistent data across sites. We will compare each participant's clinical management to WHO guidelines to identify any contributory factors in hospital care. Factors associated with outcomes during hospitalisation (e.g. mortality, nutritional recovery) will be determined for each group using multivariate analysis (Cox models for time-to-event data, linear models for continuous outcomes). Factors associated with outcomes over 48 weeks postdischarge (hospital re-admission, morbidity and mortality, relapse, anthropometry, body composition and response to ART) will be determined for each group using multivariate analysis (Cox models for time-to-event data, linear models for continuous outcomes). We will evaluate the ability of MUAC at discharge to predict

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long-term outcomes using receiver-operator-characteristic (ROC) analysis, in the whole cohort and within the HIV-SAM and SAM subgroups. We will then evaluate whether addition of other variables improves the predictive capacity of MUAC for each group, including body composition, haemoglobin, albumin and CRP, plus CD4%, viral load and timing of ART initiation (HIV-SAM only). We will construct multivariable models and compare them with MUAC alone using the net-reclassification index.

Body composition analysis

Previous work in body composition by bio-electrical impedance in Ethiopian infants and children with SAM has shown that the conventional approach, predicting total body weight from height-adjusted impedance, fails due to confounding by oedema⁷². The same project validated an alternative approach, known as Bio-electrical Impedance Vector Analysis (BIVA), and described significant differences between each of three groups: healthy controls, oedematous-SAM and non-oedematous SAM. Vector analysis splits impedance into two height-adjusted components, resistance and reactance, which are further linked through phase angle (PA). Variability in these components is associated with biochemical parameters⁷³. These variables will be explored using graphical analysis, or transformed into age- and sexadjusted z-scores for statistical comparison, including longitudinal analyses. Higher phase angle indicates better nutritional status, while declining height-adjusted resistance over time indicates loss of oedema.

Enteropathy substudy

The primary comparison for the enteropathy substudy will be between children with HIV-SAM (group A) and SAM (group C), stratified by presence or absence of oedema. Control groups (B and D) are well-nourished children with or without HIV, to provide normative data for biomarkers. For each continuous outcome, simple

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descriptive analysis will be used to compare groups (HIV-SAM versus HIV, and HIV-SAM versus SAM) during hospitalisation using t-tests on appropriately transformed data. For any outcome with moderate (p<0.05) evidence of difference between either group a regression model will be constructed including groups A, B, C, D to directly test (using interactions) whether there is a synergistic effect of HIV-SAM versus SAM versus HIV versus neither. These models will also be used to explore whether there is any evidence for heterogeneity in effects between oedematous and nonoedematous SAM. Associations between enrolment factors (e.g. intestinal permeability and microbial translocation) will be explored using pairwise Spearman correlations and principal components analysis. Mean changes at the follow-up timepoints in each group will be estimated, and groups compared (as above) using generalised estimating equations. For outcomes that differ across SAM groups over time, multilevel models will be used to explore possible predictors from the other factors measured. Time to nutritional recovery will be compared using Kaplan-Meier and log-rank tests.

Microbiota and metabolomics substudy

The primary comparison will be between HIV-uninfected children with oedematous and non-oedematous SAM, with a separate comparison between children with HIV-SAM and SAM. Analyses will examine: (i) differences in metagenomic/metabolomic variables between groups at each time-point; (ii) differences in metagenomic/metabolomic variables within groups over time; (iii) correlations between metagenomic and metabolomic variables; and (iv) correlations between metagenomic/metabolomic variables and clinical outcomes. A systematic analysis will be undertaken to reduce high-dimensional data, integrate the multi-omics datasets and minimise false discovery.

Compositional metagenomic data will be compared between groups for indices of alpha and beta diversity. Principal coordinate analysis and partial least squares discriminant analysis will be performed on metabolomics data to identify overall differences between groups. High-dimensional datasets will be reduced using random forest models to identify taxa, microbial gene families and metabolites that most strongly contribute to differences between groups, corrected by Benjamani-Hochburg false discovery rate detection. Targeted analysis by qRT-PCR will validate differential abundance or expression of candidate microbial genes. Longitudinal comparisons will be performed within and between groups using multilevel simultaneous component analysis. Orthogonal projections to latent structures models will integrate metabolomic and metagenomic data whilst linear regression, canonical correlation and hierarchal clustering analysis will measure correlations between - omics datasets. Finally, ROC analysis will identify the ability of different analytes to predict long-term nutritional and clinical outcomes.

Immunology substudy

Integrated profiles of innate immune cell function will be generated for each child using principal components analysis followed by hierarchical clustering^{74 75}. This data-reduction method identifies whether absolute levels of specific markers or relative differences between markers differentiate children into groups. The resulting innate immune profiles will be compared between SAM, HIV-SAM and well-nourished groups using univariate tests and multivariate analysis of variance (MANOVA) of the principal components.

To address the relationship between immune function and infections, regression analyses will determine whether baseline innate immune profiles (or the individual parameters defining them) are associated with the infectious morbidity or mortality during hospitalisation, using logistic models for binary outcomes and linear models BMJ Open: first published as 10.1136/bmjopen-2018-023077 on 1 February 2019. Downloaded from http://bmjopen.bmj.com/ on May 15, 2025 at Department GEZ-LTA Erasmushogeschool . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

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for duration. Key clinical characteristics, including age, sex, oedema and baseline WHZ, will be added to models to investigate their confounding effects. Multivariate stacked regression methods will be used to compare the impact of different factors on severe bacterial infections based on heterogeneity tests.

To determine whether treatment for SAM restores innate immune cell antibacterial function, mixed effects regression models will compare longitudinal changes in individual immune parameters, and the principal components calculated from the weights identified at baseline (which include healthy controls). Similarities and differences in longitudinal immune profiles will be compared between groups using nonmetric multi-dimensional scaling^{74 76 77}. This approach will group children according to their composite innate immune function, allowing the duration and variability of immune restoration to be evaluated over the course of nutritional rehabilitation. Binary logistic regression will determine whether innate immune profiles at discharge are associated with morbidity or mortality during follow-up.

PATIENT AND PUBLIC INVOLVEMENT

Patients and their caregivers were not involved in the design of the study. During recruitment, all caregivers of children admitted to hospital were given information about the study; those whose children had severe acute malnutrition were approached to give written informed consent. A meeting to disseminate results of the study to participants and their caregivers will be held at the end of the study. An interactive game to engage caregivers in the science underlying malnutrition is being developed in collaboration with experts from the Centre of the Cell, a unique science education centre based at Queen Mary University of London (https://www.centreofthecell.org/).

SAFETY REPORTING

For all adverse events, the study team will assess expectedness and relatedness to study activities. Since this is an observational study without interventions, we anticipate that the risk is minimal; however, serious adverse events will be reported to local ethical review boards (Medical Research Council of Zimbabwe, and University of Zambia Biomedical Research Ethics Committee) and the study sponsor (Queen Mary University of London) according to their respective guidelines.

DATA COLLECTION AND MONITORING

Clinical and demographic data are recorded on paper case report forms. All data are checked for completeness and plausibility before data entry and problems flagged for resolution by the clinical team. All data are double-entered onto a dedicated password-protected study database, and any discrepancies resolved. Study participants are identified on electronic databases only by study numbers (assigned at enrolment); no personal identifiers are entered.

ETHICS AND DISSEMINATION

The study complies with the principles of the Declaration of Helsinki (2008) and is conducted in compliance with the principles of Good Clinical Practice (GCP) and local regulatory requirements in each country. Ethical approval was obtained from the University of Zambia Biomedical Research Ethics Committee, the Joint Research Ethics Committee of the University of Zimbabwe and the Medical Research Council of Zimbabwe. The ethical review board of the Sponsor, Queen Mary University of London, provided an advisory review of the study. Since this is an observational study, there is no Data and Safety Monitoring Board.

Results will be disseminated through conference abstracts and peer-reviewed publications and discussed with relevant policymakers and programmers. Study findings will be disseminated to families of participants at face-to-face meetings.

TIME FRAME AND STUDY STATUS

Enrolment into the study began in July 2016 and is expected to end in March 2018. All participants will be followed for 48 weeks, with an expected study completion date of March 2019.

DISCUSSION

HOPE-SAM aims to document the short- and long-term outcomes of children with SAM and HIV-SAM, and to identify the factors at presentation and at discharge from hospital that independently predict these outcomes. Mechanistic substudies aim to evaluate the contribution of enteropathy, microbiota, metabolome and innate immune cell function to these clinical outcomes. The prevalence of malnutrition in HIVinfected children is as high as 40% in some settings and the challenges of managing this population are well recognised⁷⁸. The WHO protocol on management of SAM aims to reduce case fatality below 10%, but rates as high as 35% are still reported among HIV-infected children^{79 80}. No studies have systematically and longitudinally collected morbidity data in HIV-SAM, or documented repeat hospitalisations and mortality after discharge from hospital, particularly in the current era where ART is available upon diagnosis. HOPE-SAM will provide a unique opportunity to enrol and follow a cohort of children managed for SAM in three large hospitals across two sub-Saharan African countries at several time-points over a one-year period. Nested longitudinal laboratory substudies aim to better characterise the pathogenesis of SAM and HIV-SAM, to determine whether pathogenic processes are normalised during nutritional rehabilitation and follow-up, and to identify potential mechanistic pathways for new intervention approaches.

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AUTHOR CONTRIBUTIONS

Designed study: MB-D, BA, CDB, RCR, BM, KC, CK, KCh, DN, PC, NC, FM, JW, ARM, JS, ASW, KJN, PK, AJP Sought funding: MB-D, BA, CDB, RCR, JHH, ARM, JS, ASW, KJN, PK, AJP Undertaking study: BM, KC, CK, KCh, FM, DN, PC, NC, FM, IM, EB, KM, SM, TR Study oversight: MB-D, BA, JHH, KJN, PK, AJP Analysis: MB-D, BA, CDB, RCR, RN, JW, ARM, JS, ASW, KJN, PK, AJP Wrote first draft of manuscript: MB-D, CDB, RCR, AJP Critically revised manuscript: All

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COMPETING INTERESTS

None of the authors have any competing interests to declare.

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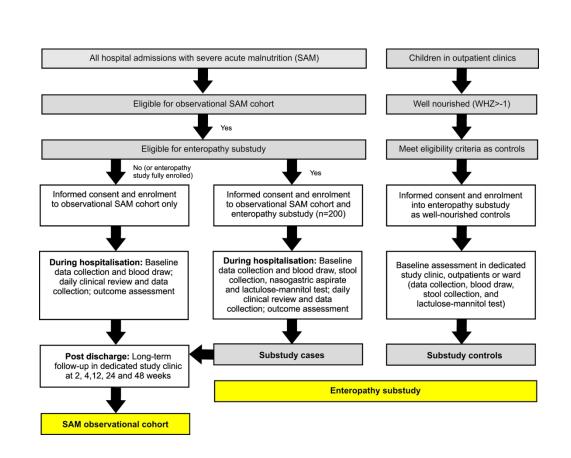
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FIGURE LEGENDS

Figure 1: Study flow chart.

All hospital admissions are screened for eligibility for the observational cohort and enteropathy substudy, with procedures undertaken as shown in the flow chart during hospitalisation and post-discharge. Well-nourished children from outpatient clinics meeting eligibility criteria as healthy controls are enrolled and undergo a single baseline assessment as shown. for oper texics only



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Supplementary Table 1: Assays undertaken on stored samples for children in the observational cohort.

| Sample type | Assay (method) | Location of work | Study subjects | Time-points |
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| Blood | HIV testing (rapid antibody test algorithm if >18 mo old or HIV DNA | TROPGAN, Zvitambo or clinical sites | All | Baseline |
| Blood | PCR <18 mo old) ¹ CD4 count (flow cytometry or PIMA) ¹ | TROPGAN, Zvitambo or clinical sites | All HIV- positive | Baseline, wk 12, 24, 48 |
| Plasma | HIV viral load (real-time PCR) ¹ | TROPGAN, Zvitambo or clinical sites | All HIV- positive | Baseline, wk 12, 24, 48 |
| Plasma | C-reactive protein (ELISA) | TROPGAN, Zvitambo | All | Baseline, discharge, wk 12, 24, 4 |
| Plasma | Albumin (ELISA) | TROPGAN, Zvitambo | All | Baseline, discharge, wk 12, 24, 4 |
| Plasma | Lipopolysaccharide (LAL assay) | TROPGAN and Zvitambo | Subgroup ³ | Baseline, discharge, wk 12, 24, 4 |
| Plasma | Lipopolysaccharide binding protein (LBP) | TROPGAN and Zvitambo | Subgroup ³ | Baseline, discharge, wk 12, 24, 4 |
| Plasma | sCD14 (ELISA) | TROPGAN and Zvitambo | Subgroup ³ | Baseline, discharge, wk 12, 24, 4 |
| Plasma | sCD163 (ELISA) | TROPGAN and Zvitambo | Subgroup ³ | Baseline, discharge, wk 12, 24, 4 |
| Plasma | IL-6, TNF-alpha, IL-1β (ELISA) and/or multiplex cytokines | TROPGAN and Zvitambo | Subgroup ³ | Baseline, discharge, wk 12, 24, 4 |
| Plasma | Total PAMP activity (THP1 reporter cell line ²) | TROPGAN and Zvitambo | Subgroup ³ | Baseline, discharge, wk 12, 24, 4 |
| Whole blood | Molecular techniques for bacterial detection (broad-range and specific PCR and next- generation sequencing) | QMUL, London ² | Subgroup ³ | Baseline, discharge, wk 12, 24, 4 |
| Whole blood | <i>In vitro</i> binding to bacterial products, cytokine expression and cellular responses to PAMP stimulation ⁴ | TROPGAN, Zvitambo | All | Baseline, discharge, wk 12, 24, 4 |
| Plasma | Co-culture with healthy immune cells ⁵ | Blizard Institute | All | Baseline, discharge, wk 12, 24, 4 |

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¹If HIV test, CD4 and viral load have already been conducted as part of routine clinical care, they will not be repeated on the research sample.

²THP1 reporter cells are derived from THP1, a human monocytic cell line that naturally expresses many pattern recognition receptors (PRR). The cell line stably expresses an NF- κ B/AP-1 inducible reporter (SEAP) system to facilitate the monitoring of PRR-induced NF- κ B/AP-1 activation. ³Assays will be undertaken in a subgroup of children, using a case-control or case-cohort design

³Assays will be undertaken in a subgroup of children, using a case-control or case-cohort design to evaluate the impact of biomarkers on immune activation and mortality.

⁴Whole blood will be stimulated with pathogen-associated molecular patterns (PAMP) in culture plates and bacterial antigens labelled with fluorescent tags in test tubes, and incubated for 1-24hr. Supernatant will be removed and stored at -80C for subsequent analysis of pro- and anti-inflammatory cytokines, and cells will be fixed as described in section 12.2.2 for subsequent analysis of bacterial binding, cellular activation, proliferation and cytokine elaboration by flow cytometry.

⁵To determine the effect of the systemic milieu on healthy immune cell function, plasma samples will be transported to the Blizard Institute and co-cultured with healthy immune cells, which will be functionally analysed via multi-parameter flow cytometry in the Flow Cytometry Core Facility.

IFABP: Intestinal fatty acid binding protein; ELISA: Enzyme-linked immunosorbent assay; GLP-2: glucagon-like peptide 2; sCD14: soluble CD14; sCD163: soluble CD163; PAMP: pathogenassociated molecular pattern; QMUL: Queen Mary University of London; CRP: C-reactive protein; LAL: limulus amoebocyte lysate assay.

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Supplementary table 2: Additional laboratory analyses for enteropathy substudy

| Sample type | Assay (method) | Location of work | Study groups ¹ | Time- points |
|--|---|---|---|--|
| Urine | Lactulose-mannitol ratio (mass spectrometry) | Orgeon Analytics, USA ² | Ā, B, C, D (all) | Baseline, discharge, wk 12 and 48 |
| Stool | Neopterin, myeloperoxidase, alpha-1 antitrypsin and REG-1B (ELISA) | TROPGAN and Zvitambo | A, B, C, D (all) | Baseline, discharge, wk 12, 24, 48 |
| Plasma | I-FABP (ELISA) | TROPGAN and Zvitambo | A, B, C, D (all) | Baseline, discharge, wk 12, 24, 48 |
| Plasma | GLP-2 | TROPGAN and Zvitambo | A, B, C, D (all) | Baseline, discharge, wk 12, 24, 48 |
| Plasma | Citrulline (mass spectrometry) | Imperial College London | A, B, C, D (all) | Baseline, discharge, wk 12, 24, 48 |
| Plasma | Kynurenine:tryptophan ratio and metabolites along tryptophan pathway (mass spectrometry) | Imperial College London | A, B, C, D (all) | Baseline, discharge, wk 12, 24, 48 |
| Stool | Microbiome analysis ³ | BCCDC, Vancouver ² | A, B, C, D (all) | Baseline, discharge, wk 12, 24, 48 |
| Stool | Helicobacter pylori antigen | TROPGAN and Zvitambo | A, B, C, D (all) | Baseline |
| Gastric juice | Culture and molecular techniques for bacterial detection (broad-range and specific PCR and next-generation sequencing) | QMUL, London ² | Subgroup of A, C (n=50 per group) | Baseline |
| RNA extracted from PAXGene tubes | Gene expression analysis (RNASeq) | QMUL, London ² | A, B, C, D (all) | Baseline and discharge |
| Plasma and urine | Targeted and untargeted metabolic phenotyping | Imperial College London ² | A, B, C, D (all) | Baseline, discharge, wk 12, 24 and 48 |

¹Enteropathy substudy groups are shown in **Table 1**. Note that controls only have blood taken at baseline as per Table 4.

²Assay methodology not available in country. See section 12.1.4 for details of shipment to UK/USA/Canada.

³For microbiome analyses,total DNA and/or RNA will be extracted from stool samples and used as template for next generation sequencing library preparation and for quantitative polymerase chain reaction (qPCR), then sequenced via whole metagenome shotgun sequencing (see section 12.3.3.2).

QMUL: Queen Mary University of London; BCCDC: British Columbia Centre for Disease Control.

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Health Outcomes, Pathogenesis and Epidemiology of Severe Acute Malnutrition (HOPE-SAM): rationale and methods of a longitudinal observational study

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| 1 | Health Outcomes, Pathogenesis and Epidemiology of Severe Acute |
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| 2 | Malnutrition (HOPE-SAM): rationale and methods of a longitudinal |
| 3 | observational study |
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| 5 | Mutsa Bwakura-Dangarembizi ¹ , Beatrice Amadi ² , Claire D Bourke ³ , Ruairi C |
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1 ABSTRACT

2 Introduction

Mortality among children hospitalised for complicated severe acute malnutrition
(SAM) remains high despite the implementation of WHO guidelines, particularly in
settings of high HIV prevalence. Children continue to be at high risk of morbidity,
mortality and relapse after discharge from hospital although long-term outcomes are
not well documented. Better understanding the pathogenesis of SAM and the factors
associated with poor outcomes may inform new therapeutic interventions.
Methods and analysis

- 11 The Health Outcomes, Pathogenesis and Epidemiology of Severe Acute Malnutrition
- 12 (HOPE-SAM) study is a longitudinal observational cohort that aims to evaluate the
- 13 short- and long-term clinical outcomes of HIV-positive and HIV-negative children with
- 14 complicated SAM, and to identify the risk factors at admission and discharge from
- 15 hospital that independently predict poor outcomes. Children aged 0-59mo
- 16 hospitalised for SAM are being enrolled at three tertiary hospitals in Harare,
- 17 Zimbabwe, and Lusaka, Zambia. Longitudinal mortality, morbidity and nutritional data
- 18 are being collected at admission, discharge and for 48 weeks post-discharge. Nested
- 19 laboratory substudies are exploring the role of enteropathy, gut microbiota,
- 20 metabolomics and cellular immune function in the pathogenesis of SAM using stool,
- 21 urine and blood collected from participants and from well-nourished controls.
- 22

23 Ethics and dissemination

- 24 The study is approved by the local and international institutional review boards in the
- 25 participating countries (the Joint Research Ethics Committee of the University of
- 26 Zimbabwe, Medical Research Council of Zimbabwe and University of Zambia
- 27 Biomedical Research Ethics Committee) and the study sponsor (Queen Mary
- 28 University of London). Caregivers provide written informed consent for each

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| 1 | participant. Findings will be disseminated through peer-reviewed journals, |
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| 2 | conference presentations and to caregivers at face-to-face meetings. |
| 3 | |
| 4 | Strengths and limitations of this study |
| 5 | Strengths: |
| 6 | The primary strength of this study is the rigorous collection of longitudinal |
| 7 | data on morbidity, mortality and nutritional status during inpatient care and for |
| 8 | 48 weeks after initial admission for SAM. |
| 9 | Laboratory sub-studies investigating enteropathy, microbiota, metabolomics |
| 10 | and immune cell function provide a unique opportunity to understand which |
| 11 | pathogenic pathways contribute to SAM and whether these processes |
| 12 | normalise with nutritional rehabilitation, capitalising on a well-characterised |
| 13 | cohort with inclusion of well-nourished controls. |
| 14 | This study will compare longitudinal clinical outcomes among HIV-negative |
| 15 | and HIV-positive children with SAM in the current ART era. |
| 16 | |
| 17 | Potential limitations: |
| 18 | High loss to follow-up due to participants returning to home settings following |
| 19 | hospital discharge. A dedicated clinical study team is in place to maximise |
| 20 | follow-up through phone reminders and community visits. |
| 21 | The clinical heterogeneity of the study participants, including comorbidities |
| 22 | such as infections, may make it challenging to identify the specific causes of |
| 23 | |
| 23 | clinical outcomes. However, the embedded sub-studies will enable multiple |
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| | clinical outcomes. However, the embedded sub-studies will enable multiple |
| 24 | clinical outcomes. However, the embedded sub-studies will enable multiple pathways to be explored within the same cohort. |
| 24 25 | clinical outcomes. However, the embedded sub-studies will enable multiple pathways to be explored within the same cohort. Potential bias in recruiting well-nourished controls only from hospitals will be |

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| 1 | | |
|----------------|----|---|
| 2 3 | 1 | INTRODUCTION |
| 4 5 | 2 | Malnutrition underlies almost half of all childhood deaths in developing countries ¹ . |
| 6 7 | 3 | Severe acute malnutrition (SAM) is defined by a weight-for-height Z-score <-3, mid- |
| 8 9 | 4 | upper arm circumference (MUAC) <115mm and/or bilateral pitting oedema ² . Current |
| 10 11 | 5 | treatment guidelines distinguish two groups: i) children with uncomplicated SAM who |
| 12 13 | 6 | can be managed in the community; and ii) children with complicated SAM, who are |
| 14 15 | 7 | hospitalised and undergo resuscitation, stabilisation and nutritional rehabilitation. In- |
| 16 17 | 8 | hospital mortality in children with complicated SAM remains high despite the |
| 18 19 | 9 | implementation of WHO guidelines ³ . Furthermore, SAM presents as two major |
| 20 21 | 10 | clinical phenotypes: non-oedematous SAM (marasmus), characterised by severe |
| 22 23 | 11 | wasting, and oedematous SAM (kwashiorkor), a more complex syndrome |
| 24 25 | 12 | characterised by bilateral pitting oedema, steatosis and diarrhea ⁴⁵ . Despite differing |
| 26 27 | 13 | clinical outcomes, treatment protocols are the same for both oedematous and non- |
| 28 29 | 14 | oedematous SAM. |
| 30 31 | 15 | |
| 32 33 | 16 | A contributory factor to high in-patient mortality is the co-occurrence of HIV infection |
| 34 35 | 17 | in around one-third of children hospitalised for SAM in sub-Saharan Africa ⁶⁷ . While |
| 36 37 | 18 | new HIV infections in children have declined ⁸ , a substantial number of infected |
| 38 39 | 19 | children are diagnosed late and present with malnutrition. There is also a growing |
| 40 41 | 20 | population of HIV-exposed uninfected (HEU) children who have immune |
| 41 42 43 | 21 | abnormalities, poor growth and higher risk of mortality and infectious morbidity ⁹ . |
| 43 44 45 | 22 | Hence, HIV has transformed the epidemiology and outcomes of SAM ¹⁰ . Even with |
| 46 | 23 | standardised treatment approaches, inpatient deaths are almost four-fold higher |
| 47 48 | 24 | among HIV-positive children with SAM (herein termed HIV-SAM), compared to HIV- |
| 49 50 | 25 | negative children with SAM (30.4% vs 8.4%), for reasons that remain unclear ¹⁰ ; this |
| 51 52 | 26 | mortality is three-fold higher than would be expected from anthropometric |
| 53 54 | 27 | parameters alone ¹⁰ . Management of HIV-SAM is particularly challenging because |
| 55 56 | 28 | HIV fundamentally alters the clinical presentation of malnutrition and the response to |
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| 1 | treatment. Children with HIV-SAM are more stunted and wasted; have a higher |
|----|--|
| 2 | frequency of persistent diarrhoea; tend to have delayed nutritional recovery and have |
| 3 | a more complicated clinical course than HIV-negative children with SAM ¹⁰ . |
| 4 | |
| 5 | Long-term outcomes of SAM |
| 6 | Following resolution of complications and return of appetite, children are discharged |
| 7 | from hospital to continue therapeutic feeds at home. However, emerging data |
| 8 | indicate high post-discharge mortality following in-hospital management of SAM ¹¹⁻¹³ . |
| 9 | Malnutrition together with young age, HIV infection and pneumonia have been |
| 10 | associated with higher post-discharge mortality ¹⁴ . One of the largest prospective |
| 11 | studies of growth and mortality in children with SAM (FuSAM), conducted in Malawi |
| 12 | from July 2006 to March 2007, collected 12-month outcome data on 87% of 1024 |
| 13 | children admitted to the nutrition ward ¹¹ . A total of 427 (42%) died and 44% of these |
| 14 | deaths occurred after discharge from hospital. Survival was greatest among those |
| 15 | who were nutritionally cured upon discharge from outpatient therapeutic feeding |
| 16 | centres, defined as two consecutive visits with >80% expected weight-for-height, no |
| 17 | oedema and clinically stable. The risk of mortality after hospital discharge was four- |
| 18 | fold higher for HIV-SAM compared to HIV-negative children with SAM, but the |
| 19 | outcomes among HEU children were not reported. The loss to follow-up was high in |
| 20 | the FuSAM study because there was only one follow-up visit, one year after |
| 21 | discharge from outpatient-feeding centres. A recent study from Kenya identified |
| 22 | malnutrition and HIV infection as key drivers for post-discharge mortality, with 52% of |
| 23 | deaths attributable to MUAC <11.5cm and 11% to HIV infection ¹⁵ . |
| 24 | |
| 25 | The impact of SAM appears to persist beyond the first year after discharge from |
| 26 | hospital. The ChroSAM study, which followed children with SAM seven years post- |
| 27 | discharge, showed that children had poorer growth, body composition and physical |
| 28 | function compared to siblings and community controls, which are all indicators of |

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1 future cardiovascular and metabolic disease¹².

While anthropometry is used to assess nutritional recovery after discharge from hospital, the pattern and quality of growth recovery following SAM is poorly understood. The observation that children treated for SAM have a deficit in lean tissue despite regaining weight suggests that assessing body composition in addition to anthropometry may help to identify children who have not completely recovered and are at potential risk of long-term metabolic diseases¹². Children with HIV-SAM appear to have potential for catch-up growth in weight-for-age and/or weight-for-height, which have been shown to normalise with treatment even prior to widespread availability of ART¹⁶; by contrast, height-for-age shows less potential for catch-up growth¹⁷. However, the body composition of children with HIV-SAM compared to HIV-negative children with SAM has not been described. Whether children recover fat mass at the expense of lean mass is unknown, but differences in tissue accretion patterns may have implications for survival and long-term metabolic health^{18 19}. There is also a need to consider the effect of SAM on the size of body parts which grow at different rates: relatively shorter legs, for example, are associated with epidemiologic risk of overweight, coronary artery disease, liver dysfunction and diabetes^{20 21}. Taken together, there is clearly an elevated risk of mortality among HIV-positive children with SAM compared to HIV-negative children with SAM, and an ongoing mortality risk among all children with SAM that persists after discharge from hospital. There are several gaps in our understanding of the long-term outcomes: (i) causes of death have not been clearly defined; (ii) no studies have systematically and longitudinally collected morbidity and mortality data or documented repeat hospitalisations post-discharge; and, (iii) the long-term outcomes of HIV-positive children with SAM in the era of ART availability are unclear.

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Pathogenesis of SAM

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Better understanding the pathogenesis of SAM may help to explain the high mortality

of children both during and after hospitalisation and identify new targets for

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| or children both during and alter hospitalisation and identity new targets for |
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| interventions to supplement existing treatment strategies. Consistent evidence that |
| immune mediators are altered in malnutrition ²² and that systemic and intestinal |
| inflammation are associated with poor outcomes in SAM ²³ , suggest that immune |
| dysfunction contributes to infectious susceptibility ²⁴ . Malnutrition is also characterised |
| by a complex derangement in gut microbial ²⁵ metabolic, ²⁶ immune ²⁷ and hormonal |
| pathways, organ dysfunction and micronutrient deficiencies in the context of co- |
| infections, enteropathy and chronic inflammation. Several studies have recently |
| provided insights into these perturbations using new tools ^{25 26 28 29} , including |
| metabolomics and metagenomics, but we still lack a clear understanding of many of |
| the pathogenic pathways driving malnutrition, the interactions between these |
| pathways, and which are the most tractable targets for intervention. |
| |
| SAM shares several pathological and clinical features with HIV, which may explain |
| clinical outcomes in these co-occuring conditions: 1) both are characterised by |
| intestinal damage, leading to impairment of the mucosal barrier and increased |
| intestinal permeability; 2) both have underlying systemic immune activation; and 3) |
| both are frequently complicated by persistent diarrhoea, pneumonia and sepsis that |
| may plausibly arise due to loss of intestinal barrier function ³⁰ . Understanding the |
| overlapping impact of HIV and SAM is critical to inform additional interventions to |
| improve outcomes of children with HIV-SAM. |
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| |
| OBJECTIVES OF HOPE-SAM |
| OBJECTIVES OF HOPE-SAM The Health Outcomes, Pathogenesis and Epidemiology of Severe Acute Malnutrition |
| |

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1 2

To describe the short- and long-term clinical outcomes of children with
 complicated SAM, with and without HIV infection, and to identify the risk
 factors at admission and discharge from hospital that independently predict
 these outcomes.

5 2) To better characterise the pathogenesis of SAM through nested laboratory
 sub-studies evaluating enteropathy, gut microbiota, metabolomics and
 immune cell function.

8

9 STUDY DESIGN

10 HOPE-SAM is a longitudinal observational cohort study, enrolling between 600-800 11 children aged 0-59 months admitted with complicated SAM to the tertiary pediatric 12 wards at two sites in Zimbabwe (Parirenyatwa Hospital and Harare Children's 13 Hospital) and one in Zambia (University Teaching Hospital, Lusaka). Both HIV-14 positive and HIV-negative children will be enrolled. Throughout this paper, 'SAM' 15 refers to all children, regardless of HIV status; where analyses specifically compare 16 children by HIV status, groups are identified as HIV-positive children with SAM (or 17 HIV-SAM) and HIV-negative children with SAM. All participants with SAM are 18 followed for 48 weeks post-discharge, with longitudinal data collection and blood 19 sampling. The study contains four nested sub-studies as shown in Figure 1. A 20 subgroup of children will be recruited to the enteropathy substudy for which they will 21 have the same follow-up procedures but more intensive biological specimen 22 collection including stool (all time-points), urine after lactulose-mannitol (LM) 23 challenge as an assessment of intestinal permeability, and nasogastric aspirate 24 (baseline only); these children are also included in microbiota and metabolomics 25 substudies. Children with SAM for whom blood samples are available are included in 26 the immunology substudy, for which circulating inflammatory mediators will be 27 assayed; functional cellular immunology assays will be conducted for all children in 28 the immunology sub-study with sufficient sample volume (> 2mL) recruited after June

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| 1 | 2017. A group of healthy children recruited from the same hospitals and |
|----|--|
| 2 | communities, who are well-nourished and matched to children in the enteropathy |
| 3 | substudy by age and HIV status, will have data and specimens collected to provide |
| 4 | normative data for the laboratory substudies; these well-nourished controls will not |
| 5 | be followed longitudinally. |
| 6 | |
| 7 | The study protocol, data collection forms and standard operating procedures are |
| 8 | available online at osf.io/29uaw. |
| 9 | |
| 10 | RECRUITMENT |
| 11 | Screening: Caregivers of all hospitalised children are sensitised about the study. All |
| 12 | new admissions aged 0-59 months are screened for SAM, which is defined |
| 13 | according to WHO criteria as any of: weight-for-height Z-score (WHZ) <-3, MUAC |
| 14 | <115 mm (if aged 6-59mo) and/or bilateral pitting oedema. All children with SAM are |
| 15 | recruited from hospital and this study therefore focuses on complicated SAM; |
| 16 | children with uncomplicated SAM will not be enrolled. |
| 17 | |
| 18 | Eligibility for observational cohort: All children with SAM whose caregivers are willing |
| 19 | to provide written informed consent and to learn their child's HIV status are offered |
| 20 | enrolment. Any children who die prior to study enrolment and those with a known |
| 21 | malignancy are ineligible. |
| 22 | |
| 23 | Eligibility for enteropathy substudy: Children with SAM aged 6-59 months with a |
| 24 | nasogastric tube in place (or due to be placed) are categorized into 4 groups (HIV- |
| 25 | positive oedematous (Group A-I); HIV-positive non-oedematous (Group A-II); HIV- |
| 26 | negative oedematous (Group C-I) and HIV-negative non-oedematous (Group C-II), |
| 27 | as shown in Table 1. Children meeting eligibility criteria will be enrolled throughout |
| 28 | the study recruitment period until sufficient specimens have been collected from the |
| | |

- groups shown in Table 1. Children in the enteropathy substudy are stratified into age
- bands (6-11 months; 12-23 months and 24-59 months) to enable age-matching of
- well-nourished controls. Children with underlying chronic gastrointestinal disease or a
- known malignancy are ineligible.

Table 1: Enteropathy substudy groups

| Children aged 6-59 months | Severe acute ma | Well nourished | | |
|---|-------------------------|----------------------|---------------------------------|--|
| | Oedematous ² | Non-oedematous | controls WHZ>-1 | |
| HIV-positive (HIV PCR+ if <18mo; HIV antibody + if >18mo) | N=50 (Group A-I) | N=50 (Group A-II) | N=100 ³ (Group B) | |
| HIV-negative (HIV PCR- if <18mo; HIV antibody - if >18mo) | N=50 (Group C-I) | N=50 (Group C-II) | N=100 ⁴ (Group D) | |

¹SAM defined according to WHO criteria

²Presence of bilateral pitting pedal oedema.

Note that children below 6 months of age are excluded from the enteropathy substudy to avoid

interrupting exclusive breastfeeding during the lactulose-mannitol test.

WHZ: Weight-for-height Z score; PCR: polymerase chain reaction.

Eligibility for microbiota and metabolomics substudies: Children enrolled into the

enteropathy substudy are also included in the microbiota and metabolomics

substudies, since these substudies utilize the stool, urine and plasma samples

collected for enteropathy analyses.

Eligibility for immunology substudy: The immunology substudy comprises all children

with SAM (drawn from both the observational cohort and the enteropathy substudy,

- as shown in Figure 1) providing a blood sample of sufficient volume (>2ml) for
- cellular assays after 1st June 2017.

- Well-nourished controls: Controls are children drawn from the same hospitals and
- communities as cases with SAM (including well-nourished sibling controls), who are

Baseline data

Daily clinical review⁵

CD4 count and viral load

Blood collection⁶

HIV testing⁷

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| 1 | aged 6-59 months (match | ed to enterop | athy substudy | childre | n withiı | n age ba | ands), | | |
|----------|---|-------------------------|------------------------|----------|----------|-------------------|-----------|----------|--|
| 2 | well-nourished (weight-for-height Z-score >-1) and clinically well (no acute illness or | | | | | | | | |
| 3 | current infections) with known HIV status. Controls are categorized into two groups: | | | | | | | | |
| 4 | well-nourished HIV-positiv | /e (Group B) a | and well-nouris | hed HI | V-nega | ative (G | roup D) | , | |
| 5 | as shown in Table 1. Child | dren with unde | erlying chronic | gastro | intestir | al disea | ase or a | | |
| 6 | known malignancy are ine | eligible. Well-r | ourished contr | ols pro | vide co | omparis | on | | |
| 7 | biomarker data for all the | laboratory sub | ostudies. | | | | | | |
| 8 | | | | | | | | | |
| 9 | Informed consent procedu | <i>ures:</i> Written ir | nformed conse | nt is ob | tained | from the | e primar | y | |
| 10 | caregiver using consent for | orms translated | d into local lang | luages | ; where | possib | le, other | | |
| 11 | family members are includ | | - | - | | • | | | |
| 12 | understood a verbal expla | | | | - | | | | |
| 13 | presence of a witness. As | | | | | | | hd | |
| 13 | | | | , ni 600 | | | youro e | <i>.</i> | |
| 15 | STUDY PROCEDURES | | | | | | | | |
| | | lined in Teble | | | | | | | |
| 16 | Study procedures are out | | | | _ | | | | |
| 17 18 | Table 2: Summary of pro | ocedures in c | observational | cohor | t | | | | |
| | Assessment | Hospitaliza | tion | Post | -disch | arae ³ | | | |
| | | Baseline ¹ | Discharge ² | 2w | 4w | 12w | 24w | 48 | |
| | Caregiver informed consent to join observational cohort | x | | | | | | | |
| | Summary checklist | X | | | | | | | |
| | Locator information ⁴ | Х | | | | | | | |
| | Acute admission information | X | | | | | | | |

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Daily during

hospitalisation

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| (HIV-infected children | | | | | | | |
|---------------------------------|---|---|-------|--------|----------|----------|------|
| only) | | | | | | | |
| Full blood count ⁸ | Х | Х | | | х | х | Х |
| Anthropometry | х | х | х | х | х | х | х |
| Skinfold thickness ⁹ | | Х | х | х | Х | Х | Х |
| Body composition ¹⁰ | Х | Х | Х | х | Х | Х | х |
| Discharge data collection | | х | | | | | |
| Daily morbidity diary | | | Daily | during | follow-u | ip perio | d by |
| | | | careg | ivers | | | |
| Follow-up clinic: history, | | | Х | х | Х | х | х |
| examination, morbidity | | | | | | | |
| and mortality data | | | | | | | |
| | | | | | | | |

| 1 | |
|--|---|
| 234567890112314516718902122345678901123145167189021223245678903132 | ¹Children will be enrolled as soon as possible after hospitalisation and will undergo baseline investigations as soon as possible after enrolment. This is to provide a window of opportunity to time collection of research specimens with clinical specimens, and to ensure that the child is clinically stable before undertaking research investigations. ²The discharge procedures will be undertaken on the day of discharge, or as close as possible to that date. ³Windows will be created around these post-discharge time-points to maximize follow-up for caregivers who miss visits or are unavailable, as follows: 2 weeks (1-3 weeks); 4 weeks (3-5 weeks); 12 weeks (10-14 weeks); 24 weeks (20-28 weeks); 48 weeks (44-52 weeks). ⁴Locator information will updated at subsequent visits if caregivers have moved or changed contact details. ⁵A clinical review will be undertaken every day between admission and discharge by the study clinician. ⁶5.4 mL of blood (depending on child weight; amount will not exceed 2 mL/kg total over 2 week period) will be collected by a study nurse into endotoxin-free EDTA tubes. Samples will be used to store whole blood, PBMC and plasma for subsequent measurement of CRP and albumin. Where blood sample volumes allow (≥2mL sample), bacterial binding assays and whole blood stimulations will be conducted and culture supernatants and cells stored for subsequent assessment of immune cell function at each time-point. ⁸Full blood count results will not be collected from children with known haemoglobin <6 g/dL. ⁷HIV testing is conducted as part of routine clinical practice, but if it has not been undertaken, the study sample will be used to measure FBC in clinical laboratories at each site ⁹Skinfold thickness (triceps, subscapular, supra-iliac) and mid-thigh circumference will be measured using Holtain calipers or tape measure. ¹⁰Body composition will be assessed by |
| 33 | Baseline procedures: |
| 34 | Baseline data on maternal and household characteristics, the child's past medical |
| 35 | history and current illness are collected by a study nurse. Anthropometry, including |
| 36 | body composition measured by whole-body (wrist-ankle) bio-electrical impedance |
| 37 | analysis (BodyStat 1500MD; BodyStat Ltd., Douglas, Isle of Man), leg length using |
| 38 | an electronic knemometer (Zimbabwe only) and triceps, subscapular and supra-iliac |
| | |

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| 1 | skinfold thickness using calipers (Holtain Ltd., Crymych, UK) are undertaken at |
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| 2 | baseline. Blood (1mL/kg up to 5.4mL maximum) is collected at baseline into an |
| 3 | endotoxin-free EDTA tube for all children and, in the enteropathy substudy, |
| 4 | additionally into a PAXgene tube (PreAnalytiX GmbH, Hombrechtikon, Switzerland) |
| 5 | for subsequent transcriptomic analysis. Blood is not collected from children with |
| 6 | severe anaemia (known haemoglobin <6 g/dL). HIV testing is carried out in |
| 7 | accordance with national guidelines as part of routine clinical practice; where it has |
| 8 | not been done, the child's HIV status is ascertained using a rapid test antibody |
| 9 | algorithm for children over 18 months, or HIV DNA PCR for children under 18 |
| 10 | months. CD4 count/percentage and viral load are measured in HIV-positive children. |
| 11 | Maternal HIV status is documented where available, so that HIV-exposed uninfected |
| 12 | children can be identified. Blood samples are sent to research laboratories at each |
| 13 | site to conduct whole blood stimulation and bacterial binding assays (as described in |
| 14 | the immunology substudy) and to store aliquots of whole blood, peripheral blood cells |
| 15 | and plasma at -80°C ³¹ . In the enteropathy substudy, nasogastric aspirate, stool and |
| 16 | urine (after an oral dose of lactulose and mannitol) are also collected. Lactulose and |
| 17 | mannitol are ingested by the child after fasting and urine is collected over a two-hour |
| 18 | period to measure recovery of lactulose and mannitol, a measure of intestinal |
| 19 | absorptive capacity and permeability, as previously described ³² . |
| 20 | |
| 21 | Daily procedures: Routine inpatient management is undertaken by ward clinical |
| 22 | teams according to local hospital protocols, which are based on WHO guidelines ^{2,33} |
| 23 | In addition, the HOPE-SAM study clinician at each hospital site collects daily data |
| 24 | until discharge on clinical parameters (including daily examination), resolution of |
| 25 | acute infections, nutritional recovery (loss of oedema, restoration of appetite, weight |
| 26 | gain), and treatment/nutritional supplements received; this will allow us to evaluate |
| | |

27 differences in management between countries. Children with HIV-SAM who are ART-

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naïve start ART according to national guidelines, which are based on WHO
 recommendations^{2 34}.

3

4 Discharge: The clinical team decides when the child is ready to be discharged, which 5 is generally when their medical complications are resolving and the child has a good appetite and is clinically well and alert². Children receive ready-to-use therapeutic 6 7 feeds (RUTF) to take at home according to local guidelines. At discharge, the study 8 nurse collects data and a repeat blood sample (including full blood count) and 9 undertakes discharge anthropometry, body composition, leg length (Zimbabwe only) 10 and triceps, subscapular and supra-iliac skinfold thickness measurements (Table 2). 11 The caregiver is given a daily morbidity diary and pre-prepared stickers 12 corresponding to different illnesses and shown how to complete the diary. The 13 caregiver is provided with the date of the first follow-up appointment and contact 14 details of the study nurse. 15 16 Follow-up: Children attend follow-up appointments at dedicated study clinics at 2, 4, 17 12, 24 and 48 weeks post-discharge. At each visit, the study physician undertakes a 18 clinical assessment and the study nurse captures illness, medication and feeding 19 data. Clinic data are transcribed from handheld medical records if available and the 20 morbidity diary is reviewed and a new diary and stickers supplied. Anthropometry, 21 body composition, leg length (Zimbabwe only) and triceps, subscapular and supra-22 iliac skinfold thicknesses are measured at each visit. Acute illnesses are treated in 23 the study clinic, or the child is referred to hospital if necessary. Children with relapsed 24 malnutrition are provided with nutritional supplements or RUTF according to local 25 guidelines, or readmitted to hospital if they develop complicated SAM. Transport 26 reimbursement for clinic attendance is provided to caregivers for each visit. 27 28 Blood is collected at weeks 12, 24 and 48 post-discharge into endotoxin-free EDTA

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| 1 | tubes to measure full blood count, CD4 count and viral load (HIV-positive children |
|---|--|
| 2 | only), conduct whole blood stimulation and bacterial binding assays (where blood |
| 3 | volumes >2mL), and store peripheral blood cells and plasma aliquots for subsequent |
| 4 | analysis (all blood samples), including soluble and cellular markers of immune |
| 5 | activation, as outlined in Supplementary Table 1. Children in the enteropathy |
| 6 | substudy have additional stool and urine collection following lactulose-mannitol |
| 7 | dosing as shown in Table 3. |
| | |

| 9 | Table 3: Summa | ry o | f procedures for cases in the enteropathy substudy |
|----|----------------|------|--|
| 10 | | | |

| Assessment | Hospitaliza | Post-discharge ³ | | | | | |
|--|-----------------------|-----------------------------|----|----|-----|-----|-----|
| | Baseline ¹ | Discharge ² | 2w | 4w | 12w | 24w | 48w |
| Caregiver informed consent to join observational cohort and enteropathy substudy | x | 6 | | | | | |
| Summary checklist | Х | | | | | | |
| Locator information ⁴ | Х | | | | | | |
| Acute admission information | x | | | | | | |
| Baseline data | X | | | | | | |
| Daily clinical review ⁵ | Daily during | | | | | | |
| Blood collection ⁶ | x | х | | | х | x | х |
| HIV testing ⁷ | Х | | | | | | |
| CD4 count and viral load (HIV-infected children only) | X | | | | x | x | x |
| Full blood count ⁸ | Х | х | | | х | х | х |
| Gastric aspirate9 | x | | | | | | |
| Stool collection ¹⁰ | x | х | | | х | x | x |
| Lactulose-mannitol testing ¹¹ | x | x | | | X | | x |
| Anthropometry | Х | х | Х | х | Х | х | х |
| Skinfold thickness ¹² | | х | Х | х | Х | Х | Х |
| Body composition ¹³ | Х | х | х | х | х | х | х |
| Discharge data collection | | x | | | | | |

| Daily | morbidity diary | Daily during follow-up period by caregivers |
|---|--|---|
| Follow-up clinic: history, examination, morbidity and mortality data | | |
| $\begin{array}{c}1\\2\\3\\4\\5\\6\\7\\8\\9\\0\\1\\1\\2\\1\\2\\2\\2\\2\\2\\2\\2\\2\\2\\2\\2\\2\\2\\2\\2$ | within 72h of hospitalization. Tresearch specimens with clinic undertaking research investiga ²The discharge procedures will that date. ³Windows will be created arou caregivers who miss visits or a weeks); 12 weeks (10-14 weel ⁴Locator information will updat details. ⁵Daily clinical review will be coclinician. ⁶During hospitalisation, 5.4 mL mL/kg total over 2 week period EDTA tube and a 2.7 mL PAX analysis. After discharge (wee amount will not exceed 2 mL/k 2.7 mL endotoxin-free EDTA tu ⁷HIV testing is conducted as p study sample will be used to the since HIV status is required to ⁸Full blood count results will be nasogastric tube with a sterile be instilled and a sample of ga (section 7.5.2) ¹⁰Stool collection will be under ¹¹Lactulose-mannitol testing w by a 2hr urine collection post-L clinically stable by the study period to the since HIV status is (triceps, s measured using Holtain calipe ¹³Body composition will be assisted to the since HIV stable study period to the study period to the study period to the section 7.5.2 | I be undertaken on the day of discharge, or as close as possible to and these post-discharge time-points to maximize follow-up for re unavailable, as follows: 2 weeks (1-3 weeks); 4 weeks (3-5 (s); 24 weeks (20-28 weeks); 48 weeks (44-52 weeks). ed at subsequent visits if caregivers have moved or changed conta- nducted every day between admission and discharge by the study of blood (depending on child weight; amount will not exceed 2) will be collected by a study nurse into a 2.7 mL endotoxin-free Gene tube, for subsequent isolation of RNA and gene expression ks 12, 24 and 48), 5.4 mL of blood (depending on child weight; g total over 2 week period) will be collected by a study nurse into the st for HIV (see section 9.4), as stated in the informed consent form allocate children to study groups. e transcribed from clinical records; if not done by clinical teams, the neasure FBC in clinical laboratories at each site collected at the same time as the blood draw by aspirating the feeding syringe, to test for gastric pH; sterile water or saline will the stric juice collected for storage for subsequent PCR and culture taken at the same time as the blood draw II be conducted, with collection of a baseline urine sample, followe. M ingestion. This test will be deferred until children are judged to b sysician during daily reviews. In general, this will be a child in the who has no cardiorespiratory compromise. |
| 43 | - | aregivers do not attend follow-up appointments, |
| 44 | attempts are made to contac | t them by phone and home visits are made if feasible, |
| 45 | particularly for those defaulti | ng the 48-week visit, so that long-term outcome data c |
| 46 | be collected. For post-discha | rge deaths, a home visit is undertaken by study nurses |
| | | verbal autopsy. Children who are readmitted to one of |

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| 1 | the study sites with relapsed SAM have data collected during the new episode of |
|----|---|
| 2 | hospitalisation. The study ends for each participant at the week 48 visit. |
| 3 | |
| 4 | SUBSTUDIES |
| 5 | As outlined in Figure 1, four nested substudies will utilise biological specimens to |
| 6 | address mechanistic questions related to enteropathy, microbiota, metabolomics and |
| 7 | immune function. |
| 8 | |
| 9 | Enteropathy substudy |
| 10 | The gut, which acts as an internal interface between humans and the environment, |
| 11 | must contain the nutrient stream and the symbiotic microbiota while allowing |
| 12 | molecular intimacy to permit absorption. The mechanism underlying this duality is the |
| 13 | integrity of the gastrointestinal barrier; intestinal damage (enteropathy) can impair |
| 14 | this critical barrier function. A spectrum of enteropathies affect children in developing |
| 15 | countries ³⁰ . Environmental enteric dysfunction (EED), characterised by small |
| 16 | intestinal inflammation, blunted villi and increased intestinal permeability, is almost |
| 17 | universal and is morphologically indistinguishable from HIV enteropathy ³⁰ . Children in |
| 18 | resource-poor settings also suffer from frequent diarrhoea, food insecurity and |
| 19 | micronutrient deficiencies, which all exacerbate enteropathy ³⁰ . As a result, a cycle of |
| 20 | intestinal infection, impaired mucosal function and malnutrition commonly arises, |
| 21 | which may ultimately precipitate SAM, especially in the context of HIV infection ^{35 36} . |
| 22 | It is not yet established if the enteropathy seen in children with SAM ³⁷ , which we here |
| 23 | refer to as malnutrition enteropathy ³⁷ , is qualitatively or quantitatively distinguishable |
| 24 | from EED. In addition to local intestinal pathology, enteropathies may cause systemic |
| 25 | pathology due to persistent immune activation arising from enteric inflammation and |
| 26 | microbial translocation across the damaged gut wall ³⁰ . It is becoming apparent that |
| 27 | chronic inflammation may be particularly deleterious in malnourished individuals ²³ ; in |
| | |

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| 1 | children with SAM, systemic inflammation arising from underlying enteropathy may |
|----|--|
| 2 | further increase morbidity and mortality. |
| 3 | |
| 4 | We hypothesize that i) the degree of enteropathy during hospitalisation differs |
| 5 | between oedematous and non-oedematous SAM and is independently associated |
| 6 | with morbidity, mortality and nutritional recovery during hospitalization; ii) the degree |
| 7 | of enteropathy at discharge is independently associated with morbidity, mortality and |
| 8 | relapse of SAM; and iii) children with HIV-SAM have more severe enteropathy than |
| 9 | HIV-negative children with SAM, which contributes to their poorer outcomes. |
| 10 | |
| 11 | Using stored samples, a longitudinal series of investigations will compare gastric and |
| 12 | small intestinal barrier function, using a range of biomarkers to capture the domains |
| 13 | of malnutrition enteropathy (Supplementary Table 2). To understand better the |
| 14 | extra-intestinal consequences of enteropathy, we will first compare the microbial |
| 15 | composition of the upper gut and plasma using deep sequencing in a subgroup of |
| 16 | children with paired gastric and blood samples. Secondly, we will undertake |
| 17 | transcriptomics using PAXGene blood samples to determine i) whether there are |
| 18 | differences in gene expression profiles between well-nourished controls, HIV- |
| 19 | negative children with SAM and HIV-positive children with SAM (including |
| 20 | comparison of oedematous and non-oedematous types); and ii) whether specific |
| 21 | patterns of gene expression are associated with morbidity and mortality in SAM. |
| 22 | |
| 23 | Microbiota substudy |
| 24 | Normal assembly of the gut microbiota in early life is critical for many aspects of |
| 25 | physiological, neurological and immune development ³⁸ . Recent evidence suggests |
| 26 | that an immature or pathogenic microbiota plays a causative role in the pathogenesis |
| 27 | of SAM ²⁵ . For example, a number of microbial taxa have been identified, including |
| 28 | Faecalibacteium prausnitzii, which discriminate and predict gut microbiota maturity |
| | |

| 1 | and child growth ²⁸ . Other pathogenic microorganisms, including IgA-targeted |
|----|---|
| 2 | Enterobacteriaceae, are associated with impaired growth and may contribute to |
| 3 | SAM ³⁹ . Nutritional rehabilitation with RUTF induces temporary recovery of a |
| 4 | disturbed microbiota; however, the microbiota appears to revert back to an immature |
| 5 | diseased state following nutritional recovery ²⁹ . HIV infection is also associated with a |
| 6 | disturbed gut microbiota ⁴⁰ , which may further compound enteropathy phenotypes. |
| 7 | Furthermore, there is some evidence that differences exist in malnutrition |
| 8 | enteropathy between oedematous and non-oedematous SAM ⁴¹ ; however, few |
| 9 | studies have investigated differences in the gut microbiota between the two forms of |
| 10 | the disease. |
| 11 | |
| 12 | We hypothesize that (i) a unique gut microbial signature exists in HIV-SAM, |
| 13 | compared with HIV-negative children with SAM, that is independently associated with |
| 14 | morbidity, mortality, nutritional recovery and degree of enteropathy during |
| 15 | hospitalisation; (ii) a unique gut microbial signature exists in oedematous compared |
| 16 | with non-oedematous SAM; (iii) specific microorganisms or gut microbial diversity |
| 17 | indices are independently associated with morbidity, mortality, nutritional recovery |
| 18 | and degree of enteropathy during hospitalisation; and (iv) the gut microbiota is |
| 19 | partially restored to a healthy state with nutritional rehabilitation but reverts to a |
| 20 | dysbiotic state during follow-up, which predicts morbidity, mortality and relapse of |
| 21 | SAM. |
| 22 | |
| 23 | Using stored stool samples collected at baseline, a cross-sectional investigation will |
| 24 | determine differences in the gut microbial composition and predicted function |
| 25 | between: HIV-negative children with SAM versus HIV-SAM, oedematous versus non- |
| 26 | oedematous SAM, and well-nourished controls. Gut microbial composition and |
| 27 | predicted function will be compared between groups at discharge and at 12, 24 and |
| 28 | 48 weeks post-discharge. Briefly, total DNA and/or RNA will be extracted from stool |
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samples and used as template for next-generation sequencing library preparation and for quantitative polymerase chain reaction (qPCR). Whole metagenome shotgun sequencing will be performed using the HiSeq 2500 system. Raw metagenomic sequencing data will be quality-filtered and analysed through a well-validated bioinformatics pipeline using MetaPhIAn⁴² and HUMAnN⁴³. The compositional and predicted functional metagenomic data generated will be used to identify signatures of SAM and to investigate associative links between specific gut microbial signatures and clinical outcomes. Metabolomics substudy During SAM, metabolic processes are altered in response to a starved environment, and may plausibly contribute to long-term clinical outcomes. Previous studies suggest that amino acid turnover, lipid metabolism, oxidative stress and other metabolic pathways are disrupted in SAM and may be associated with disease state and clinical outcome^{26 44 45}; however, little is known about how the metabolic phenotype responds to nutritional therapy. It is hypothesised that disturbed gut microbiota composition and function may drive microbial metabolic dysregulation in addition to host-derived dysregulation. Of particular interest are differences in the metabolic phenotype between oedematous and non-oedematous SAM. The 'reductive adaptation' seen in non-oedematous SAM (utilisation of fat and muscle stores) is disrupted in oedematous SAM, which may contribute to differences in clinical outcomes. Specifically, protein turnover, inflammation, oxidative stress and bile acid metabolism are disrupted in oedematous-SAM, which may contribute to co-morbidities including diarrhoea, steatosis and enteropathy^{46 47}. We hypothesize that: (i) a unique plasma and urine metabolic phenotype exists in oedematous compared with non-oedematous SAM during hospitalisation, which is

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| 1 | independently associated with morbidity, mortality and nutritional recovery; (ii) the | | |
|----|--|--|--|
| 2 | metabolic phenotype is partially restored to a healthy state with nutritional | | |
| 3 | rehabilitation but reverts to a disturbed state during follow-up, which predicts | | |
| 4 | morbidity, mortality and relapse; and (iii) both host-derived and gut microbial-driven | | |
| 5 | metabolic dysregulation underlie clinical outcomes. | | |
| 6 | | | |
| 7 | Using stored urine and plasma samples collected during hospitalisation, a cross- | | |
| 8 | sectional investigation will determine differences in the metabolic phenotype betweer | | |
| 9 | children with oedematous SAM, non-oedematous SAM and well-nourished controls. | | |
| 10 | Urine and plasma metabolic phenotypes will be compared between groups at | | |
| 11 | discharge and 12, 24 and 48 weeks post-discharge. Briefly, global untargeted | | |
| 12 | metabolomic phenotyping will be performed via ¹ H nuclear magnetic resonance | | |
| 13 | (NMR) spectroscopy using a 700 MHz Bruker NMR spectrometer to identify | | |
| 14 | metabolic signatures of SAM. Targeted analysis via ultra-performance liquid | | |
| 15 | chromatography-mass spectrometry will be performed to examine specific pathways | | |
| 16 | of interest, including tryptophan and bile acid metabolism. | | |
| 17 | | | |
| 18 | Immunology substudy | | |
| 19 | Bacterial infections are common among children hospitalised for SAM ^{23 48-50} and | | |
| 20 | mortality is driven by a range of species ^{48 50-53} , consistent with generalised defects in | | |
| 21 | innate anti-bacterial defence. Increased infectious morbidity and mortality persist | | |
| 22 | after discharge from hospital ^{10 17 54} , suggesting that restoration of anti-bacterial | | |
| 23 | immune responses may lag behind nutritional rehabilitation. A recent randomised | | |
| 24 | trial in children with SAM confirmed that deaths following hospitalisation were | | |
| 25 | predominantly due to bacterial infections but were not prevented by daily co- | | |
| 26 | trimoxazole prophylaxis ¹⁷ . Collectively, these observations highlight that children | | |
| 27 | remain vulnerable to infection despite current treatment approaches; targeting | | |

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| 2 3 4 | 1 | persistent im |
| 4 5 | 2 | discharge ²⁴ . |
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| 1 | persistent immune dysfunction could plausibly reduce infectious mortality after |
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| 2 | discharge ²⁴ . |
| 3 | |
| 4 | Multiple innate and adaptive immune mediators are dysregulated in malnutrition ^{24 27} |
| 5 | ⁵⁵ . However, few studies have assessed cellular immune function in malnourished |
| 6 | children; most existing studies were undertaken decades ago on small cross- |
| 7 | sectional cohorts without the benefit of recent advances in immunology techniques ²⁷ . |
| 8 | Immune dysfunction in SAM likely reflects both intrinsic defects, whereby immune |
| 9 | cells lack capacity to adequately respond to infection, and extrinsic defects, where |
| 0 | cells have intact anti-bacterial capacity but are chronically modulated by the systemic |
| 1 | pro-inflammatory environment which characterises SAM (i.e. heightened pro- |
| 2 | inflammatory cytokines ⁴⁴ and circulating bacterial antigens ^{23 56 57}). Systemic |
| 3 | inflammation is directly associated with mortality in SAM ²³ and driven by multiple |
| 4 | comorbidities, including bacterial translocation from the damaged gut into the blood, |
| 5 | sub-clinical infections and metabolic dysregulation ^{44 58 59} . The implications of innate |
| 6 | immune cell dysfunction for subsequent acquisition of infections and infectious |
| 7 | mortality have not been investigated. |
| 8 | |
| 9 | We hypothesise that: (i) anti-bacterial functions of innate immune cells are |
| 20 | compromised in SAM due to a combination of intrinsic and extrinsic defects; ii) innate |
| 21 | immune cell function is independently associated with infectious morbidity and |
| 22 | mortality during hospitalisation for SAM; and iii) nutritional rehabilitation only partly |
| 23 | restores innate immune cell function, leading to an ongoing risk of bacterial infections |
| 24 | post-discharge. |
| 25 | |
| 26 | Using blood samples collected at baseline, discharge and 12, 24 and 48 weeks post- |
| 27 | discharge, the longitudinal relationship between circulating innate immune cell |
| 28 | function and bacterial infections will be assessed. The intrinsic phagocytic capacity, |
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| 2 3 | 1 | secreted cytokine response and maturation state of innate immune cells after culture |
| 4 5 | 2 | with bacterial antigens will be assessed. Plasma concentrations of endotoxin and |
| 6 7 | 3 | pro-inflammatory mediators will be quantified at each time-point and the degree to |
| 8 9 | 4 | which these extrinsic factors influence innate immune cell antibacterial function will |
| 10 11 | 5 | be assessed via plasma co-culture with innate immune cells from healthy donors. |
| 12 13 | 6 | Bacterial infections during hospitalisation will be diagnosed using clinical criteria and |
| 14 15 | 7 | blood culture, stool culture and urinalysis where available. |
| 16 17 | 8 | |
| 18 19 | 9 | SAMPLE SIZES |
| 20 21 | 10 | Observational study: The observational cohort will recruit as many children with SAM |
| 22 23 | 11 | as possible during the period of enrolment (July 2016 to March 2018), estimated at |
| 24 25 | 12 | 600-800 children (capped at 800 maximum), to assess clinical and nutritional |
| 26 27 | 13 | outcomes among HIV-positive and HIV-negative children hospitalised with SAM. |
| 28 29 | 14 | Assuming mortality of 15%, overall loss to follow-up of 15% and recruitment target of |
| 30 31 | 15 | 800 children, there would be 560 evaluable children at 48 weeks, of whom 224 would |
| 32 33 | 16 | have HIV-SAM based on an estimated inpatient HIV prevalence of 40%. This will |
| 34 35 | 17 | provide >80% power to detect absolute differences of 17% in binary outcomes |
| 36 37 | 18 | between HIV-SAM and HIV-negative children with SAM, and of 0.33 times the |
| 38 39 | 19 | standard deviation in continuous outcomes. |
| 40 41 | 20 | standard deviation in continuous outcomes. |
| 42 43 | 21 | |
| 44 45 | 22 | Enteropathy substudy: The sample size was estimated using previously reported |
| 46 47 | 23 | values for LM ratios, which remain a widely used non-invasive marker of |
| 48 49 | 24 | enteropathy. Comparing 100 versus 100 children with two-sided alpha=0.025 (to |
| 50 51 | 25 | allow for two primary comparisons, i.e. HIV-SAM versus HIV-negative children with |
| 52 53 | 26 | SAM, and HIV-SAM versus well-nourished HIV-positive children) provides >80% |
| 55 55 56 57 | 27 | power to detect differences in mean LM ratio during hospitalisation of at least 0.16 |
| 58 | | |

| 1 | (assuming SD=0.36), a difference which would be clinically relevant given the LM |
|----|--|
| 2 | ratios previously reported for well-nourished children (0.42), malnourished children |
| 3 | (1.3) and children with persistent diarrhoea (2.85) in the Gambia ⁶⁰ . It also provides |
| 4 | >80% power to detect differences of at least 0.1 in the mean change in LM ratio from |
| 5 | enrolment (assuming SD for change=0.23 and 7% missing samples). For |
| 6 | inflammatory markers, comparing 100 versus 100 children with two-sided |
| 7 | alpha=0.025 provides >80% power to detect differences in mean log_{10} concentrations |
| 8 | of at least 0.44 times their standard deviation, or 2.75-fold differences between |
| 9 | groups. Inclusion of well-nourished controls provides an indication of normal ranges |
| 10 | in young African children. HIV-positive and HIV-negative SAM groups will be |
| 11 | stratified to include approximately 50 children with and without oedematous |
| 12 | malnutrition, if possible. |
| 13 | |
| 14 | Microbiota and metabolomics substudy |
| 15 | Power calculations are difficult in metagenomics and metabolomic analyses due to |
| 16 | the large number of observed outcomes and unknown effect sizes and variance. |
| 17 | Previous studies using smaller sample sizes have identified significant taxonomic |
| 18 | differences in twin pairs discordant for oedematous-SAM $(n=13)^{25}$ and metabolic |
| 19 | differences between the two forms of SAM $(n=40)^{26}$. These studies suggest that a |
| 20 | difference of 50% in metabolites could be expected. Using ANCOVA, setting α =0.05 |
| 21 | and assuming either low (ρ =0.1) or high (ρ =0.7) correlation, the study would require |
| 22 | 95-126 subjects to achieve 80% power ⁶¹ . False discovery rate (FDR) multiple |
| 23 | correction testing will be applied to reduce the high-dimensionality of the data and |
| 24 | limit false-positives. |
| 25 | |
| 26 | Immunology substudy |
| 27 | Up to 200 children with SAM and 200 well-nourished controls will be included in a |
| 28 | cross-sectional analysis of innate immune cell function during hospitalisation. |
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| 1 | Assuming similar infectious mortality to a recent Kenyan study (15%) ¹⁷ , a cohort of |
|----|--|
| 2 | 200 provides 80% power to detect associations between immune profiles and |
| 3 | infectious mortality at an odds ratio of 1.7 and 2-sided alpha of 0.05. We will aim for |
| 4 | 100 children with longitudinal analysis of innate immune cell function at discharge, |
| 5 | 12, 24 and 48 weeks post-discharge ¹⁷ . |
| 6 | |
| 7 | STUDY OUTCOMES AND RISK FACTORS |
| 8 | The main study outcomes are clinical (mortality, morbidity and relapse of |
| 9 | malnutrition) and nutritional (weight, height, mid-upper arm circumference, leg length, |
| 10 | head circumference, mid-thigh circumference, skin-fold thickness and body |
| 11 | composition by bioimpedance vector analysis) assessed over 48 weeks of follow-up. |
| 12 | Mortality is assessed in hospital by daily physician review and, post-discharge, |
| 13 | through study visits and by telephone where possible for children who are lost to |
| 14 | follow-up. Morbidity during hospitalization is assessed through daily clinical |
| 15 | assessments and available hospital laboratory tests. Morbidity after discharge is |
| 16 | assessed, first, using daily morbidity diaries, in which caregivers record episodes of |
| 17 | illness (lethargy interfering with feeding; respiratory distress; diarrhoea; oedema and |
| 18 | fever); second, from caregiver recall and review of handheld medical records at each |
| 19 | follow-up visit; and, third, from data collected during hospitalization for children who |
| 20 | are readmitted during the follow-up period. Time-to-recovery from malnutrition will be |
| 21 | evaluated during hospitalization; relapse of malnutrition during follow-up will be |
| 22 | categorized as moderate acute malnutrition, uncomplicated SAM and complicated |
| 23 | SAM, according to WHO definitions. Nutritional outcomes will be expressed both as |
| 24 | continuous variables (attained Z-score and change in Z-score between visits), and as |
| 25 | categorical variables (moderate wasting, WHZ<-2; severe wasting, WHZ<-3; |
| 26 | stunting, HAZ<-2; severe stunting, HAZ<-3; underweight, WAZ <-2; and |
| 27 | microcephaly, head circumference-for-age <-2). |
| 28 | |
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| 1 | Risk factors will be evaluated at baseline, hospital discharge and over the period of |
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| 2 | follow-up for associations with clinical and nutritional outcomes. In addition to |
| 3 | baseline clinical and demographic factors, the following laboratory parameters will be |
| 4 | evaluated: haemoglobin, serum albumin, C-reactive protein, CD4 count and HIV viral |
| 5 | load (for HIV-positive children). Haemoglobin, CD4 and HIV-viral load will be |
| 6 | measured in real time and the results reviewed during follow-up clinics. |
| 7 | |
| 8 | Data on potential confounders are collected at baseline, discharge and during the |
| 9 | follow-up period, including child feeding practices, household socioeconomic status |
| 10 | (defined by household income and cooking method), maternal employment and |
| 11 | education, and household factors such as water, sanitation and hygiene practices, |
| 12 | availability of electricity, location (rural, peri-urban or urban) and household size. |
| 13 | |
| 14 | ANALYSIS |
| 15 | All analyses will be interpreted exploratively since HOPE-SAM is an observational |
| 16 | study with multiple risk factors, outcomes and substudies. For all analyses, P values |
| 17 | will not be artificially adjusted, but interpreted as exploring the strength of evidence |
| 18 | supporting any association. The only exception is the use of approaches to minimise |
| 19 | false discovery when analysing high-dimensional data from the microbiota and |
| 20 | metabolomics substudies, as described. |
| 21 | |
| 22 | Observational Cohort |
| 23 | The primary comparison will be the clinical and nutritional outcomes of children with |
| 24 | HIV-SAM compared to HIV-negative children with SAM. We will review all deaths |
| 25 | and adjudicate clinical diagnoses and causes of death to ensure robust and |
| 26 | consistent data across sites. We will compare each participant's clinical management |
| 27 | to WHO guidelines to identify any contributory factors in hospital care. Factors |
| 28 | associated with outcomes during hospitalisation (e.g. mortality, nutritional recovery) |
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| 1 | will be determined for each group (HIV-SAM and HIV-negative children with SAM) |
|----|---|
| 2 | using multivariable analysis (Cox models for time-to-event data, linear models for |
| 3 | continuous outcomes). Factors associated with outcomes over 48 weeks post- |
| 4 | discharge (hospital re-admission, morbidity and mortality, relapse, anthropometry, |
| 5 | body composition and response to ART) will be determined for each group (HIV-SAM |
| 6 | and HIV-negative children with SAM) using multivariable analysis (Cox models for |
| 7 | time-to-event data, linear models for continuous outcomes). HIV-positive children |
| 8 | with SAM and HIV-negative children with SAM will be included in one model together |
| 9 | with the risk factors, and interaction tests will be used to investigate whether |
| 10 | associations between risk factors and outcomes differ between the two groups of |
| 11 | children. We will evaluate the ability of mid-upper arm circumference (MUAC) at |
| 12 | discharge to predict long-term outcomes using receiver-operator-characteristic |
| 13 | (ROC) analysis, in the whole cohort and within the subgroups of HIV-SAM and HIV- |
| 14 | negative children with SAM. We will then evaluate whether addition of other variables |
| 15 | improves the predictive capacity of MUAC (using WHO criteria in those >6 months |
| 16 | old, and published data for children <6 months ⁶²) for each group, including body |
| 17 | composition, haemoglobin, albumin and CRP, plus CD4%, viral load and timing of |
| 18 | ART initiation (HIV-SAM only). We will construct multivariable models and compare |
| 19 | them with MUAC alone using the net-reclassification index. |
| 20 | |

21 Body composition analysis

Previous work in body composition by bio-electrical impedance in Ethiopian infants
and children with SAM has shown that the conventional approach, predicting total
body weight from height-adjusted impedance, fails due to confounding by oedema⁶³.
The same project validated an alternative approach, known as Bio-electrical
Impedance Vector Analysis (BIVA), and described significant differences between
each of three groups: healthy controls, oedematous-SAM and non-oedematous
SAM. Vector analysis splits impedance into two height-adjusted components,

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resistance and reactance, which are further linked through phase angle (PA). Variability in these components is associated with biochemical parameters⁶⁴. These variables will be explored using graphical analysis, or transformed into age- and sex-adjusted Z-scores for statistical comparison, including longitudinal analyses. Higher phase angle indicates better nutritional status, while declining height-adjusted resistance over time indicates loss of oedema. Enteropathy substudy The primary comparison for the enteropathy substudy will be between HIV-positive children with SAM (group A) and HIV-negative children with SAM (group C), stratified by presence or absence of oedema. Control groups (B and D) are well-nourished children with or without HIV, to provide normative data for biomarkers and to evaluate the impact of SAM within each HIV group. Thus, biomarkers among HIV-positive children with SAM will first be compared to HIV-negative children with SAM (to evaluate the impact of HIV) and, second, to well-nourished HIV-positive children (to evaluate the impact of SAM). Biomarkers among HIV-negative children with SAM will be compared to well-nourished HIV-negative children. For each continuous outcome, simple descriptive analysis will be used to compare groups during hospitalisation using t-tests on appropriately transformed data. For any outcome with moderate (p<0.05) evidence of difference between either group a regression model will be constructed including groups A, B, C, D to directly test (using interactions) whether there is a synergistic effect of HIV-SAM versus HIV-negative SAM versus HIV alone versus neither. These models will also be used to explore whether there is any evidence for heterogeneity in effects between oedematous and non-oedematous SAM. Associations between enrolment factors (e.g. intestinal permeability and microbial translocation) will be explored using pairwise Spearman correlations and principal components analysis. Mean changes at the follow-up time-points in each group will be estimated, and groups compared (as above) using generalised

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| 1 | estimating equations. For outcomes that differ across SAM groups over time, |
|----|--|
| 2 | multilevel models will be used to explore possible predictors from the other factors |
| 3 | measured. Time to nutritional recovery will be compared using Kaplan-Meier and log- |
| 4 | rank tests, and Cox models to adjust for baseline differences between groups. |
| 5 | |
| 6 | Microbiota and metabolomics substudy |
| 7 | The primary comparison will be between HIV-negative children with oedematous and |
| 8 | non-oedematous SAM, with a separate comparison between HIV-positive children |
| 9 | with SAM and HIV-negative children with SAM. Analyses will examine: (i) differences |
| 10 | in metagenomic/metabolomic variables between groups at each time-point; (ii) |
| 11 | differences in metagenomic/metabolomic variables within groups over time; (iii) |
| 12 | correlations between metagenomic and metabolomic variables; and (iv) correlations |
| 13 | between metagenomic/metabolomic variables and clinical outcomes. A systematic |
| 14 | analysis will be undertaken to reduce high-dimensional data, integrate the multi- |
| 15 | omics datasets and minimise false discovery. |
| 16 | |
| 17 | Compositional metagenomic data will be compared between groups for indices of |
| 18 | alpha and beta diversity. Principal coordinate analysis and partial least squares |
| 19 | discriminant analysis will be performed on metabolomics data to identify overall |
| 20 | differences between groups. High-dimensional datasets will be reduced using |
| 21 | random forest models to identify taxa, microbial gene families and metabolites that |
| 22 | most strongly contribute to differences between groups, corrected by Benjamani- |
| 23 | Hochburg false discovery rate detection. Targeted analysis by qRT-PCR will validate |
| 24 | differential abundance or expression of candidate microbial genes. Longitudinal |
| 25 | comparisons will be performed within and between groups using multilevel |
| 26 | simultaneous component analysis. Orthogonal projections to latent structures models |
| 27 | will integrate metabolomic and metagenomic data whilst linear regression, canonical |
| 28 | correlation and hierarchal clustering analysis will measure correlations between - |
| | |

| 1 | omics datasets. Finally, ROC analysis will identify the ability of different analytes to |
|----|---|
| 2 | predict long-term nutritional and clinical outcomes. |
| 3 | |
| 4 | Immunology substudy |
| 5 | Integrated profiles of innate immune cell function will be generated for each child |
| 6 | using principal components analysis followed by hierarchical clustering ^{65 66} . This |
| 7 | data-reduction method identifies whether absolute levels of specific markers or |
| 8 | relative differences between markers differentiate children into groups. The resulting |
| 9 | innate immune profiles will be compared between HIV-SAM, HIV-negative children |
| 10 | with SAM and well-nourished groups using univariable tests and multivariable |
| 11 | analysis of variance (MANOVA) of the principal components. |
| 12 | |
| 13 | To address the relationship between immune function and infections, regression |
| 14 | analyses will determine whether baseline innate immune profiles (or the individual |
| 15 | parameters defining them) are associated with the infectious morbidity or mortality |
| 16 | during hospitalisation, using logistic models for binary outcomes and linear models |
| 17 | for duration. Key clinical characteristics, including age, sex, oedema and baseline |
| 18 | WHZ, will be added to models to investigate their confounding effects. Multivariable |
| 19 | stacked regression methods will be used to compare the impact of different factors |
| 20 | on severe bacterial infections based on heterogeneity tests. |
| 21 | |
| 22 | To determine whether treatment for SAM restores innate immune cell antibacterial |
| 23 | function, mixed effects regression models will compare longitudinal changes in |
| 24 | individual immune parameters, and the principal components calculated from the |
| 25 | weights identified at baseline (which include well-nourished controls). Similarities and |
| 26 | differences in longitudinal immune profiles will be compared between groups using |
| 27 | nonmetric multi-dimensional scaling ^{65 67 68} . This approach will group children |
| 28 | according to their composite innate immune function, allowing the duration and |
| | |

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| 2 3 | 1 | variability of immune restoration to be evaluated over the course of nutritional |
| 4 5 | 2 | rehabilitation. Binary logistic regression will determine whether innate immune |
| 6 7 | 3 | profiles at discharge are associated with morbidity or mortality during follow-up. |
| 8 9 | 4 | |
| 10 11 | 5 | PATIENT AND PUBLIC INVOLVEMENT |
| 12 13 | 6 | Patients and their caregivers were not involved in the design of the study. During |
| 14 15 | 7 | recruitment, all caregivers of children admitted to hospital were given information |
| 16 17 | 8 | about the study; those whose children had severe acute malnutrition were |
| 18 19 | 9 | approached to give written informed consent. A meeting to disseminate results of the |
| 20 21 | 10 | study to participants and their caregivers will be held at the end of the study. An |
| 22 23 | 11 | interactive game to engage caregivers in the science underlying malnutrition is being |
| 24 25 | 12 | developed in collaboration with experts from the Centre of the Cell, a unique science |
| 26 27 | 13 | education centre based at Queen Mary University of London |
| 28 29 | 14 | (https://www.centreofthecell.org/). |
| 30 31 | 15 | |
| 32 33 | 16 | |
| 34 35 | 17 | (https://www.centreofthecell.org/). SAFETY REPORTING |
| 36 37 | 18 | For all adverse events, the study team will assess expectedness and relatedness to |
| 38 39 | 19 | study activities. Since this is an observational study without interventions, we |
| 40 41 | 20 | anticipate that the risk is minimal; however, serious adverse events will be reported |
| 42 43 | 21 | to local ethical review boards (Medical Research Council of Zimbabwe, and |
| 44 45 | 22 | University of Zambia Biomedical Research Ethics Committee) and the study sponsor |
| 46 47 | 23 | (Queen Mary University of London) according to their respective guidelines. |
| 48 49 | 24 | |
| 50 51 | 25 | DATA COLLECTION AND MONITORING |
| 52 53 | 26 | Clinical and demographic data are recorded on paper case report forms. All data are |
| 54 55 | 27 | checked for completeness and plausibility before data entry and problems flagged for |
| 56 57 | 28 | resolution by the clinical team. All data are double-entered onto a dedicated |
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| 60 | | For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml |

| 1 | password-protected online study database, and any discrepancies resolved. Study |
|----|---|
| 2 | participants are identified on electronic databases only by study numbers (assigned |
| 3 | at enrolment); no personal identifiers are entered. |
| 4 | |
| 5 | ETHICS AND DISSEMINATION |
| 6 | The study complies with the principles of the Declaration of Helsinki (2008) and is |
| 7 | conducted in compliance with the principles of Good Clinical Practice (GCP) and |
| 8 | local regulatory requirements in each country. Ethical approval was obtained from the |
| 9 | University of Zambia Biomedical Research Ethics Committee, the Joint Research |
| 10 | Ethics Committee of the University of Zimbabwe and the Medical Research Council of |
| 11 | Zimbabwe. The ethical review board of the Sponsor, Queen Mary University of |
| 12 | London, provided an advisory review of the study. Since this is an observational study, |
| 13 | there is no Data and Safety Monitoring Board. |
| 14 | |
| 15 | Results will be disseminated through conference abstracts and peer-reviewed |
| 16 | publications and discussed with relevant policymakers and programmers. Study |
| 17 | findings will be disseminated to families of participants at face-to-face meetings. |
| 18 | |
| 19 | TIME FRAME AND STUDY STATUS |
| 20 | Enrolment into the study began in July 2016 and is expected to end in March 2018. |
| 21 | All participants will be followed for 48 weeks, with an expected study completion date |
| 22 | of March 2019. |
| 23 | |
| 24 | DISCUSSION |
| 25 | HOPE-SAM aims to document the short- and long-term clinical and nutritional |
| 26 | outcomes of HIV-positive and HIV-negative children with SAM, and to identify the |
| 27 | factors at presentation and at discharge from hospital that independently predict |
| 28 | these outcomes. Mechanistic substudies aim to evaluate the contribution of |
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| 1 | enteropathy, microbiota, metabolome and innate immune cell function to these |
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| 2 | clinical outcomes. The prevalence of malnutrition in HIV-positive children is as high |
| 3 | as 40% in some settings and the challenges of managing this population are well |
| 4 | recognised ⁶⁹ . The WHO protocol on management of SAM aims to reduce case |
| 5 | fatality below 10%, but rates as high as 35% are still reported among HIV-positive |
| 6 | children ^{5 70} . No studies have systematically and longitudinally collected morbidity |
| 7 | data in HIV-SAM, or documented repeat hospitalisations and mortality after |
| 8 | discharge from hospital, particularly in the current era where ART is available upon |
| 9 | diagnosis. HOPE-SAM will provide a unique opportunity to enrol and follow a cohort |
| 10 | of children managed for SAM in three large hospitals across two sub-Saharan |
| 11 | African countries at several time-points over a one-year period. Nested longitudinal |
| 12 | laboratory substudies aim to better characterise the pathogenesis of SAM in HIV- |
| 13 | positive and HIV-negative children, to determine whether pathogenic processes are |
| 14 | normalised during nutritional rehabilitation and follow-up, and to identify potential |
| 15 | mechanistic pathways. Our ultimate goal is to utilise the findings generated in this |
| 16 | study to inform new intervention approaches that can be evaluated in clinical trials to |
| 17 | improve outcomes among children with SAM. |
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| 1 | | |
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| 6 7 | 3 | ARM, JS, ASW, KJN, PK, AJP |
| 8 9 | 4 | Sought funding: MB-D, BA, CDB, RCR, JHH, ARM, JS, ASW, KJN, PK, AJP |
| 10 11 | 5 | Undertaking study: BM, KC, CK, KCh, FM, DN, PC, NC, FM, IM, EB, KM, SM, TR |
| 12 13 | 6 | Study oversight: MB-D, BA, JHH, KJN, PK, AJP |
| 14 15 | 7 | Analysis: MB-D, BA, CDB, RCR, RN, JW, ARM, JS, ASW, KJN, PK, AJP |
| 16 17 | 8 | Wrote first draft of manuscript: MB-D, CDB, RCR, AJP |
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| 38 39 | 19 | None of the authors have any competing interests to declare. |
| 40 41 | 20 | |
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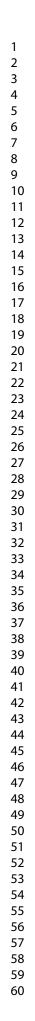
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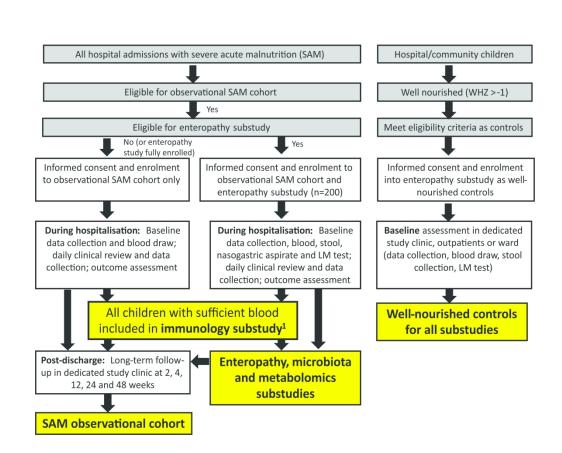
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| 2 3 | 1 | FIGURE LEGENDS |
| 4 5 | 2 3 | Figure 1: Study flow chart. |
| 6 7 | 4 | All hospital admissions are screened for eligibility for the observational cohort and |
| 8 9 | 5 | enteropathy sub-study, with procedures undertaken as shown in the flow chart during |
| 10 11 | 6 | hospitalisation and post-discharge. Well-nourished children from outpatient clinics |
| 12 13 | 7 | and the community meeting eligibility criteria as well-nourished controls are enrolled |
| 14 15 | 8 | and undergo a single baseline assessment as shown. The immunology, microbiota |
| 16 17 | 9 | and metabolomics sub-studies enrol children as shown. All children with SAM, |
| 18 19 | 10 | regardless of which arm of the study they are enrolled into, are followed for 48 weeks |
| 20 21 | 11 | post-discharge. |
| 22 23 | 12 | ¹ The immunology substudy started from 1 st June 2017 and required children to have |
| 24 25 26 | 13 | a blood sample >2mL to conduct cellular assays. |
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| Sı | upplementary Table 1: A | ssays undertakeı | n on stored s | samples for | [.] children in tl | ne observati | 6/bmjopen-2018-08307 I by copyright, incedudin all | and immunology s |
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| Sample type | Assay (method) | Location of work | Study subjects | Baseline | Discharge | Week 12 | ig foek 24 Weeuse | Week 48 |
| Blood | HIV testing (rapid antibody test algorithm if >18 mo old or HIV DNA PCR <18 mo old) ¹ | TROPGAN, Zvitambo or clinical sites | All | X | | | abruary 2019. Downloaded from Erasmushogeschool . es related to text and data minin. X X | |
| Blood | CD4 count (flow cytometry or PIMA) ¹ | TROPGAN, Zvitambo or clinical sites | All HIV- positive | X | X | X | bownloade digeschoo t and dat | X |
| Plasma | HIV viral load (real- time PCR) ¹ | TROPGAN, Zvitambo or clinical sites | All HIV- positive | x | X | X | <u> </u> | X |
| Plasma | C-reactive protein (ELISA) | TROPGAN, Zvitambo | All | x | x | X | Al tra | X |
| Plasma | Albumin (ELISA) | TROPGAN, Zvitambo | All | Х | Х | X | mjope X | X |
| Plasma | Lipopolysaccharide (LAL assay) | TROPGAN and Zvitambo | Subgroup ³ | Х | Х | X | n.bmj and s X | X |
| Plasma | Lipopolysaccharide binding protein (LBP) | TROPGAN and Zvitambo | Subgroup ³ | х | Х | X | .com/ imilar | X |
| Plasma | sCD14 (ELISA) | TROPGAN and Zvitambo | Subgroup ³ | Х | Х | X | on May techne | X |
| Plasma | sCD163 (ELISA) | TROPGAN and Zvitambo | Subgroup ³ | Х | Х | X | v 15, vlogi | X |
| Plasma | IL-6, TNF-alpha, IL-1β (ELISA) and/or multiplex cytokines | TROPGAN and Zvitambo | Subgroup ³ | X | X | X | х. Х | X |
| Plasma | Total PAMP activity (THP1 reporter cell line ²) | TROPGAN and Zvitambo | Subgroup ³ | X | X | X | at Department | X |
| Whole | Molecular techniques | QMUL, London ² | Subgroup ³ | Х | Х | X | X GEZ-LTA | Х |

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| blood | for bacterial detection (broad-range and specific PCR and next-generation sequencing) | | | | | | | 8-023077 on 1 Fe including for use | | |
| | | | | · | · | · | · | bruar Ei s rela | · | |
| Whole blood | ogy substudy only In vitro binding to bacterial products, cytokine expression and cellular responses to PAMP stimulation ⁴ | TROPGAN, Zvitambo | All | X | X | X | X | y 2019. Downlo asmushogescl ted to text and | X | - |
| Plasma | Co-culture with healthy immune cells ⁵ | QMUL, London | All | Х | Х | Х | X | oaded hool . Idata I | Х | - |
| | ⁴ Whole blood will be stimul and incubated for 1-24hr. S subsequent analysis of bac ⁵ To determine the effect of healthy immune cells, whic IFABP: Intestinal fatty acid CD163; PAMP: pathogen- | Supernatant will be rer cterial binding, cellular the systemic milieu or h will be functionally a binding protein; ELISA | moved and s activation, p healthy im nalysed via A: Enzyme-l | stored at -80C proliferation and mune cell func multi-paramete inked immunos | for subsequent a d cytokine elabo tion, plasma san er flow cytometry sorbent assay; G | analysis of pro- an ration by flow cyto nples will be trans y in the Flow Cyton GLP-2: glucagon-lil | nd anti-infl ometry. ported to metry Cor ke peptide | lanaman the Blizard renatacion e 2; sogD1 rogen; aAl | v cytokines, and c d Institute, QMUL 4: soluble CD14; | cells will be fixed fo _ and co-cultured w ; sCD163: soluble |
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| Supple | ementary table 2: Addition | al laboratory anal | yses for enterop | athy substue | dy | 6/bmjopen-2018-023077 on 1 I by copyright, including for | | |
| Sample type | Assay (method) | Location of work | Study groups ¹ | Baseline | Discharge | 1 Februar UseekFuar Warela | Week 24 | Week 4 |
| Urine | Lactulose-mannitol ratio (mass spectrometry) | Orgeon Analytics, USA | A, B, C, D (all) | X | X | y 2019. D asmushc rted to tey X | | X |
| Stool | Neopterin, myeloperoxidase, alpha-1 antitrypsin and REG-1B (ELISA) | TROPGAN and Zvitambo | A, B, C, D (all) | X | X | Fuary 2019. Downloaded f Erasmushogeschool . Fuelated to text and data m | X | X |
| Plasma | I-FABP (ELISA) | TROPGAN and Zvitambo | A, B, C, D (all) | X | X | rom h | X | Х |
| Plasma | GLP-2 | TROPGAN and Zvitambo | A, B, C, D (all) | X | X | Al tra | X | Х |
| Plasma | Citrulline (mass spectrometry) | Imperial College London | A, B, C, D (all) | X | X | mjope ining, X | X | Х |
| Plasma | Kynurenine:tryptophan ratio and metabolites along tryptophan pathway (mass spectrometry) | Imperial College London | A, B, C, D (all) | x | X | n.bmj.com/ on and similar tec × | X | X |
| Stool | Microbiome analysis ² | BCCDC, Vancouver | A, B, C, D (all) | X | X | May Xhno | Х | Х |
| Stool | Helicobacter pylori antigen | TROPGAN and Zvitambo | A, B, C, D (all) | X | | 15, 202! logies. | | |
| Gastric juice | Culture and molecular techniques for bacterial detection (broad-range and specific PCR and next-generation sequencing) | QMUL, London | Subgroup of A, C (n=50 per group) | X | | 5 at Department G | | |

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 Gene expression analysis (RNASeq)
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 Inducting for uses restruction of the sequence of the sequen RNA extracted from PAXGene tubes Plasma and urine guantitative polymerase chain reaction (gPCR), then sequenced via whole metagenome shotgun sequencing ttp://bmjopen.bmj.com/ on May 15, 2025 at Department GEZ-LTA Al training, and similar technologies QMUL: Queen Mary University of London; BCCDC: British Columbia Centre for Disease Control.

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Health Outcomes, Pathogenesis and Epidemiology of Severe Acute Malnutrition (HOPE-SAM): rationale and methods of a longitudinal observational study

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| | Wells, Jonathan C. K.; UCL Great Ormond Street Institute of Child Health Manges, Amee; University of British Columbia Swann, Jon; Imperial College London Walker, Sarah; MRC Clinical Trials Unit at UCL Nathoo, Kusum; University of Zimbabwe, College of Health Sciences Kelly, Paul; Barts and The London School of Medicine Prendergast, Andrew ; Queen Mary University of London, |
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| Keywords: | Malnutrition, HIV, Africa, mortality, microbiota, enteropathy |

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| 1 | Health Outcomes, Pathogenesis and Epidemiology of Severe Acute Malnutrition |
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| 2 | (HOPE-SAM): rationale and methods of a longitudinal observational study |
| 3 | |
| 4 | Mutsa Bwakura-Dangarembizi ¹ , Beatrice Amadi ² , Claire D Bourke ³ , Ruairi C Robertson ³ , |
| 5 | Benjamin Mwapenya ⁴ , Kanta Chandwe ² , Chanda Kapoma ² , Kapula Chifunda ² , Florence |
| 6 | Majo ⁴ , Deophine Ngosa ² , Pamela Chakara ⁴ , Nivea Chulu ² , Faithfull Masimba ⁴ , Idah |
| 7 | Mapurisa ⁴ , Ellen Besa ² , Kuda Mutasa ⁴ , Simutanyi Mwakamui ² , Thompson Runodamoto ⁴ , |
| 8 | Jean H Humphrey ⁴ , Robert Ntozini ⁴ , Jonathan Wells ⁵ , Amee R Manges ⁶ , Jonathan |
| 9 | Swann ⁷ , A Sarah Walker ⁸ , Kusum J Nathoo ¹ , Paul Kelly ^{2,3} , Andrew J Prendergast ^{3,4} for |
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| 19 | ⁷ Imperial College London, UK |
| 20 | ⁸ MRC Clinical Trials Unit at UCL, London, UK |
| 21 | |
| 22 | ⁹ Other members of the HOPE-SAM study team are listed in the Acknowledgements |
| 23 | section. |
| 24 | |
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| 2 | | |
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| 3 4 | 1 | ABSTRACT |
| 5 6 | 2 | Introduction |
| 7 8 | 3 | Mortality among children hospitalised for complicated severe acute malnutrition (SAM) |
| 9 10 | 4 | remains high despite the implementation of WHO guidelines, particularly in settings of |
| 11 12 | 5 | high HIV prevalence. Children continue to be at high risk of morbidity, mortality and |
| 13 14 | 6 | relapse after discharge from hospital although long-term outcomes are not well |
| 15 16 | 7 | documented. Better understanding the pathogenesis of SAM and the factors associated |
| 17 18 | 8 | with poor outcomes may inform new therapeutic interventions. |
| 19 20 21 | 9 | |
| 21 22 23 | 10 | Methods and analysis |
| 24 25 | 11 | The Health Outcomes, Pathogenesis and Epidemiology of Severe Acute Malnutrition |
| 26 27 | 12 | (HOPE-SAM) study is a longitudinal observational cohort that aims to evaluate the short- |
| 28 29 | 13 | and long-term clinical outcomes of HIV-positive and HIV-negative children with |
| 30 31 | 14 | complicated SAM, and to identify the risk factors at admission and discharge from |
| 32 33 | 15 | hospital that independently predict poor outcomes. Children aged 0-59mo hospitalised |
| 34 35 | 16 | for SAM are being enrolled at three tertiary hospitals in Harare, Zimbabwe, and Lusaka, |
| 36 37 | 17 | Zambia. Longitudinal mortality, morbidity and nutritional data are being collected at |
| 38 39 40 | 18 | admission, discharge and for 48 weeks post-discharge. Nested laboratory substudies |
| 40 41 42 | 19 | are exploring the role of enteropathy, gut microbiota, metabolomics and cellular immune |
| 42 43 44 | 20 | function in the pathogenesis of SAM using stool, urine and blood collected from |
| 45 46 | 21 | participants and from well-nourished controls. |
| 47 48 | 22 | |
| 49 50 | 23 | Ethics and dissemination |
| 51 52 | 24 | The study is approved by the local and international institutional review boards in the |
| 53 54 | 25 | participating countries (the Joint Research Ethics Committee of the University of |
| 55 56 | 26 | Zimbabwe, Medical Research Council of Zimbabwe and University of Zambia Biomedical |
| 57 58 | | |
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| 1 | Research Ethics Committee) and the study sponsor (Queen Mary University of London). |
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| 2 | Caregivers provide written informed consent for each participant. Findings will be |
| 3 | disseminated through peer-reviewed journals, conference presentations and to |
| 4 | caregivers at face-to-face meetings. |
| 5 | |
| 6 | Strengths and limitations of this study |
| 7 | Strengths: |
| 8 | Rigorous collection of longitudinal data on morbidity, mortality and nutritional |
| 9 | status during inpatient care and for 48 weeks after initial admission for SAM in |
| 10 | HIV-positive and HIV-negative children. |
| 11 | Laboratory sub-studies investigating enteropathy, microbiota, metabolomics and |
| 12 | immune cell function provide a unique opportunity to understand which |
| 13 | pathogenic pathways contribute to SAM and whether these processes normalise |
| 14 | with nutritional rehabilitation. |
| 15 | |
| 16 | Potential limitations: |
| 17 | High loss to follow-up due to participants returning to home settings following |
| 18 | hospital discharge. |
| 19 | The clinical heterogeneity of the study participants, including comorbidities such |
| 20 | as infections, may make it challenging to identify the specific causes of clinical |
| 21 | outcomes. |
| 22 | Potential bias in recruiting well-nourished controls only from hospitals will be |
| 23 | reduced by inclusion of community-based controls, including well-nourished |
| 24 | siblings of children with SAM. |
| 25 | INTRODUCTION |
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| 1 | Malnutrition underlies almost half of all childhood deaths in developing countries ¹ . |
|----|--|
| 2 | Severe acute malnutrition (SAM) is defined by a weight-for-height Z-score <-3, mid- |
| 3 | upper arm circumference (MUAC) <115mm and/or bilateral pitting oedema ² . Current |
| 4 | treatment guidelines distinguish two groups: i) children with uncomplicated SAM who |
| 5 | can be managed in the community; and ii) children with complicated SAM, who are |
| 6 | hospitalised and undergo resuscitation, stabilisation and nutritional rehabilitation. In- |
| 7 | hospital mortality in children with complicated SAM remains high despite the |
| 8 | implementation of WHO guidelines ³ . Furthermore, SAM presents as two major clinical |
| 9 | phenotypes: non-oedematous SAM (marasmus), characterised by severe wasting, and |
| 10 | oedematous SAM (kwashiorkor), a more complex syndrome characterised by bilateral |
| 11 | pitting oedema, steatosis and diarrhea ^{4 5} . Despite differing clinical outcomes, treatment |
| 12 | protocols are the same for both oedematous and non-oedematous SAM. |
| 13 | |
| 14 | A contributory factor to high in-patient mortality is the co-occurrence of HIV infection in |
| 15 | around one-third of children hospitalised for SAM in sub-Saharan Africa ⁶⁷ . While new |
| 16 | HIV infections in children have declined ⁸ , a substantial number of infected children are |
| 17 | diagnosed late and present with malnutrition. There is also a growing population of HIV- |
| 18 | exposed uninfected (HEU) children who have immune abnormalities, poor growth and |
| 19 | higher risk of mortality and infectious morbidity ⁹ . Hence, HIV has transformed the |
| 20 | epidemiology and outcomes of SAM ¹⁰ . Even with standardised treatment approaches, |
| 21 | inpatient deaths are almost four-fold higher among HIV-positive children with SAM |
| 22 | (herein termed HIV-SAM), compared to HIV-negative children with SAM (30.4% vs |
| 23 | 8.4%), for reasons that remain unclear ¹⁰ ; this mortality is three-fold higher than would be |
| 24 | expected from anthropometric parameters alone ¹⁰ . Management of HIV-SAM is |
| 25 | particularly challenging because HIV fundamentally alters the clinical presentation of |
| 26 | malnutrition and the response to treatment. Children with HIV-SAM are more stunted |
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and wasted; have a higher frequency of persistent diarrhoea; tend to have delayed nutritional recovery and have a more complicated clinical course than HIV-negative children with SAM¹⁰. Long-term outcomes of SAM Following resolution of complications and return of appetite, children are discharged from hospital to continue therapeutic feeds at home. However, emerging data indicate high post-discharge mortality following in-hospital management of SAM¹¹⁻¹³. Malnutrition together with young age, HIV infection and pneumonia have been associated with higher post-discharge mortality¹⁴. One of the largest prospective studies of growth and mortality in children with SAM (FuSAM), conducted in Malawi from July 2006 to March 2007, collected 12-month outcome data on 87% of 1024 children admitted to the nutrition ward¹¹. A total of 427 (42%) died and 44% of these deaths occurred after discharge from hospital. Survival was greatest among those who were nutritionally cured upon discharge from outpatient therapeutic feeding centres, defined as two consecutive visits with >80% expected weight-for-height, no oedema and clinically stable. The risk of mortality after hospital discharge was four-fold higher for HIV-SAM compared to HIV-negative children with SAM, but the outcomes among HEU children were not reported. The loss to follow-up was high in the FuSAM study because there was only one follow-up visit, one year after discharge from outpatient-feeding centres. A recent study from Kenya identified malnutrition and HIV infection as key drivers for post-discharge mortality, with 52% of deaths attributable to MUAC <11.5cm and 11% to HIV infection¹⁵. The impact of SAM appears to persist beyond the first year after discharge from hospital. The ChroSAM study, which followed children with SAM seven years post-discharge, showed that children had poorer growth, body composition and physical function

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compared to siblings and community controls, which are all indicators of future
 cardiovascular and metabolic disease¹².

While anthropometry is used to assess nutritional recovery after discharge from hospital, the pattern and quality of growth recovery following SAM is poorly understood. The observation that children treated for SAM have a deficit in lean tissue despite regaining weight suggests that assessing body composition in addition to anthropometry may help to identify children who have not completely recovered and are at potential risk of long-term metabolic diseases¹². Children with HIV-SAM appear to have potential for catch-up growth in weight-for-age and/or weight-for-height, which have been shown to normalise with treatment even prior to widespread availability of ART¹⁶; by contrast, height-for-age shows less potential for catch-up growth¹⁷. However, the body composition of children with HIV-SAM compared to HIV-negative children with SAM has not been described. Whether children recover fat mass at the expense of lean mass is unknown, but differences in tissue accretion patterns may have implications for survival and long-term metabolic health¹⁸¹⁹. There is also a need to consider the effect of SAM on the size of body parts which grow at different rates: relatively shorter legs, for example, are associated with epidemiologic risk of overweight, coronary artery disease, liver dysfunction and diabetes^{20 21}.

Taken together, there is clearly an elevated risk of mortality among HIV-positive children with SAM compared to HIV-negative children with SAM, and an ongoing mortality risk among all children with SAM that persists after discharge from hospital. There are several gaps in our understanding of the long-term outcomes: (i) causes of death have not been clearly defined; (ii) no studies have systematically and longitudinally collected morbidity and mortality data or documented repeat hospitalisations post-discharge; and,

| | 1 | (iii) the long-term outcomes of HIV-positive children with SAM in the era of ART |
|---|----|--|
| | 2 | availability are unclear. |
| | 3 | |
| | 4 | Pathogenesis of SAM |
| | 5 | Better understanding the pathogenesis of SAM may help to explain the high mortality of |
| | 6 | children both during and after hospitalisation and identify new targets for interventions to |
| | 7 | supplement existing treatment strategies. Consistent evidence that immune mediators |
| | 8 | are altered in malnutrition ²² and that systemic and intestinal inflammation are associated |
| | 9 | with poor outcomes in SAM ²³ , suggest that immune dysfunction contributes to infectious |
| 1 | 10 | susceptibility ²⁴ . Malnutrition is also characterised by a complex derangement in gut |
| 1 | 11 | microbial ²⁵ metabolic, ²⁶ immune ²⁷ and hormonal pathways, organ dysfunction and |
| 1 | 12 | micronutrient deficiencies in the context of co-infections, enteropathy and chronic |
| 1 | 13 | inflammation. Several studies have recently provided insights into these perturbations |
| 1 | 14 | using new tools ^{25 26 28 29} , including metabolomics and metagenomics, but we still lack a |
| 1 | 15 | clear understanding of many of the pathogenic pathways driving malnutrition, the |
| 1 | 16 | interactions between these pathways, and which are the most tractable targets for |
| 1 | 17 | intervention. |
| 1 | 18 | |
| 1 | 19 | SAM shares several pathological and clinical features with HIV, which may explain |
| 2 | 20 | clinical outcomes in these co-occuring conditions: 1) both are characterised by intestinal |
| 2 | 21 | damage, leading to impairment of the mucosal barrier and increased intestinal |
| 2 | 22 | permeability; 2) both have underlying systemic immune activation; and 3) both are |
| 2 | 23 | frequently complicated by persistent diarrhoea, pneumonia and sepsis that may |
| 2 | 24 | plausibly arise due to loss of intestinal barrier function ³⁰ . Understanding the overlapping |
| 2 | 25 | impact of HIV and SAM is critical to inform additional interventions to improve outcomes |
| 2 | 26 | of children with HIV-SAM. |
| | | |

| 1 | |
|----|---|
| 2 | OBJECTIVES OF HOPE-SAM |
| 3 | The Health Outcomes, Pathogenesis and Epidemiology of Severe Acute Malnutrition |
| 4 | (HOPE-SAM) study has two primary objectives: |
| 5 | 1) To describe the short- and long-term clinical outcomes of children with |
| 6 | complicated SAM, with and without HIV infection, and to identify the risk factors |
| 7 | at admission and discharge from hospital that independently predict these |
| 8 | outcomes. |
| 9 | 2) To better characterise the pathogenesis of SAM through nested laboratory sub- |
| 10 | studies evaluating enteropathy, gut microbiota, metabolomics and immune cell |
| 11 | function. |
| 12 | |
| 13 | STUDY DESIGN |
| 14 | HOPE-SAM is a longitudinal observational cohort study, enrolling between 600-800 |
| 15 | children aged 0-59 months admitted with complicated SAM to the tertiary pediatric wards |
| 16 | at two sites in Zimbabwe (Parirenyatwa Hospital and Harare Children's Hospital) and |
| 17 | one in Zambia (University Teaching Hospital, Lusaka). Both HIV-positive and HIV- |
| 18 | negative children will be enrolled. Throughout this paper, 'SAM' refers to all children, |
| 19 | regardless of HIV status; where analyses specifically compare children by HIV status, |
| 20 | groups are identified as HIV-positive children with SAM (or HIV-SAM) and HIV-negative |
| 21 | children with SAM. All participants with SAM are followed for 48 weeks post-discharge, |
| 22 | with longitudinal data collection and blood sampling. The study contains four nested sub- |
| 23 | studies as shown in Figure 1. A subgroup of children will be recruited to the |
| 24 | enteropathy substudy for which they will have the same follow-up procedures but more |
| 25 | intensive biological specimen collection including stool (all time-points), urine after |
| 26 | lactulose-mannitol (LM) challenge as an assessment of intestinal permeability, and |
| | 0 |

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| 1 2 | | | |
|----------------|----|---|--|
| 3 4 | 1 | nasogastric aspirate (baseline only); these children are also included in microbiota and | |
| 5 6 | 2 | metabolomics substudies. Children with SAM for whom blood samples are available are | |
| 7 8 | 3 | included in the immunology substudy, for which circulating inflammatory mediators will | |
| 9 10 | 4 | be assayed; functional cellular immunology assays will be conducted for all children in | |
| 11 12 | 5 | the immunology sub-study with sufficient sample volume (> 2mL) recruited after June | |
| 13 14 | 6 | 2017. A group of healthy children recruited from the same hospitals and communities, | |
| 15 16 | 7 | who are well-nourished and matched to children in the enteropathy substudy by age and | |
| 17 18 | 8 | HIV status, will have data and specimens collected to provide normative data for the | |
| 19 20 21 | 9 | laboratory substudies; these well-nourished controls will not be followed longitudinally. | |
| 21 22 23 | 10 | | |
| 24 25 | 11 | The study protocol, data collection forms and standard operating procedures are | |
| 26 27 | 12 | available online at osf.io/29uaw. | |
| 28 29 | 13 | | |
| 30 31 | 14 | RECRUITMENT | |
| 32 33 | 15 | Screening: Caregivers of all hospitalised children are sensitised about the study. All new | |
| 34 35 | 16 | admissions aged 0-59 months are screened for SAM, which is defined according to | |
| 36 37 | 17 | WHO criteria as any of: weight-for-height Z-score (WHZ) <-3, MUAC <115 mm (if aged | |
| 38 39 40 | 18 | 6-59mo) and/or bilateral pitting oedema. All children with SAM are recruited from | |
| 40 41 42 | 19 | hospital and this study therefore focuses on complicated SAM; children with | |
| 43 44 | 20 | uncomplicated SAM will not be enrolled. | |
| 45 46 | 21 | | |
| 47 48 | 22 | Eligibility for observational cohort: All children with SAM whose caregivers are willing to | |
| 49 50 | 23 | provide written informed consent and to learn their child's HIV status are offered | |
| 51 52 | 24 | enrolment. Any children who die prior to study enrolment and those with a known | |
| 53 54 | 25 | malignancy are ineligible. | |
| 55 56 | 26 | | |
| 57 58 | | | |
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| L | Eligibility for enteropathy substudy: Children with SAM aged 6-59 months with a |
|---|--|
| 2 | nasogastric tube in place (or due to be placed) are categorized into 4 groups (HIV- |
| 3 | positive oedematous (Group A-I); HIV-positive non-oedematous (Group A-II); HIV- |
| ł | negative oedematous (Group C-I) and HIV-negative non-oedematous (Group C-II), as |
| 5 | shown in Table 1. Children meeting eligibility criteria will be enrolled throughout the |
| 5 | study recruitment period until sufficient specimens have been collected from the groups |
| 7 | shown in Table 1. Children in the enteropathy substudy are stratified into age bands (6- |
| 3 | 11 months; 12-23 months and 24-59 months) to enable age-matching of well-nourished |
|) | controls. Children with underlying chronic gastrointestinal disease or a known |
|) | malignancy are ineligible. |

12 Table 1: Enteropathy substudy groups

| | Severe acute ma | alnutrition ¹ | |
|---|-------------------------|--------------------------|--------------------------------------|
| Children aged 6-59 months | Oedematous ² | Non-oedematous | Well nourished controls WHZ>-1 |
| HIV-positive (HIV PCR+ if <18mo; HIV antibody + if >18mo) | N=50 (Group A-I) | N=50 (Group A-II) | N=100 ³ (Group B) |
| HIV-negative (HIV PCR- if <18mo; HIV antibody - if >18mo) | N=50 (Group C-I) | N=50 (Group C-II) | N=100⁴ (Group D) |

14 ¹SAM defined according to WHO criteria

15 ²Presence of bilateral pitting pedal oedema.

Note that children below 6 months of age are excluded from the enteropathy substudy to avoid interrupting
 exclusive breastfeeding during the lactulose-mannitol test.

18 WHZ: Weight-for-height Z score; PCR: polymerase chain reaction.

- 21 Eligibility for microbiota and metabolomics substudies: Children enrolled into the
- 22 enteropathy substudy are also included in the microbiota and metabolomics substudies,
- 23 since these substudies utilize the stool, urine and plasma samples collected for
- 24 enteropathy analyses.

| 1 | |
|----------|---|
| 2 | Eligibility for immunology substudy: The immunology substudy comprises all children |
| 3 | with SAM (drawn from both the observational cohort and the enteropathy substudy, as |
| 4 | shown in Figure 1) providing a blood sample of sufficient volume (>2ml) for cellular |
| 5 | assays after 1 st June 2017. |
| 6 | |
| 7 | Well-nourished controls: Controls are children drawn from the same hospitals and |
| 8 | communities as cases with SAM (including well-nourished sibling controls), who are |
| 9 | aged 6-59 months (matched to enteropathy substudy children within age bands), well- |
| 10 | nourished (weight-for-height Z-score >-1) and clinically well (no acute illness or current |
| 11 | infections) with known HIV status. Controls are categorized into two groups: well- |
| 12 | nourished HIV-positive (Group B) and well-nourished HIV-negative (Group D), as shown |
| 13 | in Table 1. Children with underlying chronic gastrointestinal disease or a known |
| 14 | malignancy are ineligible. Well-nourished controls provide comparison biomarker data |
| 15 | for all the laboratory substudies. |
| 16 | |
| 17 | Informed consent procedures: Written informed consent is obtained from the primary |
| 18 | caregiver using consent forms translated into local languages; where possible, other family |
| 19 | members are included in the consent process. Illiterate caregivers who have understood a |
| 20 | verbal explanation of the study can provide a thumb imprint in the presence of a witness. |
| 21 | Assent from children is not sought because all are <5 years old. |
| 22 | |
| 23 | STUDY PROCEDURES |
| 24 | Study procedures are outlined in Table 2 . |
| 25 26 | Table 2: Summary of procedures in observational cohort |
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| Hospitaliza Baseline ¹ | Discharge ² | | | | | |
|--------------------------------------|--|---------------------------------------|--|---|--|---|
| | Discillarye- | 2w | 4w | 12w | 24w | 48w |
| x | | | | | | |
| X | | | | | | |
| X | | | | | | |
| x | | | | | | |
| x | | | | | | |
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| X | X | | | X | X | X |
| x | | | | | | |
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| X | X | | | X | X | X |
| X | X | х | X | X | X | X |
| | X | х | X | X | X | X |
| x | x | х | X | X | X | X |
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¹Children will be enrolled as soon as possible after hospitalisation and will undergo baseline investigations as soon as possible after enrolment. This is to provide a window of opportunity to time collection of research specimens with clinical specimens, and to ensure that the child is clinically stable before undertaking research investigations.

²The discharge procedures will be undertaken on the day of discharge, or as close as possible to that date.

³Windows will be created around these post-discharge time-points to maximize follow-up for caregivers who miss visits or are unavailable, as follows: 2 weeks (1-3 weeks); 4 weeks (3-5 weeks); 12 weeks (10-14 weeks); 24 weeks (20-28 weeks); 48 weeks (44-52 weeks).

⁴Locator information will updated at subsequent visits if caregivers have moved or changed contact details.

⁵A clinical review will be undertaken every day between admission and discharge by the study clinician. ⁶5.4 mL of blood (depending on child weight; amount will not exceed 2 mL/kg total over 2 week period) will be collected by a study nurse into endotoxin-free EDTA tubes. Samples will be used to store whole blood, PBMC and plasma for subsequent measurement of CRP and albumin. Where blood sample volumes allow (≥2mL sample), bacterial binding assays and whole blood stimulations will be conducted and culture supernatants and cells stored for subsequent assessment of immune cell function at each time-point.

| 1 2 3 4 5 6 7 8 9 10 11 12 | Study blood samples will not be collected from children with known haemoglobin <6 g/dL. ⁷ HIV testing is conducted as part of routine clinical practice, but if it has not been undertaken, the study sample will be used to test for HIV, as stated in the informed consent form, since HIV status is required to allocate children to study groups. ⁸ Full blood count results will be transcribed from clinical records; if not done by clinical teams, the EDTA sample will be used to measure FBC in clinical laboratories at each site ⁹ Skinfold thickness (triceps, subscapular, supra-iliac) and mid-thigh circumference will be measured using Holtain calipers or tape measure. ¹⁰ Body composition will be assessed by bioimpedance vector analysis. |
|---|--|
| 13 | Baseline procedures: |
| 14 | Baseline data on maternal and household characteristics, the child's past medical history |
| 15 | and current illness are collected by a study nurse. Anthropometry, including body |
| 16 | composition measured by whole-body (wrist-ankle) bio-electrical impedance analysis |
| 17 | (BodyStat 1500MD; BodyStat Ltd., Douglas, Isle of Man), leg length using an electronic |
| 18 | knemometer (Zimbabwe only, due to availability of knemometers) and triceps, |
| 19 | subscapular and supra-iliac skinfold thickness using calipers (Holtain Ltd., Crymych, UK) |
| 20 | are undertaken at baseline. Blood (1mL/kg up to 5.4mL maximum) is collected at |
| 21 | baseline into an endotoxin-free EDTA tube for all children and, in the enteropathy |
| 22 | substudy, additionally into a PAXgene tube (PreAnalytiX GmbH, Hombrechtikon, |
| 23 | Switzerland) for subsequent transcriptomic analysis. Blood is not collected from children |
| 24 | with severe anaemia (known haemoglobin <6 g/dL). HIV testing is carried out in |
| 25 | accordance with national guidelines as part of routine clinical practice; where it has not |
| 26 | been done, the child's HIV status is ascertained using a rapid test antibody algorithm for |
| 27 | children over 18 months, or HIV DNA PCR for children under 18 months. CD4 |
| 28 | count/percentage and viral load are measured in HIV-positive children. Maternal HIV |
| 29 | status is documented where available, so that HIV-exposed uninfected children can be |
| 30 | identified. Blood samples are sent to research laboratories at each site to conduct whole |
| 31 | blood stimulation and bacterial binding assays (as described in the immunology |
| 32 | substudy) and to store aliquots of whole blood, peripheral blood cells and plasma at - |
| | |

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80°C³¹. In the enteropathy substudy, nasogastric aspirate, stool and urine (after an oral dose of lactulose and mannitol) are also collected. Lactulose and mannitol are ingested by the child after fasting and urine is collected over a two-hour period to measure recovery of lactulose and mannitol, a measure of intestinal absorptive capacity and permeability, as previously described³². Daily procedures: Routine inpatient management is undertaken by ward clinical teams according to local hospital protocols, which are based on WHO guidelines^{2,33} In addition, the HOPE-SAM study clinician at each hospital site collects daily data until discharge on clinical parameters (including daily examination), resolution of acute infections, nutritional recovery (loss of oedema, restoration of appetite, weight gain), and treatment/nutritional supplements received; this will allow us to evaluate differences in management between countries. Children with HIV-SAM who are ART-naïve start ART according to national guidelines, which are based on WHO recommendations^{2 34}. Discharge: The clinical team decides when the child is ready to be discharged, which is generally when their medical complications are resolving and the child has a good appetite and is clinically well and alert². Children receive ready-to-use therapeutic feeds (RUTF) to take at home according to local guidelines. At discharge, the study nurse collects data and a repeat blood sample (including full blood count) and undertakes discharge anthropometry, body composition, leg length (Zimbabwe only) and triceps, subscapular and supra-iliac skinfold thickness measurements (Table 2). The caregiver is given a daily morbidity diary and pre-prepared stickers corresponding to different illnesses and shown how to complete the diary. The caregiver is provided with the date of the first follow-up appointment and contact details of the study nurse.

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|---|--|--|
| 47 | | |

2 Follow-up: Children attend follow-up appointments at dedicated study clinics at 2, 4, 12, 3 24 and 48 weeks post-discharge. At each visit, the study physician undertakes a clinical 4 assessment and the study nurse captures illness, medication and feeding data. Clinic 5 data are transcribed from handheld medical records if available and the morbidity diary 6 is reviewed and a new diary and stickers supplied. Anthropometry, body composition, 7 leg length (Zimbabwe only) and triceps, subscapular and supra-iliac skinfold thicknesses 8 are measured at each visit. Acute illnesses are treated in the study clinic, or the child is 9 referred to hospital if necessary. Children with relapsed malnutrition are provided with 10 nutritional supplements or RUTF according to local guidelines, or readmitted to hospital 11 if they develop complicated SAM. Transport reimbursement for clinic attendance is 12 provided to caregivers for each visit. 13 14 Blood is collected at weeks 12, 24 and 48 post-discharge into endotoxin-free EDTA 15 tubes to measure full blood count, CD4 count and viral load (HIV-positive children only), 16 conduct whole blood stimulation and bacterial binding assays (where blood volumes 17 >2mL), and store peripheral blood cells and plasma aliquots for subsequent analysis (all 18 blood samples), including soluble and cellular markers of immune activation, as outlined

19 in **Supplementary Table 1**. Children in the enteropathy substudy have additional stool

20 and urine collection following lactulose-mannitol dosing as shown in **Table 3**.

| 22 | Table 3: Summary of procedures for cases in the enteropathy substudy |
|----|--|
| 23 | |

| Assessment | Hospitaliza | ation | Post | -discha | rge ³ | | |
|--|-----------------------|------------------------|------|---------|------------------|-----|-----|
| | Baseline ¹ | Discharge ² | 2w | 4w | 12w | 24w | 48w |
| Caregiver informed consent to join observational cohort and enteropathy substudy | X | | | | | | |

| X x Daily during hospitalisat x X X X x x x x x X X | | x x x z | | X X X X X X X X X X X X X X | X X X X X X X X X | X X X X X X X X X X X X X X X |
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| 123456789111213415671892122323 | a 2.7 mL PAXGene tube, for subsequent isolation of RNA and gene expression analysis. After discharge (weeks 12, 24 and 48), 5.4 mL of blood (depending on child weight; amount will not exceed 2 mL/kg total over 2 week period) will be collected by a study nurse into two 2.7 mL endotoxin-free EDTA tubes. ⁷ HIV testing is conducted as part of routine clinical practice, but if it has not been undertaken, the study sample will be used to test for HIV (see section 9.4), as stated in the informed consent form, since HIV status is required to allocate children to study groups. ⁸ Full blood count results will be transcribed from clinical records; if not done by clinical teams, the EDTA sample will be used to measure FBC in clinical laboratories at each site ⁹ A gastric juice sample will be collected at the same time as the blood draw by aspirating the nasogastric tube with a sterile feeding syringe, to test for gastric pH; sterile water or saline will then be instilled and a sample of gastric juice collected for storage for subsequent PCR and culture (section 7.5.2) ¹⁰ Stool collection will be undertaken at the same time as the blood draw ¹¹ Lactulose-mannitol testing will be conducted, with collection of a baseline urine sample, followed by a 2hr urine collection post-LM ingestion. This test will be deferred until children are judged to be clinically stable by the study physician during daily reviews. In general, this will be a child in the nutritional rehabilitation phase, who has no cardiorespiratory compromise. ¹³ Body composition will be assessed by bioimpedance vector analysis. |
|--------------------------------|--|
| 25 | Caregivers are reminded of follow-up visits by phone, and visit completion is tracked on |
| 26 | a dedicated database. If caregivers do not attend follow-up appointments, attempts are |
| 27 | made to contact them by phone and home visits are made if feasible, particularly for |
| 28 | those defaulting the 48-week visit, so that long-term outcome data can be collected. For |
| 29 | post-discharge deaths, a home visit is undertaken by study nurses where possible to |
| 30 | conduct a verbal autopsy. Children who are readmitted to one of the study sites with |
| 31 | relapsed SAM have data collected during the new episode of hospitalisation. The study |
| 32 | ends for each participant at the week 48 visit. |
| 33 | |
| 34 | SUBSTUDIES |
| 35 | As outlined in Figure 1, four nested substudies will utilise biological specimens to |
| 36 | address mechanistic questions related to enteropathy, microbiota, metabolomics and |
| 37 | immune function. |
| 38 | |
| 39 | Enteropathy substudy |
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| 1 | The gut, which acts as an internal interface between humans and the environment, must |
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| 2 | contain the nutrient stream and the symbiotic microbiota while allowing molecular |
| 3 | intimacy to permit absorption. The mechanism underlying this duality is the integrity of |
| 4 | the gastrointestinal barrier; intestinal damage (enteropathy) can impair this critical barrier |
| 5 | function. A spectrum of enteropathies affect children in developing countries ³⁰ . |
| 6 | Environmental enteric dysfunction (EED), characterised by small intestinal inflammation, |
| 7 | blunted villi and increased intestinal permeability, is almost universal and is |
| 8 | morphologically indistinguishable from HIV enteropathy ³⁰ . Children in resource-poor |
| 9 | settings also suffer from frequent diarrhoea, food insecurity and micronutrient |
| 10 | deficiencies, which all exacerbate enteropathy ³⁰ . As a result, a cycle of intestinal |
| 11 | infection, impaired mucosal function and malnutrition commonly arises, which may |
| 12 | ultimately precipitate SAM, especially in the context of HIV infection ^{35 36} . It is not yet |
| 13 | established if the enteropathy seen in children with SAM ³⁷ , which we here refer to as |
| 14 | malnutrition enteropathy ³⁷ , is qualitatively or quantitatively distinguishable from EED. In |
| 15 | addition to local intestinal pathology, enteropathies may cause systemic pathology due |
| 16 | to persistent immune activation arising from enteric inflammation and microbial |
| 17 | translocation across the damaged gut wall ³⁰ . It is becoming apparent that chronic |
| 18 | inflammation may be particularly deleterious in malnourished individuals ²³ ; in children |
| 19 | with SAM, systemic inflammation arising from underlying enteropathy may further |
| 20 | increase morbidity and mortality. |
| 21 | |

We hypothesize that i) the degree of enteropathy during hospitalisation differs between oedematous and non-oedematous SAM and is independently associated with morbidity, mortality and nutritional recovery during hospitalization; ii) the degree of enteropathy at discharge is independently associated with morbidity, mortality and relapse of SAM; and

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iii) children with HIV-SAM have more severe enteropathy than HIV-negative children with

SAM, which contributes to their poorer outcomes. Using stored samples, a longitudinal series of investigations will compare gastric and small intestinal barrier function, using a range of biomarkers to capture the domains of malnutrition enteropathy (Supplementary Table 2). To understand better the extra-intestinal consequences of enteropathy, we will first compare the microbial composition of the upper gut and plasma using deep sequencing in a subgroup of children with paired gastric and blood samples. Secondly, we will undertake transcriptomics using PAXGene blood samples to determine i) whether there are differences in gene expression profiles between well-nourished controls, HIV-negative children with SAM and HIV-positive children with SAM (including comparison of oedematous and non-oedematous types); and ii) whether specific patterns of gene expression are associated with morbidity and mortality in SAM. Microbiota substudy Normal assembly of the gut microbiota in early life is critical for many aspects of physiological, neurological and immune development³⁸. Recent evidence suggests that an immature or pathogenic microbiota plays a causative role in the pathogenesis of SAM²⁵. For example, a number of microbial taxa have been identified, including Faecalibacteium prausnitzii, which discriminate and predict gut microbiota maturity and child growth²⁸. Other pathogenic microorganisms, including IgA-targeted Enterobacteriaceae, are associated with impaired growth and may contribute to SAM³⁹. Nutritional rehabilitation with RUTF induces temporary recovery of a disturbed microbiota; however, the microbiota appears to revert back to an immature diseased state following nutritional recovery²⁹. HIV infection is also associated with a disturbed gut

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microbiota⁴⁰, which may further compound enteropathy phenotypes. Furthermore, there
is some evidence that differences exist in malnutrition enteropathy between oedematous
and non-oedematous SAM⁴¹; however, few studies have investigated differences in the
gut microbiota between the two forms of the disease.

6 We hypothesize that (i) a unique gut microbial signature exists in HIV-SAM, compared 7 with HIV-negative children with SAM, that is independently associated with morbidity, 8 mortality, nutritional recovery and degree of enteropathy during hospitalisation; (ii) a 9 unique gut microbial signature exists in oedematous compared with non-oedematous 10 SAM; (iii) specific microorganisms or gut microbial diversity indices are independently 11 associated with morbidity, mortality, nutritional recovery and degree of enteropathy 12 during hospitalisation; and (iv) the gut microbiota is partially restored to a healthy state 13 with nutritional rehabilitation but reverts to a dysbiotic state during follow-up, which 14 predicts morbidity, mortality and relapse of SAM.

15

5

16 Using stored stool samples collected at baseline, a cross-sectional investigation will 17 determine differences in the gut microbial composition and predicted function between: 18 HIV-negative children with SAM versus HIV-SAM, oedematous versus non-oedematous 19 SAM, and well-nourished controls. Gut microbial composition and predicted function will 20 be compared between groups at discharge and at 12, 24 and 48 weeks post-discharge. 21 Briefly, total DNA and/or RNA will be extracted from stool samples and used as template 22 for next-generation sequencing library preparation and for quantitative polymerase chain 23 reaction (qPCR). Whole metagenome shotgun sequencing will be performed using the 24 HiSeg 2500 system. Raw metagenomic sequencing data will be guality-filtered and 25 analysed through a well-validated bioinformatics pipeline using MetaPhIAn⁴² and 26 HUMAnN⁴³. The compositional and predicted functional metagenomic data generated

| 1 | will be used to identify signatures of SAM and to investigate associative links between |
|----|--|
| 2 | specific gut microbial signatures and clinical outcomes. |
| 3 | |
| 4 | |
| 5 | Metabolomics substudy |
| 6 | During SAM, metabolic processes are altered in response to a starved environment, and |
| 7 | may plausibly contribute to long-term clinical outcomes. Previous studies suggest that |
| 8 | amino acid turnover, lipid metabolism, oxidative stress and other metabolic pathways are |
| 9 | disrupted in SAM and may be associated with disease state and clinical outcome ^{26 44 45} ; |
| 10 | however, little is known about how the metabolic phenotype responds to nutritional |
| 11 | therapy. It is hypothesised that disturbed gut microbiota composition and function may |
| 12 | drive microbial metabolic dysregulation in addition to host-derived dysregulation. Of |
| 13 | particular interest are differences in the metabolic phenotype between oedematous and |
| 14 | non-oedematous SAM. The 'reductive adaptation' seen in non-oedematous SAM |
| 15 | (utilisation of fat and muscle stores) is disrupted in oedematous SAM, which may |
| 16 | contribute to differences in clinical outcomes. Specifically, protein turnover, inflammation, |
| 17 | oxidative stress and bile acid metabolism are disrupted in oedematous-SAM, which may |
| 18 | contribute to co-morbidities including diarrhoea, steatosis and enteropathy ^{46 47} . |
| 19 | |
| 20 | We hypothesize that: (i) a unique plasma and urine metabolic phenotype exists in |
| 21 | oedematous compared with non-oedematous SAM during hospitalisation, which is |
| 22 | independently associated with morbidity, mortality and nutritional recovery; (ii) the |
| 23 | metabolic phenotype is partially restored to a healthy state with nutritional rehabilitation |
| 24 | but reverts to a disturbed state during follow-up, which predicts morbidity, mortality and |
| 25 | relapse; and (iii) both host-derived and gut microbial-driven metabolic dysregulation |
| 26 | underlie clinical outcomes. |
| | |

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| 2 | Using stored urine and plasma samples collected during hospitalisation, a cross- |
| 3 | sectional investigation will determine differences in the metabolic phenotype between |
| 4 | children with oedematous SAM, non-oedematous SAM and well-nourished controls. |
| 5 | Urine and plasma metabolic phenotypes will be compared between groups at discharge |
| 6 | and 12, 24 and 48 weeks post-discharge. Briefly, global untargeted metabolomic |
| 7 | phenotyping will be performed via ¹ H nuclear magnetic resonance (NMR) spectroscopy |
| 8 | using a 700 MHz Bruker NMR spectrometer to identify metabolic signatures of SAM. |
| 9 | Targeted analysis via ultra-performance liquid chromatography-mass spectrometry will |
| 10 | be performed to examine specific pathways of interest, including tryptophan and bile |
| 11 | acid metabolism. |
| 12 | |
| 13 | Immunology substudy |
| 14 | Bacterial infections are common among children hospitalised for SAM ^{23 48-50} and |
| 15 | mortality is driven by a range of species ^{48 50-53} , consistent with generalised defects in |
| 16 | innate anti-bacterial defence. Increased infectious morbidity and mortality persist after |
| 17 | discharge from hospital ^{10 17 54} , suggesting that restoration of anti-bacterial immune |
| 18 | responses may lag behind nutritional rehabilitation. A recent randomised trial in children |
| 19 | with SAM confirmed that deaths following hospitalisation were predominantly due to |
| 20 | bacterial infections but were not prevented by daily co-trimoxazole prophylaxis ¹⁷ . |
| 21 | Collectively, these observations highlight that children remain vulnerable to infection |
| 22 | despite current treatment approaches; targeting persistent immune dysfunction could |
| 23 | plausibly reduce infectious mortality after discharge ²⁴ . |
| 24 | |
| 25 | Multiple innate and adaptive immune mediators are dysregulated in malnutrition ^{24 27 55} . |
| 26 | However, few studies have assessed cellular immune function in malnourished children; |
| | |

| | 1 | most existing studies were undertaken decades ago on small cross-sectional cohorts |
|-------------------|----|---|
| | 2 | without the benefit of recent advances in immunology techniques ²⁷ . Immune dysfunction |
| | 3 | in SAM likely reflects both intrinsic defects, whereby immune cells lack capacity to |
| 0 | 4 | adequately respond to infection, and extrinsic defects, where cells have intact anti- |
| 1 2 | 5 | bacterial capacity but are chronically modulated by the systemic pro-inflammatory |
| 3 4 | 6 | environment which characterises SAM (i.e. heightened pro-inflammatory cytokines ⁴⁴ and |
| 5 5 | 7 | circulating bacterial antigens ^{23 56 57}). Systemic inflammation is directly associated with |
| 7 8 | 8 | mortality in SAM ²³ and driven by multiple comorbidities, including bacterial translocation |
|)) 1 | 9 | from the damaged gut into the blood, sub-clinical infections and metabolic |
| 1 2 3 | 10 | dysregulation ^{44 58 59} . The implications of innate immune cell dysfunction for subsequent |
| 5 4 5 | 11 | acquisition of infections and infectious mortality have not been investigated. |
| 5 7 | 12 | |
| 3 | 13 | We hypothesise that: (i) anti-bacterial functions of innate immune cells are compromised |
|) 1 | 14 | in SAM due to a combination of intrinsic and extrinsic defects; ii) innate immune cell |
| <u>2</u> 3 | 15 | function is independently associated with infectious morbidity and mortality during |
| 4 5 | 16 | hospitalisation for SAM; and iii) nutritional rehabilitation only partly restores innate |
| 5 7 | 17 | immune cell function, leading to an ongoing risk of bacterial infections post-discharge. |
| 3 9 | 18 | |
|) | 19 | Using blood samples collected at baseline, discharge and 12, 24 and 48 weeks post- |
| 2 3 4 | 20 | discharge, the longitudinal relationship between circulating innate immune cell function |
| 5 | 21 | and bacterial infections will be assessed. The intrinsic phagocytic capacity, secreted |
| 7 3 | 22 | cytokine response and maturation state of innate immune cells after culture with |
|)) | 23 | bacterial antigens will be assessed. Plasma concentrations of endotoxin and pro- |
| 1 2 | 24 | inflammatory mediators will be quantified at each time-point and the degree to which |
| 3 4 | 25 | these extrinsic factors influence innate immune cell antibacterial function will be |
| 5 5 | 26 | assessed via plasma co-culture with innate immune cells from healthy donors. Bacterial |
| 7 3 | | |
| 9 | | 24 |

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infections during hospitalisation will be diagnosed using clinical criteria and blood
 culture, stool culture and urinalysis where available.

3

4

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SAMPLE SIZES

5 Observational study: The observational cohort will recruit as many children with SAM as 6 possible during the period of enrolment (July 2016 to March 2018), estimated at 600-800 7 children (capped at 800 maximum), to assess clinical and nutritional outcomes among 8 HIV-positive and HIV-negative children hospitalised with SAM. Assuming mortality of 9 15%, overall loss to follow-up of 15% and recruitment target of 800 children, there would be 560 evaluable children at 48 weeks, of whom 224 would have HIV-SAM based on an 10 11 estimated inpatient HIV prevalence of 40%. This will provide >80% power to detect 12 absolute differences of 17% in binary outcomes between HIV-SAM and HIV-negative 13 children with SAM, and of 0.33 times the standard deviation in continuous outcomes.

14

15

16 Enteropathy substudy: The sample size was estimated using previously reported values 17 for LM ratios, which remain a widely used non-invasive marker of enteropathy. 18 Comparing 100 versus 100 children with two-sided alpha=0.025 (to allow for two primary 19 comparisons, i.e. HIV-SAM versus HIV-negative children with SAM, and HIV-SAM 20 versus well-nourished HIV-positive children) provides >80% power to detect differences 21 in mean LM ratio during hospitalisation of at least 0.16 (assuming SD=0.36), a difference 22 which would be clinically relevant given the LM ratios previously reported for well-23 nourished children (0.42), malnourished children (1.3) and children with persistent 24 diarrhoea (2.85) in the Gambia⁶⁰. It also provides >80% power to detect differences of at 25 least 0.1 in the mean change in LM ratio from enrolment (assuming SD for change=0.23) 26 and 7% missing samples). For inflammatory markers, comparing 100 versus 100

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| 2 3 4 | 1 | children with two-sided alpha=0.025 provides >80% power to detect differences in mean |
| 5 6 | 2 | log_{10} concentrations of at least 0.44 times their standard deviation, or 2.75-fold |
| 7 8 | 3 | differences between groups. Inclusion of well-nourished controls provides an indication |
| 9 10 | 4 | of normal ranges in young African children. HIV-positive and HIV-negative SAM groups |
| 11 12 | 5 | will be stratified to include approximately 50 children with and without oedematous |
| 13 14 | 6 | malnutrition, if possible. |
| 15 16 | 7 | |
| 17 18 | 8 | Microbiota and metabolomics substudy |
| 19 20 | 9 | Power calculations are difficult in metagenomics and metabolomic analyses due to the |
| 21 22 | 10 | large number of observed outcomes and unknown effect sizes and variance. Previous |
| 23 24 | 11 | studies using smaller sample sizes have identified significant taxonomic differences in |
| 25 26 27 | 12 | twin pairs discordant for oedematous-SAM (n=13) ²⁵ and metabolic differences between |
| 27 28 29 | 13 | the two forms of SAM (n=40) ²⁶ . These studies suggest that a difference of 50% in |
| 30 31 | 14 | metabolites could be expected. Using ANCOVA, setting α =0.05 and assuming either low |
| 32 33 | 15 | (ρ =0.1) or high (ρ =0.7) correlation, the study would require 95-126 subjects to achieve |
| 34 35 | 16 | 80% power ⁶¹ . False discovery rate (FDR) multiple correction testing will be applied to |
| 36 37 | 17 | reduce the high-dimensionality of the data and limit false-positives. |
| 38 39 | 18 | |
| 40 41 | 19 | Immunology substudy |
| 42 43 | 20 | Up to 200 children with SAM and 200 well-nourished controls will be included in a cross- |
| 44 45 | 21 | sectional analysis of innate immune cell function during hospitalisation. Assuming similar |
| 46 47 48 | 22 | infectious mortality to a recent Kenyan study (15%) ¹⁷ , a cohort of 200 provides 80% |
| 48 49 50 | 23 | power to detect associations between immune profiles and infectious mortality at an |
| 51 52 | 24 | odds ratio of 1.7 and 2-sided alpha of 0.05. We will aim for 100 children with longitudinal |
| 53 54 | 25 | analysis of innate immune cell function at discharge, 12, 24 and 48 weeks post- |
| 55 56 | 26 | discharge ¹⁷ . |
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| 1 | |
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| 2 | STUDY OUTCOMES AND RISK FACTORS |
| 3 | The main study outcomes are clinical (mortality, morbidity and relapse of malnutrition) |
| 4 | and nutritional (weight, height, mid-upper arm circumference, leg length, head |
| 5 | circumference, mid-thigh circumference, skin-fold thickness and body composition by |
| 6 | bioimpedance vector analysis) assessed over 48 weeks of follow-up. Mortality is |
| 7 | assessed in hospital by daily physician review and, post-discharge, through study visits |
| 8 | and by telephone where possible for children who are lost to follow-up. Morbidity during |
| 9 | hospitalization is assessed through daily clinical assessments and available hospital |
| 10 | laboratory tests. Morbidity after discharge is assessed, first, using daily morbidity diaries |
| 11 | in which caregivers record episodes of illness (lethargy interfering with feeding; |
| 12 | respiratory distress; diarrhoea; oedema and fever); second, from caregiver recall and |
| 13 | review of handheld medical records at each follow-up visit; and, third, from data |
| 14 | collected during hospitalization for children who are readmitted during the follow-up |
| 15 | period. Time-to-recovery from malnutrition will be evaluated during hospitalization; |
| 16 | relapse of malnutrition during follow-up will be categorized as moderate acute |
| 17 | malnutrition, uncomplicated SAM and complicated SAM, according to WHO definitions. |
| 18 | Nutritional outcomes will be expressed both as continuous variables (attained Z-score |
| 19 | and change in Z-score between visits), and as categorical variables (moderate wasting, |
| 20 | WHZ<-2; severe wasting, WHZ<-3; stunting, HAZ<-2; severe stunting, HAZ<-3; |
| 21 | underweight, WAZ <-2; and microcephaly, head circumference-for-age <-2). |
| 22 | |
| 23 | Risk factors will be evaluated at baseline, hospital discharge and over the period of |
| 24 | follow-up for associations with clinical and nutritional outcomes. In addition to baseline |
| 25 | clinical and demographic factors, the following laboratory parameters will be evaluated: |
| | haemoglobin, serum albumin, C-reactive protein, CD4 count and HIV viral load (for HIV- |

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| 1 | positive children). Haemoglobin, CD4 and HIV-viral load will be measured in real time |
|----|---|
| 2 | and the results reviewed during follow-up clinics. |
| 3 | |
| 4 | Data on potential confounders are collected at baseline, discharge and during the follow- |
| 5 | up period, including child feeding practices, household socioeconomic status (defined by |
| 6 | household income and cooking method), maternal employment and education, and |
| 7 | household factors such as water, sanitation and hygiene practices, availability of |
| 8 | electricity, location (rural, peri-urban or urban) and household size. |
| 9 | |
| 10 | ANALYSIS |
| 11 | All analyses will be interpreted exploratively since HOPE-SAM is an observational study |
| 12 | with multiple risk factors, outcomes and substudies. For all analyses, P values will not be |
| 13 | artificially adjusted, but interpreted as exploring the strength of evidence supporting any |
| 14 | association. The only exception is the use of approaches to minimise false discovery |
| 15 | when analysing high-dimensional data from the microbiota and metabolomics |
| 16 | substudies, as described. |
| 17 | |
| 18 | Observational Cohort |
| 19 | The primary comparison will be the clinical and nutritional outcomes of children with HIV- |
| 20 | SAM compared to HIV-negative children with SAM. We will review all deaths and |
| 21 | adjudicate clinical diagnoses and causes of death to ensure robust and consistent data |
| 22 | across sites. We will compare each participant's clinical management to WHO guidelines |
| 23 | to identify any contributory factors in hospital care. Factors associated with outcomes |
| 24 | during hospitalisation (e.g. mortality, nutritional recovery) will be determined for each |
| 25 | group (HIV-SAM and HIV-negative children with SAM) using multivariable analysis (Cox |
| 26 | models for time-to-event data, linear models for continuous outcomes). Factors |
| | |

| 1 | associated with outcomes over 48 weeks post-discharge (hospital re-admission, |
|----|--|
| 2 | morbidity and mortality, relapse, anthropometry, body composition and response to |
| 3 | ART) will be determined for each group (HIV-SAM and HIV-negative children with SAM) |
| 4 | using multivariable analysis (Cox models for time-to-event data, linear models for |
| 5 | continuous outcomes). HIV-positive children with SAM and HIV-negative children with |
| 6 | SAM will be included in one model together with the risk factors, and interaction tests will |
| 7 | be used to investigate whether associations between risk factors and outcomes differ |
| 8 | between the two groups of children. We will evaluate the ability of mid-upper arm |
| 9 | circumference (MUAC) at discharge to predict long-term outcomes using receiver- |
| 10 | operator-characteristic (ROC) analysis, in the whole cohort and within the subgroups of |
| 11 | HIV-SAM and HIV-negative children with SAM. We will then evaluate whether addition of |
| 12 | other variables improves the predictive capacity of MUAC (using WHO criteria in those |
| 13 | >6 months old, and published data for children <6 months ⁶²) for each group, including |
| 14 | body composition, haemoglobin, albumin and CRP, plus CD4%, viral load and timing of |
| 15 | ART initiation (HIV-SAM only). We will construct multivariable models and compare them |
| 16 | with MUAC alone using the net-reclassification index. |
| 17 | |
| 18 | Body composition analysis |
| 19 | Previous work in body composition by bio-electrical impedance in Ethiopian infants and |
| 20 | children with SAM has shown that the conventional approach, predicting total body |
| 21 | weight from height-adjusted impedance, fails due to confounding by oedema ⁶³ . The |
| 22 | same project validated an alternative approach, known as Bio-electrical Impedance |
| 23 | Vector Analysis (BIVA), and described significant differences between each of three |
| 24 | groups: healthy controls, oedematous-SAM and non-oedematous SAM. Vector analysis |
| 25 | splits impedance into two height-adjusted components, resistance and reactance, which |
| 26 | are further linked through phase angle (PA). Variability in these components is |
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| 2 3 4 | 1 | associated with biochemical parameters ⁶⁴ . These variables will be explored using |
| 5 6 | 2 | graphical analysis, or transformed into age- and sex-adjusted Z-scores for statistical |
| 7 8 | 3 | comparison, including longitudinal analyses. Higher phase angle indicates better |
| 9 10 | 4 | nutritional status, while declining height-adjusted resistance over time indicates loss of |
| 11 12 | 5 | oedema. |
| 13 14 | 6 | |
| 15 16 | 7 | Enteropathy substudy |
| 17 18 | 8 | The primary comparison for the enteropathy substudy will be between HIV-positive |
| 19 20 21 | 9 | children with SAM (group A) and HIV-negative children with SAM (group C), stratified by |
| 21 22 23 | 10 | presence or absence of oedema. Control groups (B and D) are well-nourished children |
| 23 24 25 | 11 | with or without HIV, to provide normative data for biomarkers and to evaluate the impact |
| 26 27 | 12 | of SAM within each HIV group. Thus, biomarkers among HIV-positive children with SAM |
| 28 29 | 13 | will first be compared to HIV-negative children with SAM (to evaluate the impact of HIV) |
| 30 31 | 14 | and, second, to well-nourished HIV-positive children (to evaluate the impact of SAM). |
| 32 33 | 15 | Biomarkers among HIV-negative children with SAM will be compared to well-nourished |
| 34 35 | 16 | HIV-negative children. For each continuous outcome, simple descriptive analysis will be |
| 36 37 | 17 | used to compare groups during hospitalisation using t-tests on appropriately transformed |
| 38 39 | 18 | data. For any outcome with moderate (p<0.05) evidence of difference between either |
| 40 41 42 | 19 | group a regression model will be constructed including groups A, B, C, D to directly test |
| 42 43 44 | 20 | (using interactions) whether there is a synergistic effect of HIV-SAM versus HIV-negative |
| 45 46 | 21 | SAM versus HIV alone versus neither. These models will also be used to explore |
| 47 48 | 22 | whether there is any evidence for heterogeneity in effects between oedematous and |
| 49 50 | 23 | non-oedematous SAM. Associations between enrolment factors (e.g. intestinal |
| 51 52 | 24 | permeability and microbial translocation) will be explored using pairwise Spearman |
| 53 54 | 25 | correlations and principal components analysis. Mean changes at the follow-up time- |
| 55 56 | 26 | points in each group will be estimated, and groups compared (as above) using |
| 57 58 59 | | 30 |

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1 2

1 generalised estimating equations. For outcomes that differ across SAM groups over 2 time, multilevel models will be used to explore possible predictors from the other factors 3 measured. Time to nutritional recovery will be compared using Kaplan-Meier and log-4 rank tests, and Cox models to adjust for baseline differences between groups. 5 6 Microbiota and metabolomics substudy 7 The primary comparison will be between HIV-negative children with oedematous and 8 non-oedematous SAM, with a separate comparison between HIV-positive children with 9 SAM and HIV-negative children with SAM. Analyses will examine: (i) differences in 10 metagenomic/metabolomic variables between groups at each time-point; (ii) differences 11 in metagenomic/metabolomic variables within groups over time; (iii) correlations between 12 metagenomic and metabolomic variables; and (iv) correlations between 13 metagenomic/metabolomic variables and clinical outcomes. A systematic analysis will be 14 undertaken to reduce high-dimensional data, integrate the multi-omics datasets and 15 minimise false discovery. 16 17 Compositional metagenomic data will be compared between groups for indices of alpha 18 and beta diversity. Principal coordinate analysis and partial least squares discriminant 19 analysis will be performed on metabolomics data to identify overall differences between 20 groups. High-dimensional datasets will be reduced using random forest models to 21 identify taxa, microbial gene families and metabolites that most strongly contribute to 22 differences between groups, corrected by Benjamani-Hochburg false discovery rate 23 detection. Targeted analysis by gRT-PCR will validate differential abundance or

- 24 expression of candidate microbial genes. Longitudinal comparisons will be performed
- 25 within and between groups using multilevel simultaneous component analysis.
- 26 Orthogonal projections to latent structures models will integrate metabolomic and

| 1 | metagenomic data whilst linear regression, canonical correlation and hierarchal |
|----|---|
| 2 | clustering analysis will measure correlations between -omics datasets. Finally, ROC |
| 3 | analysis will identify the ability of different analytes to predict long-term nutritional and |
| 4 | clinical outcomes. |
| 5 | |
| 6 | Immunology substudy |
| 7 | Integrated profiles of innate immune cell function will be generated for each child using |
| 8 | principal components analysis followed by hierarchical clustering ^{65 66} . This data- |
| 9 | reduction method identifies whether absolute levels of specific markers or relative |
| 10 | differences between markers differentiate children into groups. The resulting innate |
| 11 | immune profiles will be compared between HIV-SAM, HIV-negative children with SAM |
| 12 | and well-nourished groups using univariable tests and multivariable analysis of variance |
| 13 | (MANOVA) of the principal components. |
| 14 | |
| 15 | To address the relationship between immune function and infections, regression |
| 16 | analyses will determine whether baseline innate immune profiles (or the individual |
| 17 | parameters defining them) are associated with the infectious morbidity or mortality |
| 18 | during hospitalisation, using logistic models for binary outcomes and linear models for |
| 19 | duration. Key clinical characteristics, including age, sex, oedema and baseline WHZ, will |
| 20 | be added to models to investigate their confounding effects. Multivariable stacked |
| 21 | regression methods will be used to compare the impact of different factors on severe |
| 22 | bacterial infections based on heterogeneity tests. |
| 23 | |
| 24 | To determine whether treatment for SAM restores innate immune cell antibacterial |
| 25 | function, mixed effects regression models will compare longitudinal changes in individual |
| 26 | immune parameters, and the principal components calculated from the weights identified |
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at baseline (which include well-nourished controls). Similarities and differences in
longitudinal immune profiles will be compared between groups using nonmetric multidimensional scaling^{65 67 68}. This approach will group children according to their composite
innate immune function, allowing the duration and variability of immune restoration to be
evaluated over the course of nutritional rehabilitation. Binary logistic regression will
determine whether innate immune profiles at discharge are associated with morbidity or
mortality during follow-up.

9 PATIENT AND PUBLIC INVOLVEMENT

Patients and their caregivers were not involved in the design of the study. During recruitment, all caregivers of children admitted to hospital were given information about the study; those whose children had severe acute malnutrition were approached to give written informed consent. A meeting to disseminate results of the study to participants and their caregivers will be held at the end of the study. An interactive game to engage caregivers in the science underlying malnutrition is being developed in collaboration with experts from the Centre of the Cell, a unique science education centre based at Queen Mary University of London (https://www.centreofthecell.org/).

20 SAFETY REPORTING

21 For all adverse events, the study team will assess expectedness and relatedness to

- 22 study activities. Since this is an observational study without interventions, we anticipate
- that the risk is minimal; however, serious adverse events will be reported to local ethical
- 24 review boards (Medical Research Council of Zimbabwe, and University of Zambia
- 25 Biomedical Research Ethics Committee) and the study sponsor (Queen Mary University
- 26 of London) according to their respective guidelines.

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| 2 3 4 | 1 | |
| 4 5 6 | 2 | DATA COLLECTION AND MONITORING |
| 7 8 | 3 | Clinical and demographic data are recorded on paper case report forms. All data are |
| 9 10 | 4 | checked for completeness and plausibility before data entry and problems flagged for |
| 11 12 | 5 | resolution by the clinical team. All data are double-entered onto a dedicated password- |
| 13 14 | 6 | protected online study database, and any discrepancies resolved. Study participants are |
| 15 16 | 7 | identified on electronic databases only by study numbers (assigned at enrolment); no |
| 17 18 19 | 8 | personal identifiers are entered. |
| 20 21 | 9 | |
| 22 23 | 10 | ETHICS AND DISSEMINATION |
| 24 25 | 11 | The study complies with the principles of the Declaration of Helsinki (2008) and is |
| 26 27 | 12 | conducted in compliance with the principles of Good Clinical Practice (GCP) and local |
| 28 29 | 13 | regulatory requirements in each country. Ethical approval was obtained from the |
| 30 31 | 14 | University of Zambia Biomedical Research Ethics Committee, the Joint Research Ethics |
| 32 33 | 15 | Committee of the University of Zimbabwe and the Medical Research Council of |
| 34 35 26 | 16 | Zimbabwe. The ethical review board of the Sponsor, Queen Mary University of London, |
| 36 37 38 | 17 | provided an advisory review of the study. Since this is an observational study, there is no |
| 39 40 | 18 | Data and Safety Monitoring Board. |
| 41 42 | 19 | |
| 43 44 | 20 | Results will be disseminated through conference abstracts and peer-reviewed publications |
| 45 46 | 21 | and discussed with relevant policymakers and programmers. Study findings will be |
| 47 48 | 22 | disseminated to families of participants at face-to-face meetings. |
| 49 50 | 23 | |
| 51 52 | 24 | TIME FRAME AND STUDY STATUS |
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Enrolment into the study began in July 2016 and is expected to end in March 2018. All
 participants will be followed for 48 weeks, with an expected study completion date of
 March 2019.

DISCUSSION

HOPE-SAM aims to document the short- and long-term clinical and nutritional outcomes of HIV-positive and HIV-negative children with SAM, and to identify the factors at presentation and at discharge from hospital that independently predict these outcomes. Mechanistic substudies aim to evaluate the contribution of enteropathy, microbiota, metabolome and innate immune cell function to these clinical outcomes. The prevalence of malnutrition in HIV-positive children is as high as 40% in some settings and the challenges of managing this population are well recognised⁶⁹. The WHO protocol on management of SAM aims to reduce case fatality below 10%, but rates as high as 35% are still reported among HIV-positive children⁵⁷⁰. No studies have systematically and longitudinally collected morbidity data in HIV-SAM, or documented repeat hospitalisations and mortality after discharge from hospital, particularly in the current era where ART is available upon diagnosis. HOPE-SAM will provide a unique opportunity to enrol and follow a cohort of children managed for SAM in three large hospitals across two sub-Saharan African countries at several time-points over a one-year period. Nested longitudinal laboratory substudies aim to better characterise the pathogenesis of SAM in HIV-positive and HIV-negative children, to determine whether pathogenic processes are normalised during nutritional rehabilitation and follow-up, and to identify potential mechanistic pathways. Our ultimate goal is to utilise the findings generated in this study to inform new intervention approaches that can be evaluated in clinical trials to improve outcomes among children with SAM.

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1 **AUTHOR CONTRIBUTIONS**

- 2 Designed study: MB-D, BA, CDB, RCR, BM, KC, CK, KCh, DN, PC, NC, FM, JW, ARM,
- 3 JS, ASW, KJN, PK, AJP
- 4 Sought funding: MB-D, BA, CDB, RCR, JHH, ARM, JS, ASW, KJN, PK, AJP
- 5 Undertaking study: BM, KC, CK, KCh, FM, DN, PC, NC, FM, IM, EB, KM, SM, TR
- 6 Study oversight: MB-D, BA, JHH, KJN, PK, AJP
- 7 Analysis: MB-D, BA, CDB, RCR, RN, JW, ARM, JS, ASW, KJN, PK, AJP
- 8 Wrote first draft of manuscript: MB-D, CDB, RCR, AJP
- 9 Critically revised manuscript: All

10

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- 16 Initiative at Queen Mary University of London.
- 17

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- 18 **COMPETING INTERESTS**
- 20/ 19 None of the authors have any competing interests to declare.

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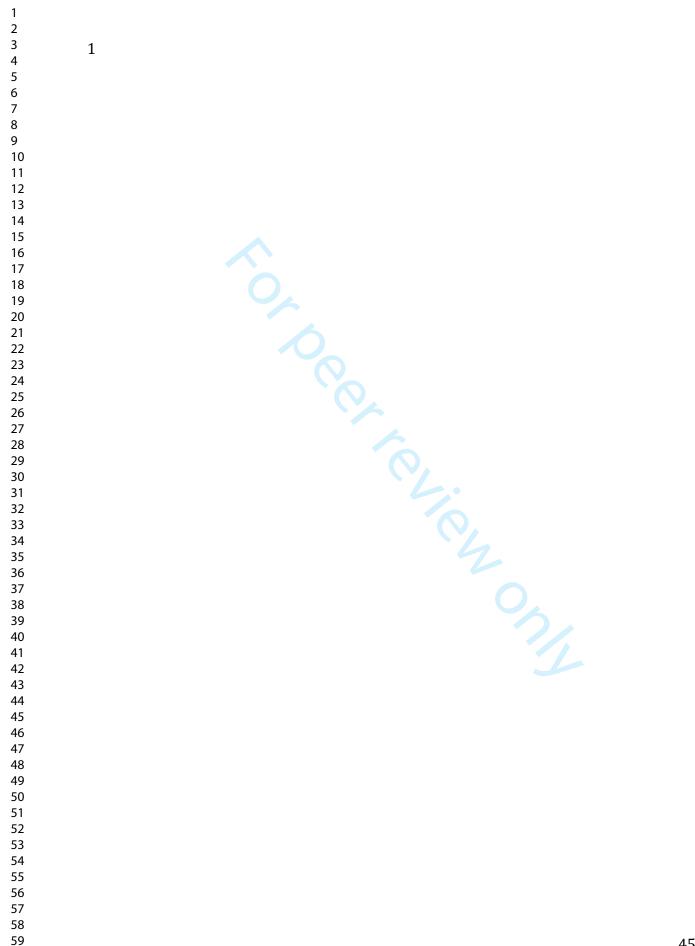
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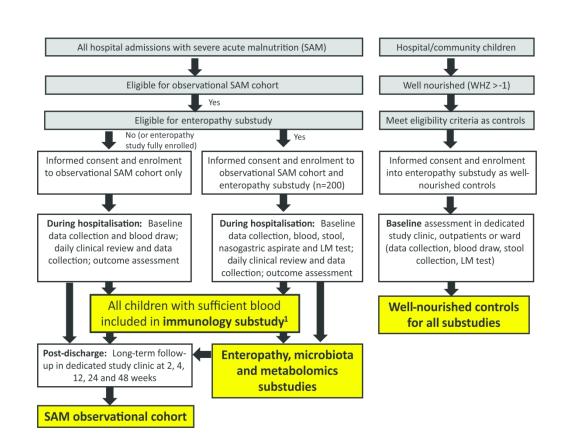
Figure 1: Study flow chart.

All hospital admissions are screened for eligibility for the observational cohort and enteropathy sub-study, with procedures undertaken as shown in the flow chart during hospitalisation and post-discharge. Well-nourished children from outpatient clinics and the community meeting eligibility criteria as well-nourished controls are enrolled and undergo a single baseline assessment as shown. The immunology, microbiota and metabolomics sub-studies enrol children as shown. All children with SAM, regardless of which arm of the study they are enrolled into, are followed for 48 weeks post-discharge. ¹The immunology substudy started from 1st June 2017 and required children to have a blood sample >2mL to conduct cellular assays.

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| Supplementary Table 1: Assays undertaken on stored sample | s for children in the observational | , inceh Cadin | କି କ୍ରିମt and immunology substudy. ବ୍ |

| Sample type | Assay (method) | Location of work | Study subjects | Baseline | Discharge | Week 12 | Week 24 | Week 48 |
|----------------|--|---|-----------------------|----------|-----------|---------|---|---------|
| Blood | HIV testing (rapid antibody test algorithm if >18 mo old or HIV DNA PCR <18 mo old) ¹ | TROPGAN, Zvitambo or clinical sites | All | Х | | | February 2019. Downloaded from http://bmjope Erasmushogeschool . ses related to text and data mining, Al training, X X X X X | |
| Blood | CD4 count (flow cytometry or PIMA) ¹ | TROPGAN, Zvitambo or clinical sites | All HIV- positive | X | X | X | ownloade geschoo t and dat X | X |
| Plasma | HIV viral load (real- time PCR) ¹ | TROPGAN, Zvitambo or clinical sites | All HIV- positive | x | X | X | baded from h hool . data mining, | X |
| Plasma | C-reactive protein (ELISA) | TROPGAN, Zvitambo | All | × | x | X | ttp://b Al tra X | X |
| Plasma | Albumin (ELISA) | TROPGAN, Zvitambo | All | X | X | X | Al training, and X X X | X |
| Plasma | Lipopolysaccharide (LAL assay) | TROPGAN and Zvitambo | Subgroup ³ | Х | X | X | n.br and | X |
| Plasma | Lipopolysaccharide binding protein (LBP) | TROPGAN and Zvitambo | Subgroup ³ | х | Х | X | | X |
| Plasma | sCD14 (ELISA) | TROPGAN and Zvitambo | Subgroup ³ | Х | Х | X | | X |
| Plasma | sCD163 (ELISA) | TROPGAN and Zvitambo | Subgroup ³ | Х | Х | X | on May 15, 20 technologies X X X | X |
| Plasma | IL-6, TNF-alpha, IL-1β (ELISA) and/or multiplex cytokines | TROPGAN and Zvitambo | Subgroup ³ | X | X | X |)25 at | X |
| Plasma | Total PAMP activity (THP1 reporter cell line ²) | TROPGAN and Zvitambo | Subgroup ³ | X | X | X | Department X | X |
| Whole | Molecular techniques | QMUL, London ² | Subgroup ³ | Х | Х | Х | GEZ-LTA | Х |

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| 2 3 4 5 6 7 8 | blood | for bacterial detection (broad-range and specific PCR and next-generation sequencing) | | | | | | | 8-023077 on 1 Fe including for use | | |
| 9 10 | Immuno | logy substudy only | | | | | | | abruar Er s rela | | |
| 10 11 12 13 14 15 | Whole blood | <i>In vitro</i> binding to bacterial products, cytokine expression and cellular responses to PAMP stimulation ⁴ | TROPGAN, Zvitambo | All | X | X | × | X | y 2019. Downlo rasmushogesc ted to text and | X | |
| 16 17 | Plasma | Co-culture with healthy immune cells ⁵ | QMUL, London | All | Х | Х | Х | Х | baded hool . data i | Х | |
| 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 | | expresses an NF-κB/AP-1 ³ Assays will be undertaken mortality. ⁴ Whole blood will be stimul and incubated for 1-24hr. 3 subsequent analysis of bac ⁵ To determine the effect of healthy immune cells, whic IFABP: Intestinal fatty acid CD163; PAMP: pathogen- | in a subgroup of child ated with pathogen-as Supernatant will be rer cterial binding, cellular the systemic milieu or h will be functionally a binding protein; ELISA | Iren, using a ssociated m moved and activation, n healthy im nalysed via A: Enzyme- | a case-control o olecular patterr stored at -80C proliferation an mune cell func multi-paramete linked immunos | or case-cohort de ns (PAMP) in cul for subsequent a d cytokine elabo tion, plasma san er flow cytometry corbent assay; G | esign to evaluate t ture plates and ba analysis of pro- an ration by flow cyto nples will be transp r in the Flow Cytor LP-2: glucagon-lik | the impact acterial anti- d anti-infla ometry. ported to t metry Cord ke peptide | tig biomi tig biomi a and set biomi tig and set | elled with fluore cytokines, and d Institute, QML 4: soluble CD14 | escent tags in test tubes I cells will be fixed for JL and co-cultured with 4; sCD163: soluble |
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| Supple Sample type | ementary table 2: Addition Assay (method) | al laboratory anal Location of work | yses for enterop Study groups ¹ | athy substue Baseline | dy Discharge | | Week 24 | Week 48 |
| Urine | Lactulose-mannitol ratio (mass spectrometry) | Orgeon Analytics, USA | A, B, C, D (all) | X | X | y 2019. D rasmushc nted to tex X | | X |
| Stool | Neopterin, myeloperoxidase, alpha-1 antitrypsin and REG-1B (ELISA) | TROPGAN and Zvitambo | A, B, C, D (all) | X | X | Fuary 2019. Downloaded for Erasmushogeschool . Welated to text and data m | X | X |
| Plasma | I-FABP (ELISA) | TROPGAN and Zvitambo | A, B, C, D (all) | Х | X | nining, | Х | Х |
| Plasma | GLP-2 | TROPGAN and Zvitambo | A, B, C, D (all) | Х | X | , Al tra | Х | Х |
| Plasma | Citrulline (mass spectrometry) | Imperial College London | A, B, C, D (all) | X | X | mjope ining, | X | Х |
| Plasma | Kynurenine:tryptophan ratio and metabolites along tryptophan pathway (mass spectrometry) | Imperial College London | A, B, C, D (all) | x | X | n.bmj.com/ on and similar teo X | X | X |
| Stool | Microbiome analysis ² | BCCDC, Vancouver | A, B, C, D (all) | Х | X | May 15, 202 hnologies. × | X | Х |
| Stool | Helicobacter pylori antigen | TROPGAN and Zvitambo | A, B, C, D (all) | Х | | May 15, 202 hnologies. | | |
| Gastric juice | Culture and molecular techniques for bacterial detection (broad-range and specific PCR and next-generation sequencing) | QMUL, London | Subgroup of A, C (n=50 per group) | X | | 5 at Department GEZ-LTA | | |

| Page 51 o | of 50 | | | BMJ | Open | | 36/bmjopen-2018-023077 d by copyright, including | | |
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| 2 3 4 5 6 7 | RNA extracted from PAXGene tubes | Gene expression analysis (RNASeq) | QMUL, London | A, B, C, D (all) | X | X | 018-023077 on 1 F nt, including for us | | |
| 8 9 10 11 | Plasma and urine | Targeted and untargeted metabolic phenotyping | Imperial College London | A, B, C, D (all) | X | X | ebruary 2 Erasr es related X | X | X |
| 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 | Not ² Fo qua | teropathy substudy groups: Gro dren with severe acute malnutr e that controls only have blood r microbiome analyses,total DN ntitative polymerase chain read UL: Queen Mary University of I | taken at baseline. IA and/or RNA will be ex ction (qPCR), then seque London; BCCDC: British | tracted from stool san | mples and used a genome shotgun Disease Control. | as template for sequencing | ided from <u>G</u> ittp://bmjopen.bmj.com/ on May 15, 2025 at Department GEZ-LTA ool . ata minin g , Al training, and similar technologies. ge ext or | n sequencing library | o C: HIV-negative preparation and for |
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