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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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3 **Health Outcomes, Pathogenesis and Epidemiology of Severe Acute**
4 **Malnutrition (HOPE-SAM): rationale and methods of a longitudinal**
5 **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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3 participant. Findings will be disseminated through peer-reviewed journals,
4 conference presentations and to caregivers at face-to-face meetings.
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8 **Strengths and limitations of this study**

9 **Strengths:**

- 10 • The primary strength of this study is the rigorous collection of longitudinal
11 data on morbidity, mortality and nutritional status during inpatient care and for
12 48 weeks after initial admission for SAM.
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- 14 • Laboratory sub-studies investigating enteropathy, microbiota, metabolomics
15 and immune cell function provide a unique opportunity to understand which
16 pathogenic pathways contribute to SAM and whether these processes
17 normalise with nutritional rehabilitation, capitalising on a well-characterised
18 cohort with appropriate controls.
- 19 • This study builds on existing studies of SAM and HIV-SAM prior to the
20 availability of antiretroviral therapy (ART) and will provide the first assessment
21 of longitudinal clinical outcomes in the current ART era.
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37 **Potential limitations:**

- 38 • High loss to follow-up due to participants returning to home settings following
39 hospital discharge. A dedicated clinical study team is in place to maximise
40 follow-up through phone reminders and community visits.
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- 43 • The clinical heterogeneity of the study participants, including comorbidities
44 such as stunting and co-infections, may make it challenging to identify the
45 specific causes of clinical outcomes. However, the embedded sub-studies will
46 enable multiple pathways to be explored within the same cohort.
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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, mid-upper 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. In-hospital 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 non-oedematous 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 Africa^{6,7}. 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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3 with HIV-SAM are more stunted and wasted; have a higher frequency of persistent
4 diarrhoea; tend to have delayed nutritional recovery and have a more complicated
5 clinical course than children with SAM¹¹.
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10 **Long-term outcomes of SAM**

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12 Following resolution of SAM complications and return of appetite, children are
13 discharged from hospital to continue therapeutic feeds at home. However, emerging
14 data indicate high post-discharge mortality following in-hospital management of
15 SAM¹²⁻¹⁴. Malnutrition together with young age, HIV infection and pneumonia have
16 been associated with higher post-discharge mortality¹⁵. One of the largest
17 prospective studies of growth and mortality in children with SAM (FuSAM),
18 conducted in Malawi from July 2006 to March 2007, collected 12-month outcome
19 data on 87% of 1024 children admitted to the nutrition ward¹². A total of 427 (42%)
20 died and 44% of these deaths occurred after discharge from hospital. Survival was
21 greatest among those who were nutritionally cured upon discharge from outpatient
22 therapeutic feeding centres, defined as two consecutive visits with >80% expected
23 weight-for-height, no oedema and clinically stable. The risk of mortality after hospital
24 discharge was four-fold higher for HIV-infected compared to HIV-uninfected children,
25 but the outcomes among HEU children were not reported. The loss to follow-up was
26 high in the FuSAM study because there was only one follow-up visit, one year after
27 discharge from outpatient-feeding centres.
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46 The impact of SAM appears to persist beyond the first year after discharge from
47 hospital. The ChroSAM study, which followed children with SAM seven years post-
48 discharge, showed that children had poorer growth, body composition and physical
49 function compared to siblings and community controls, which are all indicators of
50 future cardiovascular and metabolic disease¹³.
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3 While anthropometry is used to assess nutritional recovery following discharge from
4 hospital, the pattern and quality of growth recovery in SAM and HIV-SAM are poorly
5 understood. The observation that children treated for SAM have a deficit in lean
6 tissue despite regaining weight suggests that assessing body composition in addition
7 to anthropometry may help to identify children who have not completely recovered
8 and are at potential risk of long-term metabolic diseases¹³. Children with HIV-SAM
9 appear to have potential for catch-up growth since weight-for-age and/or weight-for-
10 height have been shown to normalise with treatment even prior to widespread
11 availability of ART¹⁶. However, the body composition of children with HIV-SAM
12 compared to SAM has not been described. Whether children with SAM (and in
13 particular HIV-SAM) recover fat mass at the expense of lean mass is unknown, but
14 differences in tissue accretion patterns may have implications for survival and long-
15 term metabolic health^{17 18}. There is also a need to consider the effect of SAM on the
16 size of body parts which grow at different rates: relatively shorter legs, for example,
17 are associated with epidemiologic risk of overweight, coronary artery disease, liver
18 dysfunction and diabetes^{19 20}.

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

29 30 31 **Pathogenesis of SAM and HIV-SAM**

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

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3 of children both during and after hospitalisation and identify new targets for
4 interventions to supplement existing treatment strategies. Consistent evidence that
5 immune mediators are altered in malnutrition²¹ and that systemic and intestinal
6 inflammation are associated with poor outcomes in SAM²², suggest that immune
7 dysfunction contributes to infectious susceptibility²³. Malnutrition is also characterised
8 by a complex derangement in gut microbial²⁴ metabolic,²⁵ immune²⁶ and hormonal
9 pathways, organ dysfunction and micronutrient deficiencies in the context of co-
10 infections, enteropathy and chronic inflammation. Several studies have recently
11 provided insights into these perturbations using new tools^{25 27-29}, including
12 metabolomics and metagenomics, but we still lack a clear understanding of many of
13 the pathogenic pathways driving malnutrition, the interactions between these
14 pathways, and which are the most tractable targets for intervention.
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28 SAM shares several pathological and clinical features with HIV, which may explain
29 clinical outcomes in these co-occurring conditions: 1) both are characterised by
30 intestinal damage, leading to impairment of the mucosal barrier and increased
31 intestinal permeability; 2) both have underlying systemic immune activation; and 3)
32 both are frequently complicated by persistent diarrhoea, pneumonia and sepsis that
33 may plausibly arise due to loss of intestinal barrier function³⁰. Understanding the
34 overlapping impact of HIV and SAM is critical to inform additional interventions to
35 improve outcomes of children with HIV-SAM.
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46 **OBJECTIVES OF HOPE-SAM**

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48 The Health Outcomes, Pathogenesis and Epidemiology of Severe Acute Malnutrition
49 (HOPE-SAM) study has two primary objectives:
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- 51 1) To describe the short- and long-term clinical outcomes of children with
52 complicated SAM and HIV-SAM, and to identify the risk factors at admission
53 and discharge from hospital that independently predict these outcomes.
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3 2) To better characterise the pathogenesis of SAM and HIV-SAM, through
4 nested laboratory sub-studies evaluating enteropathy, gut microbiota,
5 metabolomics and immune cell function.
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10 **STUDY DESIGN**

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

51 *Screening:* Caregivers of all hospitalised children are sensitised about the study. All
52 new admissions aged 0-59 months are screened for SAM, which is defined
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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), well-nourished (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 malnutrition ¹		Well nourished WHZ>-1
	Oedematous ²	Non-oedematous	
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.

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 used)	Hospitalization		Post-discharge ³				
	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 (HOPE-SAM Form_5_Baseline Form)	x						
Daily clinical review ⁵ (HOPE-SAM Form_7_Daily Follow-up Form)	Daily during hospitalisation						
Blood collection ⁶	x	x			x	x	x
HIV testing ⁷	x						
CD4 count and viral load (HIV-infected children only)	x				x	x	x
Full blood count ⁸	x	x			x	x	x
Anthropometry	x	x	x	x	x	x	x
Skinfold thickness ⁹		x	x	x	x	x	x
Body composition ¹⁰	x	x	x	x	x	x	x
Discharge data collection (HOPE-SAM Form_8_Discharge Form)		x					
Daily morbidity diary (HOPE-SAM Form_10_Morbidity Diary)			Daily during follow-up period by caregivers				
Follow-up clinic: history, examination, morbidity and mortality data (HOPE-SAM Form_11_Clinical Follow-up Form and HOPE-SAM Form_12_Nurse Follow-up Form)			x	x	x	x	x

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 (≥ 2 mL 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.

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.

¹⁰Body composition will be assessed by bioimpedance vector analysis.

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., Crymich, 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 aliquots 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

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 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 infections, 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-

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**.

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

Assessment (Form used)	Hospitalization		Post-discharge ³				
	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						

Admission Proforma)							
Baseline data (HOPE-SAM Form_5_Baseline Form)	x						
Daily clinical review ⁵ (HOPE-SAM Form_7_Daily Follow- up Form)	Daily during hospitalisation						
Blood collection ⁶	x	x			x	x	x
HIV testing ⁷	x						
CD4 count and viral load (HIV-infected children only)	x				x	x	x
Full blood count ⁸	x	x			x	x	x
Gastric aspirate ⁹	x						
Stool collection ¹⁰	x	x			x	x	x
Lactulose-mannitol testing ¹¹	x	x			x		x
Anthropometry	x	x	x	x	x	x	x
Skinfold thickness ¹²		x	x	x	x	x	x
Body composition ¹³	x	x	x	x	x	x	x
Discharge data collection (HOPE-SAM Form_8_Discharge Form)		x					
Daily morbidity diary (HOPE-SAM Form_10_Morbidity Diary)			Daily during follow-up period by caregivers				
Follow-up clinic: history, examination, morbidity and mortality data (HOPE-SAM Form_11_Clinical Follow-up Form and HOPE-SAM Form_12_Nurse Follow-up Form)			x	x	x	x	x

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.

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

⁹A 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 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.

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.

¹³Body 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.

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 cycle 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.

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

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30 Normal assembly of the gut microbiota in early life is critical for many aspects of
31 physiological, neurological and immune development³⁸. Recent evidence suggests
32 that an immature or pathogenic microbiota plays a causative role in the pathogenesis
33 of SAM²⁷. For example, a number of microbial taxa have been identified, including
34 *Faecalibacterium prausnitzii*, which discriminate and predict gut microbiota maturity
35 and child growth³⁹. Other pathogenic microorganisms, including IgA-targeted
36 *Enterobacteriaceae*, are associated with impaired growth and may contribute to
37 SAM⁴⁰. Nutritional rehabilitation with RUTF induces temporary recovery of a
38 disturbed microbiota; however, the microbiota appears to revert back to an immature
39 diseased state following nutritional recovery⁴¹. HIV infection is also associated with a
40 disturbed gut microbiota⁴², which may further compound enteropathy phenotypes.
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42 Furthermore, there is some evidence that differences exist in malnutrition
43 enteropathy between oedematous and non-oedematous SAM⁴³; however, few
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3 studies have investigated differences in the gut microbiota between the two forms of
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5 the disease.
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9 We hypothesize that (i) a unique gut microbial signature exists in HIV-SAM,
10 compared with SAM, that is independently associated with morbidity, mortality,
11 nutritional recovery and degree of enteropathy during hospitalisation; (ii) a unique gut
12 microbial signature exists in oedematous compared with non-oedematous SAM; (iii)
13 specific microorganisms or gut microbial diversity indices are independently
14 associated with morbidity, mortality, nutritional recovery and degree of enteropathy
15 during hospitalisation; and (iv) the gut microbiota is partially restored to a healthy
16 state with nutritional rehabilitation but reverts to a dysbiotic state during follow-up,
17 which predicts morbidity, mortality and relapse of SAM.
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28 Using stored stool samples collected at baseline, a cross-sectional investigation will
29 determine differences in the gut microbial composition and predicted function
30 between SAM versus HIV-SAM, oedematous versus non-oedematous SAM, and
31 healthy controls. Gut microbial composition and predicted function will be compared
32 between groups at discharge and at 12, 24 and 48 weeks post-discharge. Briefly,
33 total DNA and/or RNA will be extracted from stool samples and used as template for
34 next-generation sequencing library preparation and for quantitative polymerase chain
35 reaction (qPCR). Whole metagenome shotgun sequencing will be performed using
36 the HiSeq 2500 system. Raw metagenomic sequencing data will be quality-filtered
37 and analysed through a well-validated bioinformatics pipeline using MetaPhlAn⁴⁴ and
38 HUMAnN⁴⁵. The compositional and predicted functional metagenomic data
39 generated will be used to identify signatures of SAM and to investigate associative
40 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 cross-sectional 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

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

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18 Bacterial infections are common among children hospitalised for SAM⁵⁰⁻⁵³ and
19 mortality is driven by a range of species^{51 53-56}, consistent with generalised defects in
20 innate anti-bacterial defence. Increased infectious morbidity and mortality persist
21 after discharge from hospital⁵⁷⁻⁵⁹, suggesting that restoration of anti-bacterial immune
22 responses may lag behind nutritional rehabilitation. A recent randomised trial in
23 children with SAM confirmed that deaths following hospitalisation were predominantly
24 due to bacterial infections but were not prevented by daily co-trimoxazole
25 prophylaxis⁵⁹. Collectively, these observations highlight that children remain
26 vulnerable to infection despite current treatment approaches; targeting persistent
27 immune dysfunction could plausibly reduce infectious mortality after discharge⁶⁰.
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40 Multiple innate and adaptive immune mediators are dysregulated in malnutrition⁶⁰⁻⁶².
41 However, few studies have assessed cellular immune function in malnourished
42 children; most existing studies were undertaken decades ago on small cross-
43 sectional cohorts without the benefit of recent advances in immunology techniques⁶¹.
44 Immune dysfunction in SAM likely reflects both *intrinsic* defects, whereby immune
45 cells lack capacity to adequately respond to infection, and *extrinsic* defects, where
46 cells have intact anti-bacterial capacity but are chronically modulated by the systemic
47 pro-inflammatory environment which characterises SAM (i.e. heightened pro-
48 inflammatory cytokines^{50 63} and circulating bacterial antigens^{64 65}). Systemic
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3 inflammation is directly associated with mortality in SAM⁵⁰ and driven by multiple
4 comorbidities, including bacterial translocation from the damaged gut into the blood,
5 sub-clinical infections and metabolic dysregulation⁶³⁻⁶⁶⁻⁶⁸. The implications of innate
6 immune cell dysfunction for subsequent acquisition of infections and infectious
7 mortality have not been investigated.
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14 We hypothesise that: (i) anti-bacterial functions of innate immune cells are
15 compromised in SAM due to a combination of intrinsic and extrinsic defects; ii) innate
16 immune cell function is independently associated with infectious morbidity and
17 mortality during hospitalisation for SAM; and iii) nutritional rehabilitation only partly
18 restores innate immune cell function, leading to an ongoing risk of bacterial infections
19 post-discharge.
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28 Using blood samples collected at baseline, discharge and 12, 24 and 48 weeks post-
29 discharge, the longitudinal relationship between circulating innate immune cell
30 function and bacterial infections will be assessed. The intrinsic phagocytic capacity,
31 secreted cytokine response and maturation state of innate immune cells after culture
32 with bacterial antigens will be assessed. Plasma concentrations of endotoxin and
33 pro-inflammatory cytokines will be quantified at each time-point and the degree to
34 which these extrinsic factors influence innate immune cell antibacterial function will
35 be assessed via plasma co-culture with innate immune cells from healthy donors.
36 Bacterial infections during hospitalisation will be diagnosed using clinical criteria and
37 blood culture, stool culture and urinalysis where available.
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50 **SAMPLE SIZE**

51 *Observational study:* The observational cohort will recruit as many children with SAM
52 as possible during the period of enrolment (July 2016 to March 2018), estimated at
53 600-800 children, to provide a robust assessment of outcomes among children
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3 hospitalised with SAM. Assuming 15% mortality and 15% loss to follow-up, there
4 would be at least 420 evaluable children post-discharge, of whom up to 168 would
5 have HIV-SAM, based on an estimated inpatient HIV prevalence of 40%.
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10 *Enteropathy substudy:* The sample size was estimated using previously reported
11 values for LM ratios, which remain a widely used non-invasive marker of
12 enteropathy. Comparing 100 versus 100 children with two-sided $\alpha=0.025$ (to
13 allow for two primary comparisons, i.e. HIV-SAM versus SAM, and HIV-SAM versus
14 HIV) provides >80% power to detect differences in mean LM ratio during
15 hospitalisation of at least 0.16 (assuming $SD=0.36$), a difference which would be
16 clinically relevant given the LM ratios previously reported for well-nourished children
17 (0.42), malnourished children (1.3) and children with persistent diarrhoea (2.85) in
18 the Gambia⁶⁹. It also provides >80% power to detect differences of at least 0.1 in the
19 mean change in LM ratio from enrolment (assuming SD for change= 0.23 and 7%
20 missing samples). For inflammatory markers, comparing 100 versus 100 children
21 with two-sided $\alpha=0.025$ provides >80% power to detect differences in mean \log_{10}
22 concentrations of at least 0.44 times their standard deviation, or 2.75-fold differences
23 between groups. Inclusion of healthy controls provides an indication of normal
24 ranges in young African children. SAM groups will be stratified to include
25 approximately 50 children with and without oedematous malnutrition, if possible.
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44 *Microbiota and metabolomics substudy*

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46 Power calculations are difficult in metagenomics and metabolomic analyses due to
47 the large number of observed outcomes and unknown effect sizes and variance.
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49 Previous studies using smaller sample sizes have identified significant taxonomic
50 differences in twin pairs discordant for oedematous-SAM ($n=13$)²⁴ and metabolic
51 differences between the two forms of SAM ($n=40$)⁷⁰. These studies suggest that a
52 difference of 50% in metabolites could be expected. Using ANCOVA, setting $\alpha=0.05$
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3 and assuming either low ($\rho=0.1$) or high ($\rho=0.7$) correlation, the study would require
4 95-126 subjects to achieve 80% power⁷¹. False discovery rate (FDR) multiple
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6 correction testing will be applied to reduce the high-dimensionality of the data and
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8 limit false-positives.
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10 11 12 *Immunology substudy*

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14 Up to 200 children with SAM and 200 healthy controls will be included in a cross-
15
16 sectional analysis of innate immune cell function during hospitalisation. Assuming
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18 similar infectious mortality to a recent Kenyan study (15%)⁵⁹, a cohort of 200 provides
19
20 80% power to detect associations between immune profiles and infectious mortality
21
22 at an odds ratio of 1.7 and 2-sided alpha of 0.05. We will aim for 100 children with
23
24 longitudinal analysis of innate immune cell function at discharge, 12, 24 and 48
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26 weeks post-discharge⁵⁹.
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30 31 **ANALYSIS**

32 *Observational Cohort*

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34 The primary comparison will be the clinical and nutritional outcomes of children with
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36 SAM compared to HIV-SAM. We will review all deaths and adjudicate clinical
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38 diagnoses and causes of death to ensure robust and consistent data across sites.
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40 We will compare each participant's clinical management to WHO guidelines to
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42 identify any contributory factors in hospital care. Factors associated with outcomes
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44 during hospitalisation (e.g. mortality, nutritional recovery) will be determined for each
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46 group using multivariate analysis (Cox models for time-to-event data, linear models
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48 for continuous outcomes). Factors associated with outcomes over 48 weeks post-
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50 discharge (hospital re-admission, morbidity and mortality, relapse, anthropometry,
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52 body composition and response to ART) will be determined for each group using
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54 multivariate analysis (Cox models for time-to-event data, linear models for
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56 continuous outcomes). We will evaluate the ability of MUAC at discharge to predict
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3 long-term outcomes using receiver-operator-characteristic (ROC) analysis, in the
4 whole cohort and within the HIV-SAM and SAM subgroups. We will then evaluate
5 whether addition of other variables improves the predictive capacity of MUAC for
6 each group, including body composition, haemoglobin, albumin and CRP, plus
7 CD4%, viral load and timing of ART initiation (HIV-SAM only). We will construct
8 multivariable models and compare them with MUAC alone using the net-
9 reclassification index.
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16 17 18 *Body composition analysis*

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20 Previous work in body composition by bio-electrical impedance in Ethiopian infants
21 and children with SAM has shown that the conventional approach, predicting total
22 body weight from height-adjusted impedance, fails due to confounding by oedema⁷².
23
24 The same project validated an alternative approach, known as Bio-electrical
25 Impedance Vector Analysis (BIVA), and described significant differences between
26 each of three groups: healthy controls, oedematous-SAM and non-oedematous
27 SAM. Vector analysis splits impedance into two height-adjusted components,
28 resistance and reactance, which are further linked through phase angle (PA).
29
30 Variability in these components is associated with biochemical parameters⁷³. These
31 variables will be explored using graphical analysis, or transformed into age- and sex-
32 adjusted z-scores for statistical comparison, including longitudinal analyses. Higher
33 phase angle indicates better nutritional status, while declining height-adjusted
34 resistance over time indicates loss of oedema.
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48 *Enteropathy substudy*

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50 The primary comparison for the enteropathy substudy will be between children with
51 HIV-SAM (group A) and SAM (group C), stratified by presence or absence of
52 oedema. Control groups (B and D) are well-nourished children with or without HIV, to
53 provide normative data for biomarkers. For each continuous outcome, simple
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3 descriptive analysis will be used to compare groups (HIV-SAM versus HIV, and HIV-
4 SAM versus SAM) during hospitalisation using t-tests on appropriately transformed
5 data. For any outcome with moderate ($p < 0.05$) evidence of difference between either
6 group a regression model will be constructed including groups A, B, C, D to directly
7 test (using interactions) whether there is a synergistic effect of HIV-SAM versus SAM
8 versus HIV versus neither. These models will also be used to explore whether there
9 is any evidence for heterogeneity in effects between oedematous and non-
10 oedematous SAM. Associations between enrolment factors (e.g. intestinal
11 permeability and microbial translocation) will be explored using pairwise Spearman
12 correlations and principal components analysis. Mean changes at the follow-up time-
13 points in each group will be estimated, and groups compared (as above) using
14 generalised estimating equations. For outcomes that differ across SAM groups over
15 time, multilevel models will be used to explore possible predictors from the other
16 factors measured. Time to nutritional recovery will be compared using Kaplan-Meier
17 and log-rank tests.

34 *Microbiota and metabolomics substudy*

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

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3 Compositional metagenomic data will be compared between groups for indices of
4 alpha and beta diversity. Principal coordinate analysis and partial least squares
5 discriminant analysis will be performed on metabolomics data to identify overall
6 differences between groups. High-dimensional datasets will be reduced using
7 random forest models to identify taxa, microbial gene families and metabolites that
8 most strongly contribute to differences between groups, corrected by Benjamani-
9 Hochburg false discovery rate detection. Targeted analysis by qRT-PCR will validate
10 differential abundance or expression of candidate microbial genes. Longitudinal
11 comparisons will be performed within and between groups using multilevel
12 simultaneous component analysis. Orthogonal projections to latent structures models
13 will integrate metabolomic and metagenomic data whilst linear regression, canonical
14 correlation and hierarchal clustering analysis will measure correlations between -
15 omics datasets. Finally, ROC analysis will identify the ability of different analytes to
16 predict long-term nutritional and clinical outcomes.
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31 32 *Immunology substudy* 33

34 Integrated profiles of innate immune cell function will be generated for each child
35 using principal components analysis followed by hierarchical clustering^{74 75}. This
36 data-reduction method identifies whether absolute levels of specific markers or
37 relative differences between markers differentiate children into groups. The resulting
38 innate immune profiles will be compared between SAM, HIV-SAM and well-
39 nourished groups using univariate tests and multivariate analysis of variance
40 (MANOVA) of the principal components.
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50 To address the relationship between immune function and infections, regression
51 analyses will determine whether baseline innate immune profiles (or the individual
52 parameters defining them) are associated with the infectious morbidity or mortality
53 during hospitalisation, using logistic models for binary outcomes and linear models
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3 for duration. Key clinical characteristics, including age, sex, oedema and baseline
4 WHZ, will be added to models to investigate their confounding effects. Multivariate
5 stacked regression methods will be used to compare the impact of different factors
6 on severe bacterial infections based on heterogeneity tests.
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12 To determine whether treatment for SAM restores innate immune cell antibacterial
13 function, mixed effects regression models will compare longitudinal changes in
14 individual immune parameters, and the principal components calculated from the
15 weights identified at baseline (which include healthy controls). Similarities and
16 differences in longitudinal immune profiles will be compared between groups using
17 nonmetric multi-dimensional scaling^{74 76 77}. This approach will group children
18 according to their composite innate immune function, allowing the duration and
19 variability of immune restoration to be evaluated over the course of nutritional
20 rehabilitation. Binary logistic regression will determine whether innate immune
21 profiles at discharge are associated with morbidity or mortality during follow-up.
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34 **PATIENT AND PUBLIC INVOLVEMENT**

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

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3 Results will be disseminated through conference abstracts and peer-reviewed
4 publications and discussed with relevant policymakers and programmers. Study
5 findings will be disseminated to families of participants at face-to-face meetings.
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10 **TIME FRAME AND STUDY STATUS**

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

21 HOPE-SAM aims to document the short- and long-term outcomes of children with
22 SAM and HIV-SAM, and to identify the factors at presentation and at discharge from
23 hospital that independently predict these outcomes. Mechanistic substudies aim to
24 evaluate the contribution of enteropathy, microbiota, metabolome and innate immune
25 cell function to these clinical outcomes. The prevalence of malnutrition in HIV-
26 infected children is as high as 40% in some settings and the challenges of managing
27 this population are well recognised⁷⁸. The WHO protocol on management of SAM
28 aims to reduce case fatality below 10%, but rates as high as 35% are still reported
29 among HIV-infected children^{79 80}. No studies have systematically and longitudinally
30 collected morbidity data in HIV-SAM, or documented repeat hospitalisations and
31 mortality after discharge from hospital, particularly in the current era where ART is
32 available upon diagnosis. HOPE-SAM will provide a unique opportunity to enrol and
33 follow a cohort of children managed for SAM in three large hospitals across two sub-
34 Saharan African countries at several time-points over a one-year period. Nested
35 longitudinal laboratory substudies aim to better characterise the pathogenesis of
36 SAM and HIV-SAM, to determine whether pathogenic processes are normalised
37 during nutritional rehabilitation and follow-up, and to identify potential mechanistic
38 pathways for new intervention approaches.
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None of the authors have any competing interests to declare.

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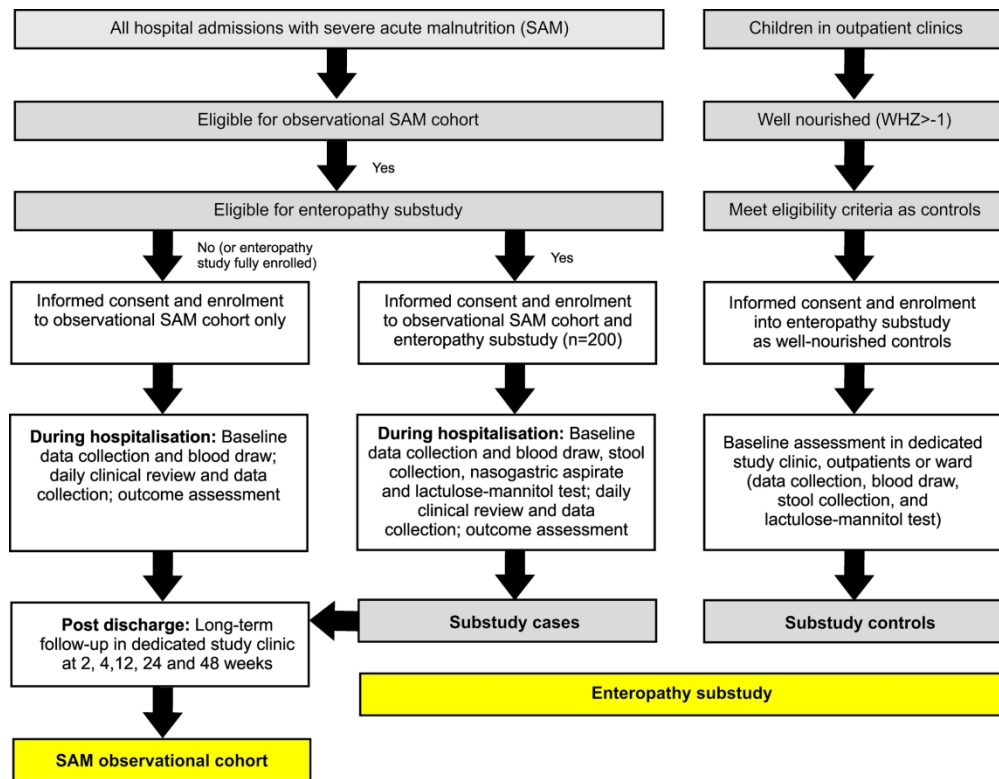
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FIGURE LEGENDS

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.

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197x152mm (300 x 300 DPI)

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
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	Baseline
Blood	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, 48
Plasma	Albumin (ELISA)	TROPGAN, Zvitambo	All	Baseline, discharge, wk 12, 24, 48
Plasma	Lipopolysaccharide (LAL assay)	TROPGAN and Zvitambo	Subgroup ³	Baseline, discharge, wk 12, 24, 48
Plasma	Lipopolysaccharide binding protein (LBP)	TROPGAN and Zvitambo	Subgroup ³	Baseline, discharge, wk 12, 24, 48
Plasma	sCD14 (ELISA)	TROPGAN and Zvitambo	Subgroup ³	Baseline, discharge, wk 12, 24, 48
Plasma	sCD163 (ELISA)	TROPGAN and Zvitambo	Subgroup ³	Baseline, discharge, wk 12, 24, 48
Plasma	IL-6, TNF-alpha, IL-1 β (ELISA) and/or multiplex cytokines	TROPGAN and Zvitambo	Subgroup ³	Baseline, discharge, wk 12, 24, 48
Plasma	Total PAMP activity (THP1 reporter cell line ²)	TROPGAN and Zvitambo	Subgroup ³	Baseline, discharge, wk 12, 24, 48
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, 48
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, 48
Plasma	Co-culture with healthy immune cells ⁵	Blizard Institute	All	Baseline, discharge, wk 12, 24, 48

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

5 ²THP1 reporter cells are derived from THP1, a human monocytic cell line that naturally expresses
6 many pattern recognition receptors (PRR). The cell line stably expresses an NF-κB/AP-1
7 inducible reporter (SEAP) system to facilitate the monitoring of PRR-induced NF-κB/AP-1
8 activation.

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

11 ⁴Whole blood will be stimulated with pathogen-associated molecular patterns (PAMP) in culture
12 plates and bacterial antigens labelled with fluorescent tags in test tubes, and incubated for 1-
13 24hr. Supernatant will be removed and stored at -80C for subsequent analysis of pro- and anti-
14 inflammatory cytokines, and cells will be fixed as described in section 12.2.2 for subsequent
15 analysis of bacterial binding, cellular activation, proliferation and cytokine elaboration by flow
16 cytometry.

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

20 IFABP: Intestinal fatty acid binding protein; ELISA: Enzyme-linked immunosorbent assay; GLP-2:
21 glucagon-like peptide 2; sCD14: soluble CD14; sCD163: soluble CD163; PAMP: pathogen-
22 associated molecular pattern; QMUL: Queen Mary University of London; CRP: C-reactive protein;
23 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	A, 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	<i>Helicobacter pylori</i> 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

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

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

8 ³For microbiome analyses, total DNA and/or RNA will be extracted from stool samples and used
9 as template for next generation sequencing library preparation and for quantitative polymerase
10 chain reaction (qPCR), then sequenced via whole metagenome shotgun sequencing (see section
11 12.3.3.2).

12 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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Primary Subject Heading:	Global health
Secondary Subject Heading:	Epidemiology, Nutrition and metabolism, Paediatrics, Immunology (including allergy)
Keywords:	Malnutrition, HIV, Africa, mortality, microbiota, enteropathy



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3 **1 Health Outcomes, Pathogenesis and Epidemiology of Severe Acute**
4 **2 Malnutrition (HOPE-SAM): rationale and methods of a longitudinal**
5 **3 observational study**
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8 Key words: Malnutrition, HIV, Africa, mortality, microbiota, enteropathy, immunology

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1 **ABSTRACT**

2 **Introduction**

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

10 **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.

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

1 participant. Findings will be disseminated through peer-reviewed journals,
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 clinical outcomes. However, the embedded sub-studies will enable multiple
24 pathways to be explored within the same cohort.
- 25 • Potential bias in recruiting well-nourished controls only from hospitals will be
26 reduced by inclusion of community-based controls, including well-nourished
27 siblings of children with SAM.

1 INTRODUCTION

2 Malnutrition underlies almost half of all childhood deaths in developing countries¹.
3 Severe acute malnutrition (SAM) is defined by a weight-for-height Z-score <-3, mid-
4 upper arm circumference (MUAC) <115mm and/or bilateral pitting oedema². Current
5 treatment guidelines distinguish two groups: i) children with uncomplicated SAM who
6 can be managed in the community; and ii) children with complicated SAM, who are
7 hospitalised and undergo resuscitation, stabilisation and nutritional rehabilitation. In-
8 hospital mortality in children with complicated SAM remains high despite the
9 implementation of WHO guidelines³. Furthermore, SAM presents as two major
10 clinical phenotypes: non-oedematous SAM (marasmus), characterised by severe
11 wasting, and oedematous SAM (kwashiorkor), a more complex syndrome
12 characterised by bilateral pitting oedema, steatosis and diarrhea^{4,5}. Despite differing
13 clinical outcomes, treatment protocols are the same for both oedematous and non-
14 oedematous SAM.
15
16 A contributory factor to high in-patient mortality is the co-occurrence of HIV infection
17 in around one-third of children hospitalised for SAM in sub-Saharan Africa^{6,7}. While
18 new HIV infections in children have declined⁸, a substantial number of infected
19 children are diagnosed late and present with malnutrition. There is also a growing
20 population of HIV-exposed uninfected (HEU) children who have immune
21 abnormalities, poor growth and higher risk of mortality and infectious morbidity⁹.
22 Hence, HIV has transformed the epidemiology and outcomes of SAM¹⁰. Even with
23 standardised treatment approaches, inpatient deaths are almost four-fold higher
24 among HIV-positive children with SAM (herein termed HIV-SAM), compared to HIV-
25 negative children with SAM (30.4% vs 8.4%), for reasons that remain unclear¹⁰; this
26 mortality is three-fold higher than would be expected from anthropometric
27 parameters alone¹⁰. Management of HIV-SAM is particularly challenging because
28 HIV fundamentally alters the clinical presentation of malnutrition and the response to

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 SAM¹⁰.

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 SAM¹¹⁻¹³.
9 Malnutrition together with young age, HIV infection and pneumonia have been
10 associated with higher post-discharge mortality¹⁴. 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 ward¹¹. 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 infection¹⁵.

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

1 future cardiovascular and metabolic disease¹².

2

3 While anthropometry is used to assess nutritional recovery after discharge from
4 hospital, the pattern and quality of growth recovery following SAM is poorly
5 understood. The observation that children treated for SAM have a deficit in lean
6 tissue despite regaining weight suggests that assessing body composition in addition
7 to anthropometry may help to identify children who have not completely recovered
8 and are at potential risk of long-term metabolic diseases¹². Children with HIV-SAM
9 appear to have potential for catch-up growth in weight-for-age and/or weight-for-
10 height, which have been shown to normalise with treatment even prior to widespread
11 availability of ART¹⁶; by contrast, height-for-age shows less potential for catch-up
12 growth¹⁷. However, the body composition of children with HIV-SAM compared to HIV-
13 negative children with SAM has not been described. Whether children recover fat
14 mass at the expense of lean mass is unknown, but differences in tissue accretion
15 patterns may have implications for survival and long-term metabolic health^{18 19}. There
16 is also a need to consider the effect of SAM on the size of body parts which grow at
17 different rates: relatively shorter legs, for example, are associated with epidemiologic
18 risk of overweight, coronary artery disease, liver dysfunction and diabetes^{20 21}.

19

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

28

1 Pathogenesis of SAM

2 Better understanding the pathogenesis of SAM may help to explain the high mortality
3 of children both during and after hospitalisation and identify new targets for
4 interventions to supplement existing treatment strategies. Consistent evidence that
5 immune mediators are altered in malnutrition²² and that systemic and intestinal
6 inflammation are associated with poor outcomes in SAM²³, suggest that immune
7 dysfunction contributes to infectious susceptibility²⁴. Malnutrition is also characterised
8 by a complex derangement in gut microbial²⁵ metabolic,²⁶ immune²⁷ and hormonal
9 pathways, organ dysfunction and micronutrient deficiencies in the context of co-
10 infections, enteropathy and chronic inflammation. Several studies have recently
11 provided insights into these perturbations using new tools^{25 26 28 29}, including
12 metabolomics and metagenomics, but we still lack a clear understanding of many of
13 the pathogenic pathways driving malnutrition, the interactions between these
14 pathways, and which are the most tractable targets for intervention.

15
16 SAM shares several pathological and clinical features with HIV, which may explain
17 clinical outcomes in these co-occurring conditions: 1) both are characterised by
18 intestinal damage, leading to impairment of the mucosal barrier and increased
19 intestinal permeability; 2) both have underlying systemic immune activation; and 3)
20 both are frequently complicated by persistent diarrhoea, pneumonia and sepsis that
21 may plausibly arise due to loss of intestinal barrier function³⁰. Understanding the
22 overlapping impact of HIV and SAM is critical to inform additional interventions to
23 improve outcomes of children with HIV-SAM.

24 OBJECTIVES OF HOPE-SAM

25 The Health Outcomes, Pathogenesis and Epidemiology of Severe Acute Malnutrition
26 (HOPE-SAM) study has two primary objectives:
27

- 1) 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.
- 2) To better characterise the pathogenesis of 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 complicated 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-positive and HIV-negative children will be enrolled. Throughout this paper, 'SAM' refers to all children, regardless of HIV status; where analyses specifically compare children by HIV status, groups are identified as HIV-positive children with SAM (or HIV-SAM) and HIV-negative children with SAM. All participants with SAM are followed for 48 weeks post-discharge, with longitudinal data collection and blood sampling. The study contains four nested sub-studies as shown in **Figure 1**. A subgroup of 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); these children are also included in microbiota and metabolomics substudies. Children with SAM for whom blood samples are available are included in the immunology substudy, for which circulating inflammatory mediators will be assayed; functional cellular immunology assays will be conducted for all children in the immunology sub-study with sufficient sample volume (> 2mL) recruited after June

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3 1 2017. A group of healthy children recruited from the same hospitals and
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5 2 communities, who are well-nourished and matched to children in the enteropathy
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7 3 substudy by age and HIV status, will have data and specimens collected to provide
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9 4 normative data for the laboratory substudies; these well-nourished controls will not
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11 5 be followed longitudinally.
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15 7 The study protocol, data collection forms and standard operating procedures are
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17 8 available online at osf.io/29uaw.
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10 **RECRUITMENT**

11 *Screening:* Caregivers of all hospitalised children are sensitised about the study. All
12
13 12 new admissions aged 0-59 months are screened for SAM, which is defined
14
15 13 according to WHO criteria as any of: weight-for-height Z-score (WHZ) <-3, MUAC
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17 14 <115 mm (if aged 6-59mo) and/or bilateral pitting oedema. All children with SAM are
18
19 15 recruited from hospital and this study therefore focuses on complicated SAM;
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21 16 children with uncomplicated SAM will not be enrolled.
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25 18 *Eligibility for observational cohort:* All children with SAM whose caregivers are willing
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27 19 to provide written informed consent and to learn their child's HIV status are offered
28
29 20 enrolment. Any children who die prior to study enrolment and those with a known
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31 21 malignancy are ineligible.
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34
35 23 *Eligibility for enteropathy substudy:* Children with SAM aged 6-59 months with a
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37 24 nasogastric tube in place (or due to be placed) are categorized into 4 groups (HIV-
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39 25 positive oedematous (Group A-I); HIV-positive non-oedematous (Group A-II); HIV-
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41 26 negative oedematous (Group C-I) and HIV-negative non-oedematous (Group C-II),
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43 27 as shown in Table 1. Children meeting eligibility criteria will be enrolled throughout
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45 28 the study recruitment period until sufficient specimens have been collected from the
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1 groups shown in Table 1. Children in the enteropathy substudy are stratified into age
 2 bands (6-11 months; 12-23 months and 24-59 months) to enable age-matching of
 3 well-nourished controls. Children with underlying chronic gastrointestinal disease or a
 4 known malignancy are ineligible.

5
 6 **Table 1: Enteropathy substudy groups**

Children aged 6-59 months	Severe acute malnutrition ¹		Well nourished controls WHZ>-1
	Oedematous ²	Non-oedematous	
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)

7
 8 ¹SAM defined according to WHO criteria

9 ²Presence of bilateral pitting pedal oedema.

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

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

13
 14
 15 *Eligibility for microbiota and metabolomics substudies:* Children enrolled into the
 16 enteropathy substudy are also included in the microbiota and metabolomics
 17 substudies, since these substudies utilize the stool, urine and plasma samples
 18 collected for enteropathy analyses.

19
 20 *Eligibility for immunology substudy:* The immunology substudy comprises all children
 21 with SAM (drawn from both the observational cohort and the enteropathy substudy,
 22 as shown in Figure 1) providing a blood sample of sufficient volume (>2ml) for
 23 cellular assays after 1st June 2017.

24
 25 *Well-nourished controls:* Controls are children drawn from the same hospitals and
 26 communities as cases with SAM (including well-nourished sibling controls), who are

aged 6-59 months (matched to enteropathy substudy children within age bands), well-nourished (weight-for-height Z-score >-1) and clinically well (no acute illness or current infections) with known HIV status. Controls are categorized into two groups: well-nourished HIV-positive (Group B) and well-nourished HIV-negative (Group D), as shown in Table 1. Children with underlying chronic gastrointestinal disease or a known malignancy are ineligible. Well-nourished controls provide comparison biomarker data for all the laboratory substudies.

Informed consent procedures: Written informed consent is obtained from the primary caregiver using consent forms translated into local languages; where possible, other family members are included 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 cohort

Assessment	Hospitalization		Post-discharge ³				
	Baseline ¹	Discharge ²	2w	4w	12w	24w	48w
Caregiver informed consent to join observational cohort	x						
Summary checklist	x						
Locator information ⁴	x						
Acute admission information	x						
Baseline data	x						
Daily clinical review ⁵	Daily during hospitalisation						
Blood collection ⁶	x	x			x	x	x
HIV testing ⁷	x						
CD4 count and viral load	x				x	x	x

(HIV-infected children only)							
Full blood count ⁸	x	x			x	x	x
Anthropometry	x	x	x	x	x	x	x
Skinfold thickness ⁹		x	x	x	x	x	x
Body composition ¹⁰	x	x	x	x	x	x	x
Discharge data collection		x					
Daily morbidity diary			Daily during follow-up period by caregivers				
Follow-up clinic: history, examination, morbidity and mortality data			x	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 (≥ 2 mL 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.
- ⁷HIV 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.
- ¹⁰Body composition will be assessed by bioimpedance vector analysis.

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

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3 1 skinfold thickness using calipers (Holtain Ltd., Crymych, UK) are undertaken at
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5 2 baseline. Blood (1mL/kg up to 5.4mL maximum) is collected at baseline into an
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7 3 endotoxin-free EDTA tube for all children and, in the enteropathy substudy,
8
9 4 additionally into a PAXgene tube (PreAnalytiX GmbH, Hombrechtikon, Switzerland)
10
11 5 for subsequent transcriptomic analysis. Blood is not collected from children with
12
13 6 severe anaemia (known haemoglobin <6 g/dL). HIV testing is carried out in
14
15 7 accordance with national guidelines as part of routine clinical practice; where it has
16
17 8 not been done, the child's HIV status is ascertained using a rapid test antibody
18
19 9 algorithm for children over 18 months, or HIV DNA PCR for children under 18
20
21 10 months. CD4 count/percentage and viral load are measured in HIV-positive children.
22
23 11 Maternal HIV status is documented where available, so that HIV-exposed uninfected
24
25 12 children can be identified. Blood samples are sent to research laboratories at each
26
27 13 site to conduct whole blood stimulation and bacterial binding assays (as described in
28
29 14 the immunology substudy) and to store aliquots of whole blood, peripheral blood cells
30
31 15 and plasma at -80°C³¹. In the enteropathy substudy, nasogastric aspirate, stool and
32
33 16 urine (after an oral dose of lactulose and mannitol) are also collected. Lactulose and
34
35 17 mannitol are ingested by the child after fasting and urine is collected over a two-hour
36
37 18 period to measure recovery of lactulose and mannitol, a measure of intestinal
38
39 19 absorptive capacity and permeability, as previously described³².

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41 20
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43 21 *Daily procedures:* Routine inpatient management is undertaken by ward clinical
44
45 22 teams according to local hospital protocols, which are based on WHO guidelines^{2, 33}.
46
47 23 In addition, the HOPE-SAM study clinician at each hospital site collects daily data
48
49 24 until discharge on clinical parameters (including daily examination), resolution of
50
51 25 acute infections, nutritional recovery (loss of oedema, restoration of appetite, weight
52
53 26 gain), and treatment/nutritional supplements received; this will allow us to evaluate
54
55 27 differences in management between countries. Children with HIV-SAM who are ART-

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3 1 naïve start ART according to national guidelines, which are based on WHO
4 2 recommendations^{2 34}.

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9 4 *Discharge:* The clinical team decides when the child is ready to be discharged, which
10 5 is generally when their medical complications are resolving and the child has a good
11 6 appetite and is clinically well and alert². Children receive ready-to-use therapeutic
12 7 feeds (RUTF) to take at home according to local guidelines. At discharge, the study
13 8 nurse collects data and a repeat blood sample (including full blood count) and
14 9 undertakes discharge anthropometry, body composition, leg length (Zimbabwe only)
15 10 and triceps, subscapular and supra-iliac skinfold thickness measurements (**Table 2**).
16 11 The caregiver is given a daily morbidity diary and pre-prepared stickers
17 12 corresponding to different illnesses and shown how to complete the diary. The
18 13 caregiver is provided with the date of the first follow-up appointment and contact
19 14 details of the study nurse.

20
21
22 15
23 16 *Follow-up:* Children attend follow-up appointments at dedicated study clinics at 2, 4,
24 17 12, 24 and 48 weeks post-discharge. At each visit, the study physician undertakes a
25 18 clinical assessment and the study nurse captures illness, medication and feeding
26 19 data. Clinic data are transcribed from handheld medical records if available and the
27 20 morbidity diary is reviewed and a new diary and stickers supplied. Anthropometry,
28 21 body composition, leg length (Zimbabwe only) and triceps, subscapular and supra-
29 22 iliac skinfold thicknesses are measured at each visit. Acute illnesses are treated in
30 23 the study clinic, or the child is referred to hospital if necessary. Children with relapsed
31 24 malnutrition are provided with nutritional supplements or RUTF according to local
32 25 guidelines, or readmitted to hospital if they develop complicated SAM. Transport
33 26 reimbursement for clinic attendance is provided to caregivers for each visit.

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56 28 Blood is collected at weeks 12, 24 and 48 post-discharge into endotoxin-free EDTA

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**.

8
 9 **Table 3: Summary of procedures for cases in the enteropathy substudy**

Assessment	Hospitalization		Post-discharge ³				
	Baseline ¹	Discharge ²	2w	4w	12w	24w	48w
Caregiver informed consent to join observational cohort and enteropathy substudy	X						
Summary checklist	X						
Locator information ⁴	X						
Acute admission information	x						
Baseline data	X						
Daily clinical review ⁵	Daily during hospitalisation						
Blood collection ⁶	x	x			x	x	x
HIV testing ⁷	X						
CD4 count and viral load (HIV-infected children only)	X				x	x	x
Full blood count ⁸	X	x			x	x	x
Gastric aspirate ⁹	x						
Stool collection ¹⁰	x	x			x	x	x
Lactulose-mannitol testing ¹¹	x	x			x		x
Anthropometry	X	x	x	x	x	x	x
Skinfold thickness ¹²		x	x	x	x	x	x
Body composition ¹³	X	x	x	x	x	x	x
Discharge data collection		x					

Daily morbidity diary			Daily during follow-up period by caregivers				
Follow-up clinic: history, examination, morbidity and mortality data			x	x	x	x	x

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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 at subsequent visits if caregivers have moved or changed contact details.

⁵Daily clinical review will be conducted every day between admission and discharge by the study clinician.

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. 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.

⁷HIV 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

⁹A 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

¹¹Lactulose-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.

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

¹³Body composition will be assessed by bioimpedance vector analysis.

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

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 countries³⁰. 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 enteropathy³⁰. Children in
18 resource-poor settings also suffer from frequent diarrhoea, food insecurity and
19 micronutrient deficiencies, which all exacerbate enteropathy³⁰. 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 SAM³⁷, which we here
23 refer to as malnutrition enteropathy³⁷, 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 wall³⁰. It is becoming apparent that
27 chronic inflammation may be particularly deleterious in malnourished individuals²³; in

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 development³⁸. Recent evidence suggests
26 that an immature or pathogenic microbiota plays a causative role in the pathogenesis
27 of SAM²⁵. For example, a number of microbial taxa have been identified, including
28 *Faecalibacterium prausnitzii*, which discriminate and predict gut microbiota maturity

1
2
3 1 and child growth²⁸. Other pathogenic microorganisms, including IgA-targeted
4 2 *Enterobacteriaceae*, are associated with impaired growth and may contribute to
5 3 SAM³⁹. Nutritional rehabilitation with RUTF induces temporary recovery of a
6 4 disturbed microbiota; however, the microbiota appears to revert back to an immature
7 5 diseased state following nutritional recovery²⁹. HIV infection is also associated with a
8 6 disturbed gut microbiota⁴⁰, which may further compound enteropathy phenotypes.
9 7 Furthermore, there is some evidence that differences exist in malnutrition
10 8 enteropathy between oedematous and non-oedematous SAM⁴¹; however, few
11 9 studies have investigated differences in the gut microbiota between the two forms of
12 10 the disease.

13
14
15 12 We hypothesize that (i) a unique gut microbial signature exists in HIV-SAM,
16 13 compared with HIV-negative children with SAM, that is independently associated with
17 14 morbidity, mortality, nutritional recovery and degree of enteropathy during
18 15 hospitalisation; (ii) a unique gut microbial signature exists in oedematous compared
19 16 with non-oedematous SAM; (iii) specific microorganisms or gut microbial diversity
20 17 indices are independently associated with morbidity, mortality, nutritional recovery
21 18 and degree of enteropathy during hospitalisation; and (iv) the gut microbiota is
22 19 partially restored to a healthy state with nutritional rehabilitation but reverts to a
23 20 dysbiotic state during follow-up, which predicts morbidity, mortality and relapse of
24 21 SAM.

25
26
27 23 Using stored stool samples collected at baseline, a cross-sectional investigation will
28 24 determine differences in the gut microbial composition and predicted function
29 25 between: HIV-negative children with SAM versus HIV-SAM, oedematous versus non-
30 26 oedematous SAM, and well-nourished controls. Gut microbial composition and
31 27 predicted function will be compared between groups at discharge and at 12, 24 and
32 28 48 weeks post-discharge. Briefly, total DNA and/or RNA will be extracted from stool

1 samples and used as template for next-generation sequencing library preparation
2 and for quantitative polymerase chain reaction (qPCR). Whole metagenome shotgun
3 sequencing will be performed using the HiSeq 2500 system. Raw metagenomic
4 sequencing data will be quality-filtered and analysed through a well-validated
5 bioinformatics pipeline using MetaPhlan⁴² and HUMAnN⁴³. The compositional and
6 predicted functional metagenomic data generated will be used to identify signatures
7 of SAM and to investigate associative links between specific gut microbial signatures
8 and clinical outcomes.

9 10 11 *Metabolomics substudy*

12 During SAM, metabolic processes are altered in response to a starved environment,
13 and may plausibly contribute to long-term clinical outcomes. Previous studies
14 suggest that amino acid turnover, lipid metabolism, oxidative stress and other
15 metabolic pathways are disrupted in SAM and may be associated with disease state
16 and clinical outcome^{26 44 45}; however, little is known about how the metabolic
17 phenotype responds to nutritional therapy. It is hypothesised that disturbed gut
18 microbiota composition and function may drive microbial metabolic dysregulation in
19 addition to host-derived dysregulation. Of particular interest are differences in the
20 metabolic phenotype between oedematous and non-oedematous SAM. The
21 'reductive adaptation' seen in non-oedematous SAM (utilisation of fat and muscle
22 stores) is disrupted in oedematous SAM, which may contribute to differences in
23 clinical outcomes. Specifically, protein turnover, inflammation, oxidative stress and
24 bile acid metabolism are disrupted in oedematous-SAM, which may contribute to co-
25 morbidities including diarrhoea, steatosis and enteropathy^{46 47}.

26
27 We hypothesize that: (i) a unique plasma and urine metabolic phenotype exists in
28 oedematous compared with non-oedematous SAM during hospitalisation, which is

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 between
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 ¹H 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 prophylaxis¹⁷. Collectively, these observations highlight that children
27 remain vulnerable to infection despite current treatment approaches; targeting

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3 1 persistent immune dysfunction could plausibly reduce infectious mortality after
4 discharge²⁴.
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9 4 Multiple innate and adaptive immune mediators are dysregulated in malnutrition^{24 27}
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11 5⁵⁵. However, few studies have assessed cellular immune function in malnourished
12
13 6 children; most existing studies were undertaken decades ago on small cross-
14
15 7 sectional cohorts without the benefit of recent advances in immunology techniques²⁷.
16
17 8 Immune dysfunction in SAM likely reflects both *intrinsic* defects, whereby immune
18
19 9 cells lack capacity to adequately respond to infection, and *extrinsic* defects, where
20
21 10 cells have intact anti-bacterial capacity but are chronically modulated by the systemic
22
23 11 pro-inflammatory environment which characterises SAM (i.e. heightened pro-
24
25 12 inflammatory cytokines⁴⁴ and circulating bacterial antigens^{23 56 57}). Systemic
26
27 13 inflammation is directly associated with mortality in SAM²³ and driven by multiple
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29 14 comorbidities, including bacterial translocation from the damaged gut into the blood,
30
31 15 sub-clinical infections and metabolic dysregulation^{44 58 59}. The implications of innate
32
33 16 immune cell dysfunction for subsequent acquisition of infections and infectious
34
35 17 mortality have not been investigated.

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39 19 We hypothesise that: (i) anti-bacterial functions of innate immune cells are
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41 20 compromised in SAM due to a combination of intrinsic and extrinsic defects; ii) innate
42
43 21 immune cell function is independently associated with infectious morbidity and
44
45 22 mortality during hospitalisation for SAM; and iii) nutritional rehabilitation only partly
46
47 23 restores innate immune cell function, leading to an ongoing risk of bacterial infections
48
49 24 post-discharge.

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51 25
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53 26 Using blood samples collected at baseline, discharge and 12, 24 and 48 weeks post-
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55 27 discharge, the longitudinal relationship between circulating innate immune cell
56
57 28 function and bacterial infections will be assessed. The intrinsic phagocytic capacity,

1 secreted cytokine response and maturation state of innate immune cells after culture
2 with bacterial antigens will be assessed. Plasma concentrations of endotoxin and
3 pro-inflammatory mediators will be quantified at each time-point and the degree to
4 which these extrinsic factors influence innate immune cell antibacterial function will
5 be assessed via plasma co-culture with innate immune cells from healthy donors.
6 Bacterial infections during hospitalisation will be diagnosed using clinical criteria and
7 blood culture, stool culture and urinalysis where available.

8 9 **SAMPLE SIZES**

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

20
21
22 *Enteropathy substudy:* The sample size was estimated using previously reported
23 values for LM ratios, which remain a widely used non-invasive marker of
24 enteropathy. Comparing 100 versus 100 children with two-sided $\alpha=0.025$ (to
25 allow for two primary comparisons, i.e. HIV-SAM versus HIV-negative children with
26 SAM, and HIV-SAM versus well-nourished HIV-positive children) provides >80%
27 power to detect differences in mean LM ratio during hospitalisation of at least 0.16

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 Gambia⁶⁰. 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 log₁₀ 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.

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 $\alpha=0.05$
21 and assuming either low ($\rho=0.1$) or high ($\rho=0.7$) correlation, the study would require
22 95-126 subjects to achieve 80% power⁶¹. False discovery rate (FDR) multiple
23 correction testing will be applied to reduce the high-dimensionality of the data and
24 limit false-positives.

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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3 1 Assuming similar infectious mortality to a recent Kenyan study (15%)¹⁷, a cohort of
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5 200 provides 80% power to detect associations between immune profiles and
6
7 3 infectious mortality at an odds ratio of 1.7 and 2-sided alpha of 0.05. We will aim for
8
9 4 100 children with longitudinal analysis of innate immune cell function at discharge,
10
11 5 12, 24 and 48 weeks post-discharge¹⁷.
12
13 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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3 1 Risk factors will be evaluated at baseline, hospital discharge and over the period of
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5 2 follow-up for associations with clinical and nutritional outcomes. In addition to
6
7 3 baseline clinical and demographic factors, the following laboratory parameters will be
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9 4 evaluated: haemoglobin, serum albumin, C-reactive protein, CD4 count and HIV viral
10
11 5 load (for HIV-positive children). Haemoglobin, CD4 and HIV-viral load will be
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13 6 measured in real time and the results reviewed during follow-up clinics.
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17 8 Data on potential confounders are collected at baseline, discharge and during the
18
19 9 follow-up period, including child feeding practices, household socioeconomic status
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21 10 (defined by household income and cooking method), maternal employment and
22
23 11 education, and household factors such as water, sanitation and hygiene practices,
24
25 12 availability of electricity, location (rural, peri-urban or urban) and household size.
26
27 13

28 14 **ANALYSIS**

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31 15 All analyses will be interpreted exploratively since HOPE-SAM is an observational
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33 16 study with multiple risk factors, outcomes and substudies. For all analyses, P values
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35 17 will not be artificially adjusted, but interpreted as exploring the strength of evidence
36
37 18 supporting any association. The only exception is the use of approaches to minimise
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39 19 false discovery when analysing high-dimensional data from the microbiota and
40
41 20 metabolomics substudies, as described.
42
43 21

44 22 *Observational Cohort*

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46 23 The primary comparison will be the clinical and nutritional outcomes of children with
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48 24 HIV-SAM compared to HIV-negative children with SAM. We will review all deaths
49
50 25 and adjudicate clinical diagnoses and causes of death to ensure robust and
51
52 26 consistent data across sites. We will compare each participant's clinical management
53
54 27 to WHO guidelines to identify any contributory factors in hospital care. Factors
55
56 28 associated with outcomes during hospitalisation (e.g. mortality, nutritional recovery)

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 months⁶²) 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.

21 *Body composition analysis*

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

1 resistance and reactance, which are further linked through phase angle (PA).
2 Variability in these components is associated with biochemical parameters⁶⁴. These
3 variables will be explored using graphical analysis, or transformed into age- and sex-
4 adjusted Z-scores for statistical comparison, including longitudinal analyses. Higher
5 phase angle indicates better nutritional status, while declining height-adjusted
6 resistance over time indicates loss of oedema.

7 8 *Enteropathy substudy*

9 The primary comparison for the enteropathy substudy will be between HIV-positive
10 children with SAM (group A) and HIV-negative children with SAM (group C), stratified
11 by presence or absence of oedema. Control groups (B and D) are well-nourished
12 children with or without HIV, to provide normative data for biomarkers and to
13 evaluate the impact of SAM within each HIV group. Thus, biomarkers among HIV-
14 positive children with SAM will first be compared to HIV-negative children with SAM
15 (to evaluate the impact of HIV) and, second, to well-nourished HIV-positive children
16 (to evaluate the impact of SAM). Biomarkers among HIV-negative children with SAM
17 will be compared to well-nourished HIV-negative children. For each continuous
18 outcome, simple descriptive analysis will be used to compare groups during
19 hospitalisation using t-tests on appropriately transformed data. For any outcome with
20 moderate ($p < 0.05$) evidence of difference between either group a regression model
21 will be constructed including groups A, B, C, D to directly test (using interactions)
22 whether there is a synergistic effect of HIV-SAM versus HIV-negative SAM versus
23 HIV alone versus neither. These models will also be used to explore whether there is
24 any evidence for heterogeneity in effects between oedematous and non-oedematous
25 SAM. Associations between enrolment factors (e.g. intestinal permeability and
26 microbial translocation) will be explored using pairwise Spearman correlations and
27 principal components analysis. Mean changes at the follow-up time-points in each
28 group will be estimated, and groups compared (as above) using generalised

1
2
3 1 estimating equations. For outcomes that differ across SAM groups over time,
4
5 2 multilevel models will be used to explore possible predictors from the other factors
6
7 3 measured. Time to nutritional recovery will be compared using Kaplan-Meier and log-
8
9 4 rank tests, and Cox models to adjust for baseline differences between groups.

10
11 5
12 6 *Microbiota and metabolomics substudy*

13
14 7 The primary comparison will be between HIV-negative children with oedematous and
15
16 8 non-oedematous SAM, with a separate comparison between HIV-positive children
17
18 9 with SAM and HIV-negative children with SAM. Analyses will examine: (i) differences
19
20 10 in metagenomic/metabolomic variables between groups at each time-point; (ii)
21
22 11 differences in metagenomic/metabolomic variables within groups over time; (iii)
23
24 12 correlations between metagenomic and metabolomic variables; and (iv) correlations
25
26 13 between metagenomic/metabolomic variables and clinical outcomes. A systematic
27
28 14 analysis will be undertaken to reduce high-dimensional data, integrate the multi-
29
30 15 omics datasets and minimise false discovery.

31
32 16
33
34 17 Compositional metagenomic data will be compared between groups for indices of
35
36 18 alpha and beta diversity. Principal coordinate analysis and partial least squares
37
38 19 discriminant analysis will be performed on metabolomics data to identify overall
39
40 20 differences between groups. High-dimensional datasets will be reduced using
41
42 21 random forest models to identify taxa, microbial gene families and metabolites that
43
44 22 most strongly contribute to differences between groups, corrected by Benjamani-
45
46 23 Hochburg false discovery rate detection. Targeted analysis by qRT-PCR will validate
47
48 24 differential abundance or expression of candidate microbial genes. Longitudinal
49
50 25 comparisons will be performed within and between groups using multilevel
51
52 26 simultaneous component analysis. Orthogonal projections to latent structures models
53
54 27 will integrate metabolomic and metagenomic data whilst linear regression, canonical
55
56 28 correlation and hierarchal clustering analysis will measure correlations between -

1
2
3 1 omics datasets. Finally, ROC analysis will identify the ability of different analytes to
4
5 2 predict long-term nutritional and clinical outcomes.

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7 3
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9 4 *Immunology substudy*

10
11 5 Integrated profiles of innate immune cell function will be generated for each child
12
13 6 using principal components analysis followed by hierarchical clustering^{65 66}. This
14
15 7 data-reduction method identifies whether absolute levels of specific markers or
16
17 8 relative differences between markers differentiate children into groups. The resulting
18
19 9 innate immune profiles will be compared between HIV-SAM, HIV-negative children
20
21 10 with SAM and well-nourished groups using univariable tests and multivariable
22
23 11 analysis of variance (MANOVA) of the principal components.

24
25 12
26
27 13 To address the relationship between immune function and infections, regression
28
29 14 analyses will determine whether baseline innate immune profiles (or the individual
30
31 15 parameters defining them) are associated with the infectious morbidity or mortality
32
33 16 during hospitalisation, using logistic models for binary outcomes and linear models
34
35 17 for duration. Key clinical characteristics, including age, sex, oedema and baseline
36
37 18 WHZ, will be added to models to investigate their confounding effects. Multivariable
38
39 19 stacked regression methods will be used to compare the impact of different factors
40
41 20 on severe bacterial infections based on heterogeneity tests.

42
43 21
44
45 22 To determine whether treatment for SAM restores innate immune cell antibacterial
46
47 23 function, mixed effects regression models will compare longitudinal changes in
48
49 24 individual immune parameters, and the principal components calculated from the
50
51 25 weights identified at baseline (which include well-nourished controls). Similarities and
52
53 26 differences in longitudinal immune profiles will be compared between groups using
54
55 27 nonmetric multi-dimensional scaling^{65 67 68}. This approach will group children
56
57 28 according to their composite innate immune function, allowing the duration and

1
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.
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9 4

10 5 **PATIENT AND PUBLIC INVOLVEMENT**

11
12 6 Patients and their caregivers were not involved in the design of the study. During
13
14 7 recruitment, all caregivers of children admitted to hospital were given information
15
16 8 about the study; those whose children had severe acute malnutrition were
17
18 9 approached to give written informed consent. A meeting to disseminate results of the
19
20 10 study to participants and their caregivers will be held at the end of the study. An
21
22 11 interactive game to engage caregivers in the science underlying malnutrition is being
23
24 12 developed in collaboration with experts from the Centre of the Cell, a unique science
25
26 13 education centre based at Queen Mary University of London
27
28 14 (<https://www.centreofthecell.org/>).
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36 17 **SAFETY REPORTING**

37
38 18 For all adverse events, the study team will assess expectedness and relatedness to
39
40 19 study activities. Since this is an observational study without interventions, we
41
42 20 anticipate that the risk is minimal; however, serious adverse events will be reported
43
44 21 to local ethical review boards (Medical Research Council of Zimbabwe, and
45
46 22 University of Zambia Biomedical Research Ethics Committee) and the study sponsor
47
48 23 (Queen Mary University of London) according to their respective guidelines.
49
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
58
59
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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

1 enteropathy, microbiota, metabolome and innate immune cell function to these
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 recognised⁶⁹. 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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2 *Designed study:* MB-D, BA, CDB, RCR, BM, KC, CK, KCh, DN, PC, NC, FM, JW,

3 ARM, 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

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17

18 COMPETING INTERESTS

19 None of the authors have any competing interests to declare.

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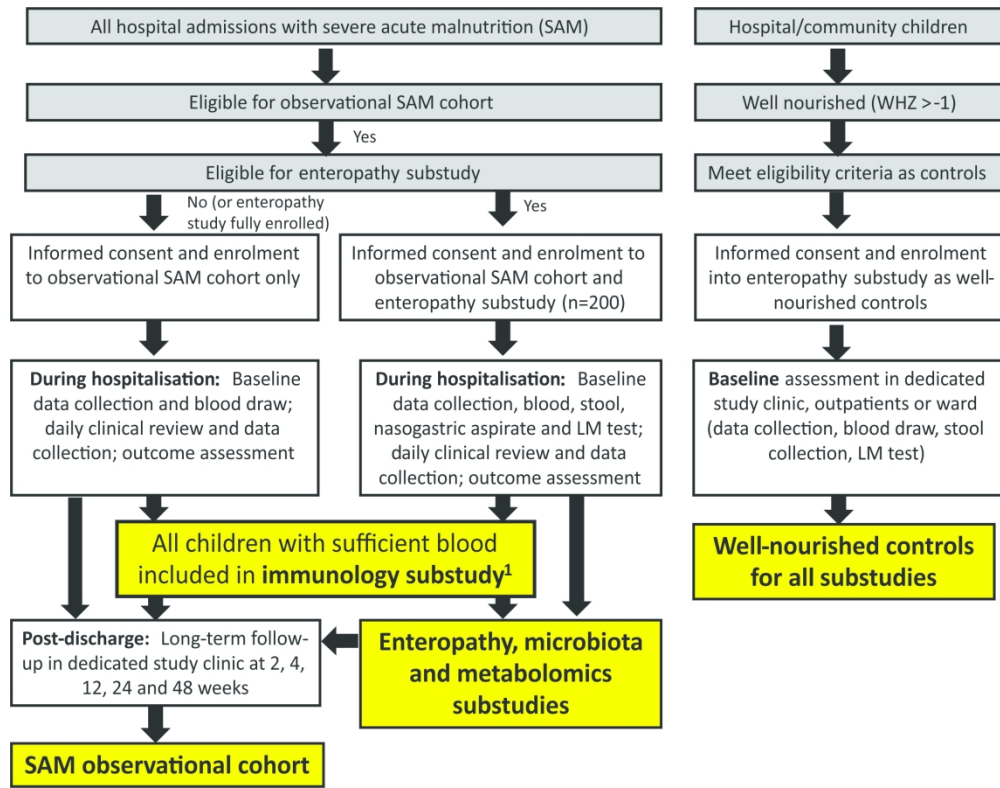
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FIGURE LEGENDS

Figure 1: Study flow chart.

All hospital admissions are screened for eligibility for the observational cohort and enteropathy 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.



204x159mm (300 x 300 DPI)

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

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	X				
Blood	CD4 count (flow cytometry or PIMA) ¹	TROPGAN, Zvitambo or clinical sites	All HIV-positive	X	X	X	X	X
Plasma	HIV viral load (real-time PCR) ¹	TROPGAN, Zvitambo or clinical sites	All HIV-positive	X	X	X	X	X
Plasma	C-reactive protein (ELISA)	TROPGAN, Zvitambo	All	X	X	X	X	X
Plasma	Albumin (ELISA)	TROPGAN, Zvitambo	All	X	X	X	X	X
Plasma	Lipopolysaccharide (LAL assay)	TROPGAN and Zvitambo	Subgroup ³	X	X	X	X	X
Plasma	Lipopolysaccharide binding protein (LBP)	TROPGAN and Zvitambo	Subgroup ³	X	X	X	X	X
Plasma	sCD14 (ELISA)	TROPGAN and Zvitambo	Subgroup ³	X	X	X	X	X
Plasma	sCD163 (ELISA)	TROPGAN and Zvitambo	Subgroup ³	X	X	X	X	X
Plasma	IL-6, TNF-alpha, IL-1 β (ELISA) and/or multiplex cytokines	TROPGAN and Zvitambo	Subgroup ³	X	X	X	X	X
Plasma	Total PAMP activity (THP1 reporter cell line ²)	TROPGAN and Zvitambo	Subgroup ³	X	X	X	X	X
Whole	Molecular techniques	QMUL, London ²	Subgroup ³	X	X	X	X	X

blood	for bacterial detection (broad-range and specific PCR and next-generation sequencing)							
Immunology substudy only								
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	X	X
Plasma	Co-culture with healthy immune cells ⁵	QMUL, London	All	X	X	X	X	X

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 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 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 Blizzard Institute, QMUL 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: pathogen-associated molecular pattern; QMUL: Queen Mary University of London; CRP: C-reactive protein; LAL: limulus amoebocyte lysate assay.

Supplementary table 2: Additional laboratory analyses for enteropathy substudy

Sample type	Assay (method)	Location of work	Study groups ¹	Baseline	Discharge	Week 12	Week 24	Week 48
Urine	Lactulose-mannitol ratio (mass spectrometry)	Orgeon Analytics, USA	A, B, C, D (all)	X	X	X		X
Stool	Neopterin, myeloperoxidase, alpha-1 antitrypsin and REG-1B (ELISA)	TROPGAN and Zvitambo	A, B, C, D (all)	X	X	X	X	X
Plasma	I-FABP (ELISA)	TROPGAN and Zvitambo	A, B, C, D (all)	X	X	X	X	X
Plasma	GLP-2	TROPGAN and Zvitambo	A, B, C, D (all)	X	X	X	X	X
Plasma	Citrulline (mass spectrometry)	Imperial College London	A, B, C, D (all)	X	X	X	X	X
Plasma	Kynurenine:tryptophan ratio and metabolites along tryptophan pathway (mass spectrometry)	Imperial College London	A, B, C, D (all)	X	X	X	X	X
Stool	Microbiome analysis ²	BCCDC, Vancouver	A, B, C, D (all)	X	X	X	X	X
Stool	<i>Helicobacter pylori</i> antigen	TROPGAN and Zvitambo	A, B, C, D (all)	X				
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				

RNA extracted from PAXGene tubes	Gene expression analysis (RNASeq)	QMUL, London	A, B, C, D (all)	X	X			
Plasma and urine	Targeted and untargeted metabolic phenotyping	Imperial College London	A, B, C, D (all)	X	X	X	X	X

¹Enteropathy substudy groups: Group A: HIV-positive children with severe acute malnutrition; Group B: HIV-positive well-nourished controls; Group C: HIV-negative children with severe acute malnutrition; Group D: HIV-negative well-nourished controls.

Note that controls only have blood taken at baseline.

²For 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

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

BMJ Open

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

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Keywords:	Malnutrition, HIV, Africa, mortality, microbiota, enteropathy

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Manuscripts

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3 **1 Health Outcomes, Pathogenesis and Epidemiology of Severe Acute Malnutrition**
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5 **2 (HOPE-SAM): rationale and methods of a longitudinal observational study**
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1 **ABSTRACT**

2 **Introduction**

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

10 **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 short-
13 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 hospitalised
16 for SAM are being enrolled at three tertiary hospitals in Harare, Zimbabwe, and Lusaka,
17 Zambia. Longitudinal mortality, morbidity and nutritional data are being collected at
18 admission, discharge and for 48 weeks post-discharge. Nested laboratory substudies
19 are exploring the role of enteropathy, gut microbiota, metabolomics and cellular immune
20 function in the pathogenesis of SAM using stool, urine and blood collected from
21 participants and from well-nourished controls.

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 Biomedical

1 Research Ethics Committee) and the study sponsor (Queen Mary University of London).
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**

1 Malnutrition underlies almost half of all childhood deaths in developing countries¹.
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 oedema². 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 guidelines³. 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^{6,7}. While new
16 HIV infections in children have declined⁸, 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 morbidity⁹. Hence, HIV has transformed the
20 epidemiology and outcomes of SAM¹⁰. 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 unclear¹⁰; this mortality is three-fold higher than would be
24 expected from anthropometric parameters alone¹⁰. 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

1 and wasted; have a higher frequency of persistent diarrhoea; tend to have delayed
2 nutritional recovery and have a more complicated clinical course than HIV-negative
3 children with SAM¹⁰.

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 indicate
8 high post-discharge mortality following in-hospital management of SAM¹¹⁻¹³. Malnutrition
9 together with young age, HIV infection and pneumonia have been associated with higher
10 post-discharge mortality¹⁴. One of the largest prospective studies of growth and mortality
11 in children with SAM (FuSAM), conducted in Malawi from July 2006 to March 2007,
12 collected 12-month outcome data on 87% of 1024 children admitted to the nutrition
13 ward¹¹. A total of 427 (42%) died and 44% of these deaths occurred after discharge from
14 hospital. Survival was greatest among those who were nutritionally cured upon
15 discharge from outpatient therapeutic feeding centres, defined as two consecutive visits
16 with >80% expected weight-for-height, no oedema and clinically stable. The risk of
17 mortality after hospital discharge was four-fold higher for HIV-SAM compared to HIV-
18 negative children with SAM, but the outcomes among HEU children were not reported.
19 The loss to follow-up was high in the FuSAM study because there was only one follow-
20 up visit, one year after discharge from outpatient-feeding centres. A recent study from
21 Kenya identified malnutrition and HIV infection as key drivers for post-discharge
22 mortality, with 52% of deaths attributable to MUAC <11.5cm and 11% to HIV infection¹⁵.

23

24 The impact of SAM appears to persist beyond the first year after discharge from hospital.
25 The ChroSAM study, which followed children with SAM seven years post-discharge,
26 showed that children had poorer growth, body composition and physical function

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

3
4 While anthropometry is used to assess nutritional recovery after discharge from hospital,
5 the pattern and quality of growth recovery following SAM is poorly understood. The
6 observation that children treated for SAM have a deficit in lean tissue despite regaining
7 weight suggests that assessing body composition in addition to anthropometry may help
8 to identify children who have not completely recovered and are at potential risk of long-
9 term metabolic diseases¹². Children with HIV-SAM appear to have potential for catch-up
10 growth in weight-for-age and/or weight-for-height, which have been shown to normalise
11 with treatment even prior to widespread availability of ART¹⁶; by contrast, height-for-age
12 shows less potential for catch-up growth¹⁷. However, the body composition of children
13 with HIV-SAM compared to HIV-negative children with SAM has not been described.
14 Whether children recover fat mass at the expense of lean mass is unknown, but
15 differences in tissue accretion patterns may have implications for survival and long-term
16 metabolic health^{18 19}. There is also a need to consider the effect of SAM on the size of
17 body parts which grow at different rates: relatively shorter legs, for example, are
18 associated with epidemiologic risk of overweight, coronary artery disease, liver
19 dysfunction and diabetes^{20 21}.

20
21 Taken together, there is clearly an elevated risk of mortality among HIV-positive children
22 with SAM compared to HIV-negative children with SAM, and an ongoing mortality risk
23 among all children with SAM that persists after discharge from hospital. There are
24 several gaps in our understanding of the long-term outcomes: (i) causes of death have
25 not been clearly defined; (ii) no studies have systematically and longitudinally collected
26 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.

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 malnutrition²² and that systemic and intestinal inflammation are associated
9 with poor outcomes in SAM²³, suggest that immune dysfunction contributes to infectious
10 susceptibility²⁴. Malnutrition is also characterised by a complex derangement in gut
11 microbial²⁵ metabolic,²⁶ immune²⁷ and hormonal pathways, organ dysfunction and
12 micronutrient deficiencies in the context of co-infections, enteropathy and chronic
13 inflammation. Several studies have recently provided insights into these perturbations
14 using new tools^{25 26 28 29}, including metabolomics and metagenomics, but we still lack a
15 clear understanding of many of the pathogenic pathways driving malnutrition, the
16 interactions between these pathways, and which are the most tractable targets for
17 intervention.

18
19 SAM shares several pathological and clinical features with HIV, which may explain
20 clinical outcomes in these co-occurring conditions: 1) both are characterised by intestinal
21 damage, leading to impairment of the mucosal barrier and increased intestinal
22 permeability; 2) both have underlying systemic immune activation; and 3) both are
23 frequently complicated by persistent diarrhoea, pneumonia and sepsis that may
24 plausibly arise due to loss of intestinal barrier function³⁰. Understanding the overlapping
25 impact of HIV and SAM is critical to inform additional interventions to improve outcomes
26 of children with HIV-SAM.

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3 14
5 2 **OBJECTIVES OF HOPE-SAM**6
7 3 The Health Outcomes, Pathogenesis and Epidemiology of Severe Acute Malnutrition8
9 4 (HOPE-SAM) study has two primary objectives:

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- 11 5 1) To describe the short- and long-term clinical outcomes of children with
-
- 12 6 complicated SAM, with and without HIV infection, and to identify the risk factors
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- 13 7 at admission and discharge from hospital that independently predict these
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- 14 8 outcomes.
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- 15 9 2) To better characterise the pathogenesis of SAM through nested laboratory sub-
-
- 16 10 studies evaluating enteropathy, gut microbiota, metabolomics and immune cell
-
- 17 11 function.

12
13 13 **STUDY DESIGN**14 14 HOPE-SAM is a longitudinal observational cohort study, enrolling between 600-800
15 15 children aged 0-59 months admitted with complicated SAM to the tertiary pediatric wards
16 16 at two sites in Zimbabwe (Parirenyatwa Hospital and Harare Children's Hospital) and
17 17 one in Zambia (University Teaching Hospital, Lusaka). Both HIV-positive and HIV-
18 18 negative children will be enrolled. Throughout this paper, 'SAM' refers to all children,
19 19 regardless of HIV status; where analyses specifically compare children by HIV status,
20 20 groups are identified as HIV-positive children with SAM (or HIV-SAM) and HIV-negative
21 21 children with SAM. All participants with SAM are followed for 48 weeks post-discharge,
22 22 with longitudinal data collection and blood sampling. The study contains four nested sub-
23 23 studies as shown in **Figure 1**. A subgroup of children will be recruited to the
24 24 enteropathy substudy for which they will have the same follow-up procedures but more
25 25 intensive biological specimen collection including stool (all time-points), urine after
26 26 lactulose-mannitol (LM) challenge as an assessment of intestinal permeability, and

1 nasogastric aspirate (baseline only); these children are also included in microbiota and
2 metabolomics substudies. Children with SAM for whom blood samples are available are
3 included in the immunology substudy, for which circulating inflammatory mediators will
4 be assayed; functional cellular immunology assays will be conducted for all children in
5 the immunology sub-study with sufficient sample volume (> 2mL) recruited after June
6 2017. A group of healthy children recruited from the same hospitals and communities,
7 who are well-nourished and matched to children in the enteropathy substudy by age and
8 HIV status, will have data and specimens collected to provide normative data for the
9 laboratory substudies; these well-nourished controls will not be followed longitudinally.

10
11 The study protocol, data collection forms and standard operating procedures are
12 available online at osf.io/29uaw.

13 14 **RECRUITMENT**

15 *Screening:* Caregivers of all hospitalised children are sensitised about the study. All new
16 admissions aged 0-59 months are screened for SAM, which is defined according to
17 WHO criteria as any of: weight-for-height Z-score (WHZ) <-3, MUAC <115 mm (if aged
18 6-59mo) and/or bilateral pitting oedema. All children with SAM are recruited from
19 hospital and this study therefore focuses on complicated SAM; children with
20 uncomplicated SAM will not be enrolled.

21
22 *Eligibility for observational cohort:* All children with SAM whose caregivers are willing to
23 provide written informed consent and to learn their child's HIV status are offered
24 enrolment. Any children who die prior to study enrolment and those with a known
25 malignancy are ineligible.

1 *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-
 4 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
 6 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-
 8 11 months; 12-23 months and 24-59 months) to enable age-matching of well-nourished
 9 controls. Children with underlying chronic gastrointestinal disease or a known
 10 malignancy are ineligible.

11
 12 **Table 1: Enteropathy substudy groups**

Children aged 6-59 months	Severe acute malnutrition ¹		Well nourished controls WHZ>-1
	Oedematous ²	Non-oedematous	
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)

13
 14 ¹SAM defined according to WHO criteria

15 ²Presence of bilateral pitting pedal oedema.

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

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

19
 20
 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.

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5 2 *Eligibility for immunology substudy:* The immunology substudy comprises all children
6
7 3 with SAM (drawn from both the observational cohort and the enteropathy substudy, as
8
9 4 shown in Figure 1) providing a blood sample of sufficient volume (>2ml) for cellular
10
11 5 assays after 1st June 2017.
12
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14 6

15
16 7 *Well-nourished controls:* Controls are children drawn from the same hospitals and
17
18 8 communities as cases with SAM (including well-nourished sibling controls), who are
19
20 9 aged 6-59 months (matched to enteropathy substudy children within age bands), well-
21
22 10 nourished (weight-for-height Z-score >-1) and clinically well (no acute illness or current
23
24 11 infections) with known HIV status. Controls are categorized into two groups: well-
25
26 12 nourished HIV-positive (Group B) and well-nourished HIV-negative (Group D), as shown
27
28 13 in Table 1. Children with underlying chronic gastrointestinal disease or a known
29
30 14 malignancy are ineligible. Well-nourished controls provide comparison biomarker data
31
32 15 for all the laboratory substudies.
33
34

35 16

36
37 17 *Informed consent procedures:* Written informed consent is obtained from the primary
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39 18 caregiver using consent forms translated into local languages; where possible, other family
40
41 19 members are included in the consent process. Illiterate caregivers who have understood a
42
43 20 verbal explanation of the study can provide a thumb imprint in the presence of a witness.
44
45 21 Assent from children is not sought because all are <5 years old.
46

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48 23 **STUDY PROCEDURES**

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51 24 Study procedures are outlined in **Table 2**.

52 25 **Table 2: Summary of procedures in observational cohort**

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Assessment	Hospitalization		Post-discharge ³				
	Baseline ¹	Discharge ²	2w	4w	12w	24w	48w
Caregiver informed consent to join observational cohort	x						
Summary checklist	x						
Locator information ⁴	x						
Acute admission information	x						
Baseline data	x						
Daily clinical review ⁵	Daily during hospitalisation						
Blood collection ⁶	x	x			x	x	x
HIV testing ⁷	x						
CD4 count and viral load (HIV-infected children only)	x				x	x	x
Full blood count ⁸	x	x			x	x	x
Anthropometry	x	x	x	x	x	x	x
Skinfold thickness ⁹		x	x	x	x	x	x
Body composition ¹⁰	x	x	x	x	x	x	x
Discharge data collection		x					
Daily morbidity diary			Daily during follow-up period by caregivers				
Follow-up clinic: history, examination, morbidity and mortality data			x	x	x	x	x

1

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 (≥ 2 mL 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 1 Study blood samples will not be collected from children with known haemoglobin <6 g/dL.
4 2 ⁷HIV testing is conducted as part of routine clinical practice, but if it has not been undertaken, the study
5 3 sample will be used to test for HIV, as stated in the informed consent form, since HIV status is required
6 4 to allocate children to study groups.
7 5 ⁸Full blood count results will be transcribed from clinical records; if not done by clinical teams, the EDTA
8 6 sample will be used to measure FBC in clinical laboratories at each site
9 7 ⁹Skinfold thickness (triceps, subscapular, supra-iliac) and mid-thigh circumference will be measured
10 8 using Holtain calipers or tape measure.
11 9 ¹⁰Body composition will be assessed by bioimpedance vector analysis.

12 10
13 11
14 12
15 13 *Baseline procedures:*

16 14 Baseline data on maternal and household characteristics, the child's past medical history
17 15 and current illness are collected by a study nurse. Anthropometry, including body
18 16 composition measured by whole-body (wrist-ankle) bio-electrical impedance analysis
19 17 (BodyStat 1500MD; BodyStat Ltd., Douglas, Isle of Man), leg length using an electronic
20 18 knemometer (Zimbabwe only, due to availability of knemometers) and triceps,
21 19 subscapular and supra-iliac skinfold thickness using calipers (Holtain Ltd., Crymych, UK)
22 20 are undertaken at baseline. Blood (1mL/kg up to 5.4mL maximum) is collected at
23 21 baseline into an endotoxin-free EDTA tube for all children and, in the enteropathy
24 22 substudy, additionally into a PAXgene tube (PreAnalytiX GmbH, Hombrechtikon,
25 23 Switzerland) for subsequent transcriptomic analysis. Blood is not collected from children
26 24 with severe anaemia (known haemoglobin <6 g/dL). HIV testing is carried out in
27 25 accordance with national guidelines as part of routine clinical practice; where it has not
28 26 been done, the child's HIV status is ascertained using a rapid test antibody algorithm for
29 27 children over 18 months, or HIV DNA PCR for children under 18 months. CD4
30 28 count/percentage and viral load are measured in HIV-positive children. Maternal HIV
31 29 status is documented where available, so that HIV-exposed uninfected children can be
32 30 identified. Blood samples are sent to research laboratories at each site to conduct whole
33 31 blood stimulation and bacterial binding assays (as described in the immunology
34 32 substudy) and to store aliquots of whole blood, peripheral blood cells and plasma at -

1 80°C³¹. In the enteropathy substudy, nasogastric aspirate, stool and urine (after an oral
2 dose of lactulose and mannitol) are also collected. Lactulose and mannitol are ingested
3 by the child after fasting and urine is collected over a two-hour period to measure
4 recovery of lactulose and mannitol, a measure of intestinal absorptive capacity and
5 permeability, as previously described³².

6
7 *Daily procedures:* Routine inpatient management is undertaken by ward clinical teams
8 according to local hospital protocols, which are based on WHO guidelines^{2,33}. In addition,
9 the HOPE-SAM study clinician at each hospital site collects daily data until discharge on
10 clinical parameters (including daily examination), resolution of acute infections,
11 nutritional recovery (loss of oedema, restoration of appetite, weight gain), and
12 treatment/nutritional supplements received; this will allow us to evaluate differences in
13 management between countries. Children with HIV-SAM who are ART-naïve start ART
14 according to national guidelines, which are based on WHO recommendations^{2,34}.

15
16 *Discharge:* The clinical team decides when the child is ready to be discharged, which is
17 generally when their medical complications are resolving and the child has a good
18 appetite and is clinically well and alert². Children receive ready-to-use therapeutic feeds
19 (RUTF) to take at home according to local guidelines. At discharge, the study nurse
20 collects data and a repeat blood sample (including full blood count) and undertakes
21 discharge anthropometry, body composition, leg length (Zimbabwe only) and triceps,
22 subscapular and supra-iliac skinfold thickness measurements (**Table 2**). The caregiver is
23 given a daily morbidity diary and pre-prepared stickers corresponding to different
24 illnesses and shown how to complete the diary. The caregiver is provided with the date
25 of the first follow-up appointment and contact details of the study nurse.

1

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**.

21

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

23

Assessment	Hospitalization		Post-discharge ³				
	Baseline ¹	Discharge ²	2w	4w	12w	24w	48w
Caregiver informed consent to join observational cohort and enteropathy substudy	X						

Summary checklist	X							
Locator information ⁴	X							
Acute admission information	x							
Baseline data	X							
Daily clinical review ⁵	Daily during hospitalisation							
Blood collection ⁶	x	x			x	x	x	
HIV testing ⁷	X							
CD4 count and viral load (HIV-infected children only)	X				x	x	x	
Full blood count ⁸	X	x			x	x	x	
Gastric aspirate ⁹	x							
Stool collection ¹⁰	x	x			x	x	x	
Lactulose-mannitol testing ¹¹	x	x			x			x
Anthropometry	X	x	x	x	x	x	x	x
Skinfold thickness ¹²		x	x	x	x	x	x	x
Body composition ¹³	X	x	x	x	x	x	x	x
Discharge data collection		x						
Daily morbidity diary			Daily during follow-up period by caregivers					
Follow-up clinic: history, examination, morbidity and mortality data			x	x	x	x	x	

1

2

3

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.

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

8

9

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).

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

13

14

⁵Daily clinical review will be conducted every day between admission and discharge by the study clinician.

15

16

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

17

1
2
3 1 a 2.7 mL PAXGene tube, for subsequent isolation of RNA and gene expression analysis. After
4 2 discharge (weeks 12, 24 and 48), 5.4 mL of blood (depending on child weight; amount will not exceed 2
5 3 mL/kg total over 2 week period) will be collected by a study nurse into two 2.7 mL endotoxin-free EDTA
6 4 tubes.

7 5 ⁷HIV testing is conducted as part of routine clinical practice, but if it has not been undertaken, the study
8 6 sample will be used to test for HIV (see section 9.4), as stated in the informed consent form, since HIV
9 7 status is required to allocate children to study groups.

10 8 ⁸Full blood count results will be transcribed from clinical records; if not done by clinical teams, the EDTA
11 9 sample will be used to measure FBC in clinical laboratories at each site

12 10 ⁹A gastric juice sample will be collected at the same time as the blood draw by aspirating the
13 11 nasogastric tube with a sterile feeding syringe, to test for gastric pH; sterile water or saline will then be
14 12 instilled and a sample of gastric juice collected for storage for subsequent PCR and culture (section
15 13 7.5.2)

16 14 ¹⁰Stool collection will be undertaken at the same time as the blood draw

17 15 ¹¹Lactulose-mannitol testing will be conducted, with collection of a baseline urine sample, followed by a
18 16 2hr urine collection post-LM ingestion. This test will be deferred until children are judged to be clinically
19 17 stable by the study physician during daily reviews. In general, this will be a child in the nutritional
20 18 rehabilitation phase, who has no cardiorespiratory compromise.

21 19 ¹²Skinfold thickness (triceps, subscapular, supra-iliac) and mid-thigh circumference will be measured
22 20 using Holtain calipers or tape measure.

23 21 ¹³Body composition will be assessed by bioimpedance vector analysis.
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25 25 Caregivers are reminded of follow-up visits by phone, and visit completion is tracked on
26 26 a dedicated database. If caregivers do not attend follow-up appointments, attempts are
27 27 made to contact them by phone and home visits are made if feasible, particularly for
28 28 those defaulting the 48-week visit, so that long-term outcome data can be collected. For
29 29 post-discharge deaths, a home visit is undertaken by study nurses where possible to
30 30 conduct a verbal autopsy. Children who are readmitted to one of the study sites with
31 31 relapsed SAM have data collected during the new episode of hospitalisation. The study
32 32 ends for each participant at the week 48 visit.
33 33
34 34

34 34 **SUBSTUDIES**

35 35 As outlined in Figure 1, four nested substudies will utilise biological specimens to
36 36 address mechanistic questions related to enteropathy, microbiota, metabolomics and
37 37 immune function.
38 38

39 39 *Enteropathy substudy*

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2
3 1 The gut, which acts as an internal interface between humans and the environment, must
4
5 2 contain the nutrient stream and the symbiotic microbiota while allowing molecular
6
7 3 intimacy to permit absorption. The mechanism underlying this duality is the integrity of
8
9 4 the gastrointestinal barrier; intestinal damage (enteropathy) can impair this critical barrier
10
11 5 function. A spectrum of enteropathies affect children in developing countries³⁰.
12
13 6 Environmental enteric dysfunction (EED), characterised by small intestinal inflammation,
14
15 7 blunted villi and increased intestinal permeability, is almost universal and is
16
17 8 morphologically indistinguishable from HIV enteropathy³⁰. Children in resource-poor
18
19 9 settings also suffer from frequent diarrhoea, food insecurity and micronutrient
20
21 10 deficiencies, which all exacerbate enteropathy³⁰. As a result, a cycle of intestinal
22
23 11 infection, impaired mucosal function and malnutrition commonly arises, which may
24
25 12 ultimately precipitate SAM, especially in the context of HIV infection^{35 36}. It is not yet
26
27 13 established if the enteropathy seen in children with SAM³⁷, which we here refer to as
28
29 14 malnutrition enteropathy³⁷, is qualitatively or quantitatively distinguishable from EED. In
30
31 15 addition to local intestinal pathology, enteropathies may cause systemic pathology due
32
33 16 to persistent immune activation arising from enteric inflammation and microbial
34
35 17 translocation across the damaged gut wall³⁰. It is becoming apparent that chronic
36
37 18 inflammation may be particularly deleterious in malnourished individuals²³; in children
38
39 19 with SAM, systemic inflammation arising from underlying enteropathy may further
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41 20 increase morbidity and mortality.
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22 We hypothesize that i) the degree of enteropathy during hospitalisation differs between
23 oedematous and non-oedematous SAM and is independently associated with morbidity,
24 mortality and nutritional recovery during hospitalization; ii) the degree of enteropathy at
25 discharge is independently associated with morbidity, mortality and relapse of SAM; and

1
2
3 1 iii) children with HIV-SAM have more severe enteropathy than HIV-negative children with
4
5 2 SAM, which contributes to their poorer outcomes.
6

7 3
8
9 4 Using stored samples, a longitudinal series of investigations will compare gastric and
10
11 5 small intestinal barrier function, using a range of biomarkers to capture the domains of
12
13 6 malnutrition enteropathy (**Supplementary Table 2**). To understand better the extra-
14
15 7 intestinal consequences of enteropathy, we will first compare the microbial composition
16
17 8 of the upper gut and plasma using deep sequencing in a subgroup of children with
18
19 9 paired gastric and blood samples. Secondly, we will undertake transcriptomics using
20
21 10 PAXGene blood samples to determine i) whether there are differences in gene
22
23 11 expression profiles between well-nourished controls, HIV-negative children with SAM
24
25 12 and HIV-positive children with SAM (including comparison of oedematous and non-
26
27 13 oedematous types); and ii) whether specific patterns of gene expression are associated
28
29 14 with morbidity and mortality in SAM.
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35 16 *Microbiota substudy*

36
37 17 Normal assembly of the gut microbiota in early life is critical for many aspects of
38
39 18 physiological, neurological and immune development³⁸. Recent evidence suggests that
40
41 19 an immature or pathogenic microbiota plays a causative role in the pathogenesis of
42
43 20 SAM²⁵. For example, a number of microbial taxa have been identified, including
44
45 21 *Faecalibacterium prausnitzii*, which discriminate and predict gut microbiota maturity and
46
47 22 child growth²⁸. Other pathogenic microorganisms, including IgA-targeted
48
49 23 *Enterobacteriaceae*, are associated with impaired growth and may contribute to SAM³⁹.
50
51 24 Nutritional rehabilitation with RUTF induces temporary recovery of a disturbed
52
53 25 microbiota; however, the microbiota appears to revert back to an immature diseased
54
55 26 state following nutritional recovery²⁹. HIV infection is also associated with a disturbed gut
56
57
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60

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

5
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
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 HiSeq 2500 system. Raw metagenomic sequencing data will be quality-filtered and
25 analysed through a well-validated bioinformatics pipeline using MetaPhlAn⁴² 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.

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.

1

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 ¹H 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 prophylaxis¹⁷.
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 discharge²⁴.

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 techniques²⁷. Immune dysfunction
3 in SAM likely reflects both *intrinsic* defects, whereby immune cells lack capacity to
4 adequately respond to infection, and *extrinsic* defects, where cells have intact anti-
5 bacterial capacity but are chronically modulated by the systemic pro-inflammatory
6 environment which characterises SAM (i.e. heightened pro-inflammatory cytokines⁴⁴ and
7 circulating bacterial antigens^{23 56 57}). Systemic inflammation is directly associated with
8 mortality in SAM²³ and driven by multiple comorbidities, including bacterial translocation
9 from the damaged gut into the blood, sub-clinical infections and metabolic
10 dysregulation^{44 58 59}. The implications of innate immune cell dysfunction for subsequent
11 acquisition of infections and infectious mortality have not been investigated.

12
13 We hypothesise that: (i) anti-bacterial functions of innate immune cells are compromised
14 in SAM due to a combination of intrinsic and extrinsic defects; ii) innate immune cell
15 function is independently associated with infectious morbidity and mortality during
16 hospitalisation for SAM; and iii) nutritional rehabilitation only partly restores innate
17 immune cell function, leading to an ongoing risk of bacterial infections post-discharge.

18
19 Using blood samples collected at baseline, discharge and 12, 24 and 48 weeks post-
20 discharge, the longitudinal relationship between circulating innate immune cell function
21 and bacterial infections will be assessed. The intrinsic phagocytic capacity, secreted
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-
24 inflammatory mediators will be quantified at each time-point and the degree to which
25 these extrinsic factors influence innate immune cell antibacterial function will be
26 assessed via plasma co-culture with innate immune cells from healthy donors. Bacterial

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

4 **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
10 be 560 evaluable children at 48 weeks, of whom 224 would have HIV-SAM based on an
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

1 children with two-sided $\alpha=0.025$ provides >80% power to detect differences in mean
2 \log_{10} concentrations of at least 0.44 times their standard deviation, or 2.75-fold
3 differences between groups. Inclusion of well-nourished controls provides an indication
4 of normal ranges in young African children. HIV-positive and HIV-negative SAM groups
5 will be stratified to include approximately 50 children with and without oedematous
6 malnutrition, if possible.

8 *Microbiota and metabolomics substudy*

9 Power calculations are difficult in metagenomics and metabolomic analyses due to the
10 large number of observed outcomes and unknown effect sizes and variance. Previous
11 studies using smaller sample sizes have identified significant taxonomic differences in
12 twin pairs discordant for oedematous-SAM ($n=13$)²⁵ and metabolic differences between
13 the two forms of SAM ($n=40$)²⁶. These studies suggest that a difference of 50% in
14 metabolites could be expected. Using ANCOVA, setting $\alpha=0.05$ and assuming either low
15 ($\rho=0.1$) or high ($\rho=0.7$) correlation, the study would require 95-126 subjects to achieve
16 80% power⁶¹. False discovery rate (FDR) multiple correction testing will be applied to
17 reduce the high-dimensionality of the data and limit false-positives.

19 *Immunology substudy*

20 Up to 200 children with SAM and 200 well-nourished controls will be included in a cross-
21 sectional analysis of innate immune cell function during hospitalisation. Assuming similar
22 infectious mortality to a recent Kenyan study (15%)¹⁷, a cohort of 200 provides 80%
23 power to detect associations between immune profiles and infectious mortality at an
24 odds ratio of 1.7 and 2-sided α of 0.05. We will aim for 100 children with longitudinal
25 analysis of innate immune cell function at discharge, 12, 24 and 48 weeks post-
26 discharge¹⁷.

1

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:
26 haemoglobin, serum albumin, C-reactive protein, CD4 count and HIV viral load (for HIV-

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 months⁶²) 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 oedema⁶³. 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

1 associated with biochemical parameters⁶⁴. These variables will be explored using
2 graphical analysis, or transformed into age- and sex-adjusted Z-scores for statistical
3 comparison, including longitudinal analyses. Higher phase angle indicates better
4 nutritional status, while declining height-adjusted resistance over time indicates loss of
5 oedema.

6

7 *Enteropathy substudy*

8 The primary comparison for the enteropathy substudy will be between HIV-positive
9 children with SAM (group A) and HIV-negative children with SAM (group C), stratified by
10 presence or absence of oedema. Control groups (B and D) are well-nourished children
11 with or without HIV, to provide normative data for biomarkers and to evaluate the impact
12 of SAM within each HIV group. Thus, biomarkers among HIV-positive children with SAM
13 will first be compared to HIV-negative children with SAM (to evaluate the impact of HIV)
14 and, second, to well-nourished HIV-positive children (to evaluate the impact of SAM).
15 Biomarkers among HIV-negative children with SAM will be compared to well-nourished
16 HIV-negative children. For each continuous outcome, simple descriptive analysis will be
17 used to compare groups during hospitalisation using t-tests on appropriately transformed
18 data. For any outcome with moderate ($p < 0.05$) evidence of difference between either
19 group a regression model will be constructed including groups A, B, C, D to directly test
20 (using interactions) whether there is a synergistic effect of HIV-SAM versus HIV-negative
21 SAM versus HIV alone versus neither. These models will also be used to explore
22 whether there is any evidence for heterogeneity in effects between oedematous and
23 non-oedematous SAM. Associations between enrolment factors (e.g. intestinal
24 permeability and microbial translocation) will be explored using pairwise Spearman
25 correlations and principal components analysis. Mean changes at the follow-up time-
26 points in each group will be estimated, and groups compared (as above) using

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.

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 Benjamini-Hochburg false discovery rate
23 detection. Targeted analysis by qRT-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

1 at baseline (which include well-nourished controls). Similarities and differences in
2 longitudinal immune profiles will be compared between groups using nonmetric multi-
3 dimensional scaling^{65 67 68}. This approach will group children according to their composite
4 innate immune function, allowing the duration and variability of immune restoration to be
5 evaluated over the course of nutritional rehabilitation. Binary logistic regression will
6 determine whether innate immune profiles at discharge are associated with morbidity or
7 mortality during follow-up.

9 **PATIENT AND PUBLIC INVOLVEMENT**

10 Patients and their caregivers were not involved in the design of the study. During
11 recruitment, all caregivers of children admitted to hospital were given information about
12 the study; those whose children had severe acute malnutrition were approached to give
13 written informed consent. A meeting to disseminate results of the study to participants
14 and their caregivers will be held at the end of the study. An interactive game to engage
15 caregivers in the science underlying malnutrition is being developed in collaboration with
16 experts from the Centre of the Cell, a unique science education centre based at Queen
17 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
23 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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45 2 **DATA COLLECTION AND MONITORING**
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7 3 Clinical and demographic data are recorded on paper case report forms. All data are
8
9 4 checked for completeness and plausibility before data entry and problems flagged for
10
11 5 resolution by the clinical team. All data are double-entered onto a dedicated password-
12
13 6 protected online study database, and any discrepancies resolved. Study participants are
14
15 7 identified on electronic databases only by study numbers (assigned at enrolment); no
16
17 8 personal identifiers are entered.
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2122 10 **ETHICS AND DISSEMINATION**
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24 11 The study complies with the principles of the Declaration of Helsinki (2008) and is
25
26 12 conducted in compliance with the principles of Good Clinical Practice (GCP) and local
27
28 13 regulatory requirements in each country. Ethical approval was obtained from the
29
30 14 University of Zambia Biomedical Research Ethics Committee, the Joint Research Ethics
31
32 15 Committee of the University of Zimbabwe and the Medical Research Council of
33
34 16 Zimbabwe. The ethical review board of the Sponsor, Queen Mary University of London,
35
36 17 provided an advisory review of the study. Since this is an observational study, there is no
37
38 18 Data and Safety Monitoring Board.
39

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43 20 Results will be disseminated through conference abstracts and peer-reviewed publications
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45 21 and discussed with relevant policymakers and programmers. Study findings will be
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47 22 disseminated to families of participants at face-to-face meetings.
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5051 24 **TIME FRAME AND STUDY STATUS**
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3 1 Enrolment into the study began in July 2016 and is expected to end in March 2018. All
4
5 2 participants will be followed for 48 weeks, with an expected study completion date of
6
7 3 March 2019.
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11 5 **DISCUSSION**

13 6 HOPE-SAM aims to document the short- and long-term clinical and nutritional outcomes
14
15 7 of HIV-positive and HIV-negative children with SAM, and to identify the factors at
16
17 8 presentation and at discharge from hospital that independently predict these outcomes.
18
19 9 Mechanistic substudies aim to evaluate the contribution of enteropathy, microbiota,
20
21 10 metabolome and innate immune cell function to these clinical outcomes. The prevalence
22
23 11 of malnutrition in HIV-positive children is as high as 40% in some settings and the
24
25 12 challenges of managing this population are well recognised⁶⁹. The WHO protocol on
26
27 13 management of SAM aims to reduce case fatality below 10%, but rates as high as 35%
28
29 14 are still reported among HIV-positive children^{5 70}. No studies have systematically and
30
31 15 longitudinally collected morbidity data in HIV-SAM, or documented repeat
32
33 16 hospitalisations and mortality after discharge from hospital, particularly in the current era
34
35 17 where ART is available upon diagnosis. HOPE-SAM will provide a unique opportunity to
36
37 18 enrol and follow a cohort of children managed for SAM in three large hospitals across
38
39 19 two sub-Saharan African countries at several time-points over a one-year period. Nested
40
41 20 longitudinal laboratory substudies aim to better characterise the pathogenesis of SAM in
42
43 21 HIV-positive and HIV-negative children, to determine whether pathogenic processes are
44
45 22 normalised during nutritional rehabilitation and follow-up, and to identify potential
46
47 23 mechanistic pathways. Our ultimate goal is to utilise the findings generated in this study
48
49 24 to inform new intervention approaches that can be evaluated in clinical trials to improve
50
51 25 outcomes among children with SAM.
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22

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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

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17

18 COMPETING INTERESTS

19 None of the authors have any competing interests to declare.

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2
3 **1 FIGURE LEGENDS**

4 **2**
5 **3 Figure 1: Study flow chart.**

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7 4 All hospital admissions are screened for eligibility for the observational cohort and
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9 5 enteropathy sub-study, with procedures undertaken as shown in the flow chart during
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11 6 hospitalisation and post-discharge. Well-nourished children from outpatient clinics and
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13 7 the community meeting eligibility criteria as well-nourished controls are enrolled and
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15 8 undergo a single baseline assessment as shown. The immunology, microbiota and
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17 9 metabolomics sub-studies enrol children as shown. All children with SAM, regardless of
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19 10 which arm of the study they are enrolled into, are followed for 48 weeks post-discharge.

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22 11 ¹The immunology substudy started from 1st June 2017 and required children to have a
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24 12 blood sample >2mL to conduct cellular assays.
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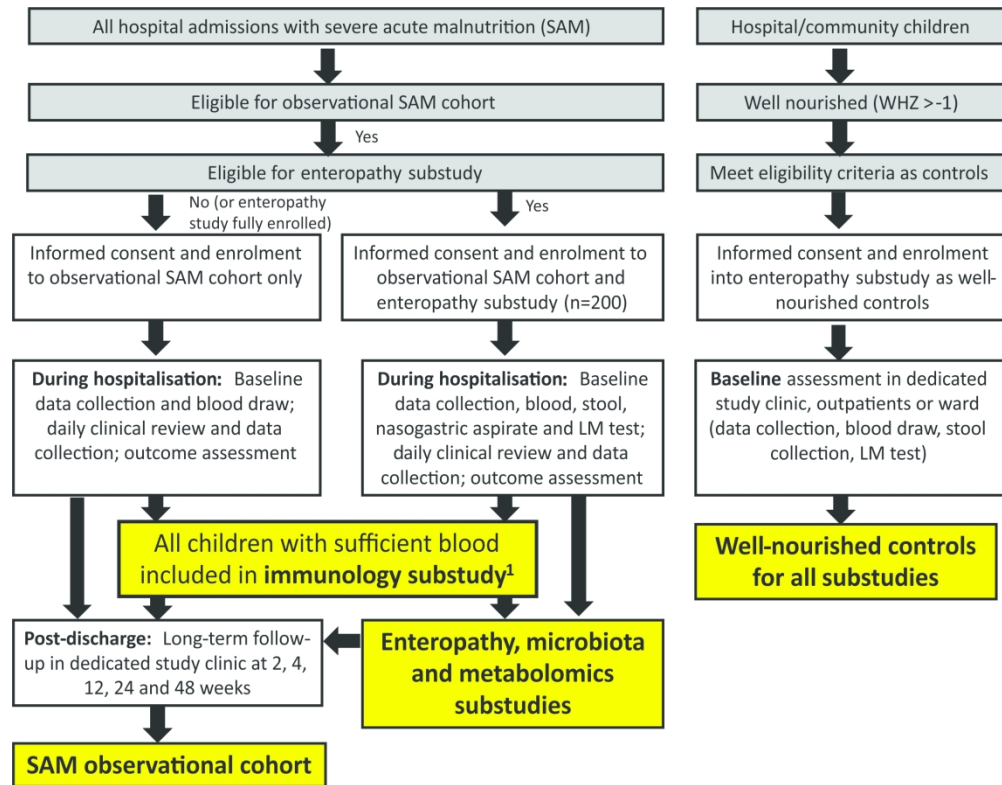
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For peer review only



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

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	X				
Blood	CD4 count (flow cytometry or PIMA) ¹	TROPGAN, Zvitambo or clinical sites	All HIV-positive	X	X	X	X	X
Plasma	HIV viral load (real-time PCR) ¹	TROPGAN, Zvitambo or clinical sites	All HIV-positive	X	X	X	X	X
Plasma	C-reactive protein (ELISA)	TROPGAN, Zvitambo	All	X	X	X	X	X
Plasma	Albumin (ELISA)	TROPGAN, Zvitambo	All	X	X	X	X	X
Plasma	Lipopolysaccharide (LAL assay)	TROPGAN and Zvitambo	Subgroup ³	X	X	X	X	X
Plasma	Lipopolysaccharide binding protein (LBP)	TROPGAN and Zvitambo	Subgroup ³	X	X	X	X	X
Plasma	sCD14 (ELISA)	TROPGAN and Zvitambo	Subgroup ³	X	X	X	X	X
Plasma	sCD163 (ELISA)	TROPGAN and Zvitambo	Subgroup ³	X	X	X	X	X
Plasma	IL-6, TNF-alpha, IL-1 β (ELISA) and/or multiplex cytokines	TROPGAN and Zvitambo	Subgroup ³	X	X	X	X	X
Plasma	Total PAMP activity (THP1 reporter cell line ²)	TROPGAN and Zvitambo	Subgroup ³	X	X	X	X	X
Whole	Molecular techniques	QMUL, London ²	Subgroup ³	X	X	X	X	X

blood	for bacterial detection (broad-range and specific PCR and next-generation sequencing)							
Immunology substudy only								
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	X	X
Plasma	Co-culture with healthy immune cells ⁵	QMUL, London	All	X	X	X	X	X

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-κB/AP-1 inducible reporter (SEAP) system to facilitate the monitoring of PRR-induced NF-κB/AP-1 activation.

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 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 Blizzard Institute, QMUL 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: pathogen-associated molecular pattern; QMUL: Queen Mary University of London; CRP: C-reactive protein; LAL: limulus amoebocyte lysate assay.

Supplementary table 2: Additional laboratory analyses for enteropathy substudy

Sample type	Assay (method)	Location of work	Study groups ¹	Baseline	Discharge	Week 12	Week 24	Week 48
Urine	Lactulose-mannitol ratio (mass spectrometry)	Orgeon Analytics, USA	A, B, C, D (all)	X	X	X		X
Stool	Neopterin, myeloperoxidase, alpha-1 antitrypsin and REG-1B (ELISA)	TROPGAN and Zvitambo	A, B, C, D (all)	X	X	X	X	X
Plasma	I-FABP (ELISA)	TROPGAN and Zvitambo	A, B, C, D (all)	X	X	X	X	X
Plasma	GLP-2	TROPGAN and Zvitambo	A, B, C, D (all)	X	X	X	X	X
Plasma	Citrulline (mass spectrometry)	Imperial College London	A, B, C, D (all)	X	X	X	X	X
Plasma	Kynurenine:tryptophan ratio and metabolites along tryptophan pathway (mass spectrometry)	Imperial College London	A, B, C, D (all)	X	X	X	X	X
Stool	Microbiome analysis ²	BCCDC, Vancouver	A, B, C, D (all)	X	X	X	X	X
Stool	<i>Helicobacter pylori</i> antigen	TROPGAN and Zvitambo	A, B, C, D (all)	X				
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				

RNA extracted from PAXGene tubes	Gene expression analysis (RNASeq)	QMUL, London	A, B, C, D (all)	X	X			
Plasma and urine	Targeted and untargeted metabolic phenotyping	Imperial College London	A, B, C, D (all)	X	X	X	X	X

¹Enteropathy substudy groups: Group A: HIV-positive children with severe acute malnutrition; Group B: HIV-positive well-nourished controls; Group C: HIV-negative children with severe acute malnutrition; Group D: HIV-negative well-nourished controls.

Note that controls only have blood taken at baseline.

²For 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

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